Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Nicotine (ISO); 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine

EC Number: 200-193-3
CAS Number: 54-11-5

CLH-O-0000001412-86-68/F

Adopted

10 September 2015

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON NICOTINE (ISO); 3-[(2S)-1-METHYLPYRROLIDIN-2-YL]PYRIDINE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.
All attachments including confidential documents received during the public consultation have been provided in full to the dossier submitter, to RAC members and to the Commission (after adoption of the RAC opinion). Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website.

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Substance name: nicotine (ISO); 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine
CAS number: 54-11-5
EC number: 200-193-3
Dossier submitter: Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	United Kingdom	Totally Wicked Ltd	BehalfOfAnOrganisation	1

Comment received

The submitter has warranted the need for action due to an increased number of accidents with nicotine containing e-cigarettes. The number of reported incidents has increased although this is logical due to the increasing popularity of nicotine containing e-liquids. There is very little, if any, toxicity data to warrant this submission. For this reason alone, reclassification of nicotine is not justified. The proposal is based upon poor scientific data, estimates and guesswork and the proposer has selected non validated studies to support their position. Credible scientific studies are ignored or dismissed as not acceptable yet the main study on which the submission is based (Lazutka et al. 1969) is 46 years old and cannot be replicated by any subsequent acute toxicity study. The scientific judgement used in selecting the most appropriate LD50 values is poor and biased towards an unjustified reclassification proposal. If nicotine was as toxic to humans as the Lazutka study states it is to mice, then we would have seen many cases of severe nicotine intoxications in humans over the decades of exposure. The proposal for reclassification should therefore not be implemented.

Dossier Submitter's Response

Thank you for your comment. For our response regarding the need for action see our response to comment 7.

In the dossier it is outlined why the studies were choosen upon which the classification is based. Age of a particular scientific study is not a reason to dismiss that study. Although the quality of the reporting of some of these studies is limited, some studies are considered acceptable given the period (pre-OECD and pre-GLP) in which they were performed. Moreover, recent GLP-compliant data from the same species were not available. However, it should be noted that in comment number 41, new studies regarding the acute toxicity of nicotine are provided. These studies are assessed and the results taken into account for the revised proposed classification in our response to comment 41.
Absence of nicotine intoxications in humans does not preclude classification, and, as outlined in the report, several cases of severe nicotine intoxication, some fatal, of both

adults and children have in fact been reported. These are described in the CLH report. Also, comment number 18 from the UK MSCA provides additional information on the increase of incidents regarding (un)intentional nicotine ingestion.
RAC's response
Thank you for the opinion. The data used for classification are evaluated based on their scientific value and according criteria of Regulation 1272/2008.

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	Finland		Individual	2
Comment received				
I am in favor of changing Acute Tox. 3* (oral) into Acute Tox. 1 (oral) based on the emergence of unregulated e-cigarette products on the market and their potential harm to toddlers. There are multiple cases of nicotine poisonings reported with infants and toddlers after accidental ingestion of e-cig liquids containing nicotine (Bassett, Osterhoudt, Brabazon, Nicotine Poisoning in an Infant N Engl J Med 2014; 370:2249-2250 June 5, 2014 DOI: 10.1056/NEJMc1403843; Cantrell, Clark More on Nicotine Poisoning in Infants N Engl J Med 2014; 371:880 August 28, 2014 DOI: 10.1056/NEJMc1407921). Also in New York, USA one-year old toddler (December 12, 2014) and earlier in Israel (May 29, 2013) 2-year old toddler have both died after ingestion of e-cig liquids containing nicotine. We need to perform every possible measure in order to avoid any more tragic events connected with toddlers and e-cigarettes, and I believe that this change is one of those measures.				
Dossier Submitter's Response				
Thank you for the support for our proposal to change the classification of nicotine for acute oral toxicity.				
RAC's response				
Thank you for the information, however in case of nicotine the human data alone are not sufficient for classification. The data used for classification will be evaluated based on their scientific value and according to criteria of Regulation 1272/2008.				

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	United Kingdom	JT International SA	BehalfOfAnOrganisation	3
Comment received				
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 LD50 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 LD50 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% (ED50) 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 LD50 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 LD50 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 LD50 determined with the classical LD50 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 LC50 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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[ECHA note: The following attachment was provided with the comment above:]
JTI comment on the RIVM proposal for a new CLP classification of nicotine

Dossier Submitter's Response

Thank you for your comment. Two points are disputed: JTI disagrees with the choice of the most relevant species for classification of acute oral toxicity (1), and disagrees with the classification for acute inhalation toxicity due to the lack of validity of the data extrapolation (2).

In response to point (1), and as outlined in the CLH proposal, the metabolism of nicotine is complex and differs between species. The available information indicates that the rat may be less relevant to humans due to differences in the main type of P450 responsible for metabolism between rats and humans. We agree that the half-live of nicotine in each species is more important than the type of P450 responsible for the metabolism. The data

on half-live indicating that rats are more relevant to humans than mice are supported by the ED50 values for seizures as determined by Matta et al., (2007). However, other factors besides half-life such as uptake and distribution are also relevant. The differences between the different tests in different species may also be caused by the method of oral administration. The gavage studies in the rat resulting in uptake via the gastro-intestinal tract cause lethalities after at least 50 minutes (Lazutka, 1969), whereas the studies by Franke and Thomas in dogs (1932) using drops into the mouth cause lethalities within a few minutes. This is probably due to the direct uptake of nicotine via the gums. This route is not possible when animals are exposed via gavage treatment. Direct uptake via the gums is considered relevant for human exposure to nicotine. An estimate of the minimal lethal dose in humans seems to be in the range of 6.5 – 13 mg/kg bw/day (Mayer, 2014), which is comparable to what was found in dogs. It can be concluded that the oral LD50 values in the rat using gavage exposure probably underestimate the human toxicity.
Here, it should be noted that in comment number 41, new studies regarding the acute toxicity of nicotine are provided. These studies are assessed and the results taken into account for the revised proposed classification in our response to comment 41.

In response to point (2); in the CLH report it is explained how the LC50 for nicotine was estimated. Indeed, the available acute inhalation data do not allow determination of an LC50 value. Based on the available data it can be estimated that the 4-hour LC50 is between 0.1 and 2.3 mg/L as an aerosol. According to the CLP criteria (footnote C to table 3.1.1), conversion of a one hour exposure to dusts and mists to a four hour exposure should be done by dividing with a factor of 4. At least this factor should be applied when extrapolating from 20 minutes to 4 hours. The use of a factor of 4 results in a LC50 value of 0.58 mg/L but probably even lower. Also the effects observed at 0.1 mg/L indicate that this exposure level is close to the LC50. Therefore, classification in category 2 (LC50 between 0.05 and 0.5 mg/L) seems justified. An LC50 value of 0.25 mg/L is suggested as ATE as this is in the middle between 0.1 and 0.5 mg/L.
The studies by Shao (2012) and Syversen (1999) are difficult to compare because no information on the external nicotine concentration is provided by Syversen. The lower nicotine blood concentration of 43.2 ng/L in the Shao publication corresponds with a 2 minute exposure to 1% nicotine solution. However, the LC50 of 2.3 mg/L corresponds with a 20 minute exposure to a 32% solution. The blood nicotine concentration was not measured but can be expected to be much higher than after 2 minutes exposure to 1% nicotine solution. Therefore, the results of this study do not contradict the LC50 determined by Shao (2012).

RAC's response

Thank you very much for the thorough analysis of the literature.

Your disagreement with the classification proposed of the Dossier Submitter has been noted as well as your justification of this disagreement.

As for many other chemicals at present it is not possible to provide sufficient proof that the toxicity data generated on mice or rats are more relevant for human hazard assessment, therefore the rule of using the most sensitive species will be followed.

It is agreed that the data on LD_{50} provided by Van den Heuvel *et al.* study (1990) on rats are reliable, however, in the RAC opinion also other data for mice and dogs have to be considered, and the principle to use the LD_{50} for the most sensitive species should be followed.

It is agreed that the Shao *et al.* study (2012) provides good data, but creates a problem

with extrapolation to 4-hour LD_{50}. Two approaches have been used to derive 4-hour LD_{50} values: one by the Dossier Submitter and one by RAC.

The comparison of data from the acute inhalation toxicity (Shao *et al.* study (2012) with data from the 2-years study (Syversen *et al.* 1999) for acute hazard assessment and classification is rather not supported due to various purposes of these studies.

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	Netherlands	Fontem Ventures	BehalfOfAnOrganisation	4

Comment received

Fontem Ventures disagrees with the proposal to change the oral acute toxicity and the proposed inhalation classification. In Fontem Ventures' scientific opinion, the current oral toxicity classification is still valid and the inhalation classification should be Category 3.

Fontem Ventures strongly disagrees with the comment in the proposal that there is a need for action at the community level.

The CLH report claims action is needed due to the increase in accidents with e-cigarette refills and its increasing popularity. Fontem believe the current oral classification is still valid as recent incidences of accidental nicotine exposure have resulted in minimal adverse findings (Ordonez, J.E. Kleinschmidt, K.C. Forrester, M.B. [2014]; Vakkalanka, J.P. Hardison, L.S. Holstege, C.P. [2014]).

Also, the misconceptions underlying the toxicological risk posed by nicotine to the human population, by the oral route, has recently been reviewed by Mayer (2014).

Please see uploaded document for Fontem Ventures' specific comments on inhalation toxicity of nicotine, and oral toxicity of nicotine.

[ECHA note: The following attachment was provided with the comment above:]
Submission challenging nicotine CLP reclassification proposal

Dossier Submitter's Response

Thank you for your comments regarding the classification of nicotine. Three points are disputed: (1) the need for action at the community level, (2) the relevant species for determining the oral LD50 and (3) the estimation of the LC50 for inhalation.

Regarding (1), please see below:
We agree that the increase in accidents is mainly caused by the increase in the use of e-cigarettes. However, we do not agree that this only results in minimal toxicity. There are many reported cases of children ingesting e-liquid resulting in more severe effects, including lethality (see comment 2). The current minimal acute oral classification in category 3 (ATE = 50 mg/kg bw) would allow marketing of refills without child protective closures at concentrations up to 167 mg/ml (50 mg/kg bw / 300 mg/kg bw * 100%). For example the case described by Bratschat (2014) shows that the intake of three 50 ml bottles containing 72 mg/ml nicotine each, and a body weight of 72.5 kg is lethal. This corresponds with an oral dose of 149 mg/kg. For a child of 10 kg bw, this would mean that 9 ml of the highest concentration without protective closure could be fatal. Probably even lower dose levels can be fatal as this may have been a clear overdose. Therefore, a more stringent classification for acute oral toxicity at the community level resulting in the requirement of child resistant fastenings at lower concentrations is considered justified.

Regarding (2) and (3), we would like to refer to the explanation we provided on these issues in comment number 3, above, where similar points were raised.
The stated inhalation exposure by Caldwell et al (2012) seems very unrealistic as 130000 mg/L with humans inhaling approximately 16 L/minute (20 m3/day) corresponds with 2080000 mg/minute or 2 kg per minute. Inhalation of such amounts of any substance would cause severe toxicity.

RAC's response

Thank you for the opinion. Your disagreement with the classification proposed by Dossier Submitter has been noted as well as your justification of this disagreement.

The data used for classification are evaluated based on their scientific value and according to the criteria of Regulation 1272/2008.

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	Finland		MemberState	5

Comment received

In general, it appears that CLH report could have been elaborated more. Only a summary on toxicokinetics is presented and the text is directly quoted from previously published reports/articles without any updates or further analysis. Most of the studies presented in the report are inadequately described. It raises a question how justified conclusions can be drawn based on the presented results. Nevertheless, the data implies that the classification for oral toxicity of nicotine may need to be reconsidered. It should be confirmed that all relevant data is included in the report.

Dossier Submitter's Response

Thank you for your comment. This CLH report is limited to classification for acute toxicity. The section on toxicokinetics was added to describe the metabolism of nicotine.
All studies available to us regarding acute toxicity were summarized; all relevant data from each study is indeed included in the report. It is noted in the CLH report when particular studies were not available to us. Those were not further reported on. Although the quality of the reporting of some of these studies is limited, some studies are considered acceptable given the period (pre-OECD and pre-GLP) in which they were performed. However, it should be noted that in comment number 41, new studies regarding the acute toxicity of nicotine are provided. These studies are assessed and the results taken into account for the revised proposed classification in our response to comment 41.

RAC's response

Thank you for the opinion. The more thorough description of studies would be appreciated, however most of them are not accessible, and therefore RAC makes its opinion based on available data taking into account original studies used in the CLH report if available and results of acute toxicity studies submitted during public consultation.

Date	Country	Organisation	Type of Organisation	Comment number
04.06.2015	France		MemberState	6

Comment received

Page 5: the minimum purity is not in agreement with the monograph submitted for the inclusion in annexe I of directive 91/414 (min 950g/kg). Moreover, only 3 impurities have been specified in the monograph: Myosmine at 0,25% ; Anatabine 0,8% and water at 4%.

Impurities reported in the CLH report should be considered as confidential.

Dossier Submitter's Response
Thank you for your comment. The information on purity and impurities comes from the European Pharmacopoeia and is therefore not considered confidential.
RAC's response
Thanks for the remark which should be taken into account in the preparation of final background document.

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2015	United Kingdom	Nicoventures Ltd.	BehalfOfAnOrganisation	7

Comment received

There is a lack of need for action at the community level.

Appropriate hazard classification is required in order to ensure appropriate protection of workers and the public. There would thus be a need to reconsider the current classification if there was evidence of harm to workers or the public as a result of inappropriate hazard classification of nicotine or its mixtures. In this context, the CLH report (in section 3) claims reclassification is required because of the growing incidence of accidental exposures to vaping products. However, although incidence is increasing with increased use, the prevailing absence of symptoms or minimal toxicity experienced as a result of the accidental nicotine exposures (Vakkalanka, Hardison, Jr. et al., 2014;Chatham-Stephens, Law et al., 2014;Ordonez, Kleinschmidt et al., 2014), indicates the current acute toxicity labelling is effective and no reclassification is required.
The misconception on the acute toxicity risk nicotine presents to humans, that is evident in the CLH report's "Justification that action is needed at the community level", is quite widely spread in the literature. The data underlying this misconception has recently been reviewed (Mayer, 2014). The actual data on human exposures does not support the perceived high acute toxicity of nicotine. As per one of the reports on accidental exposures to vaping products: "Most of our cases had no effects or mild manifestations. Considering how highly concentrated the solutions might be, this relatively low toxicity was surprising." (Ordonez, Kleinschmidt et al., 2014)

The bibliography for this general comment and the specific comments is provided in the attached file.

[ECHA note: The following attachment was provided with the comment above:]
Supporting information for Nicoventures' response to RIVM's nicotine CLH report

Dossier Submitter's Response

Thank you for your comment. We agree that the increase in accidents is mainly caused by the increase in the use of e-cigarettes. However, we do not agree that this only results in minimal toxicity. There are many reported cases of children ingesting e-liquid showing more severe effects including lethality (see comment 2). The current minimal acute oral classification in category 3 (ATE = 50 mg/kg bw) would allow marketing of refills without child protective closures at concentrations up to 167 mg/ml (50 mg/kg bw / 300 mg/kg bw * 100%). For example the case described by Bratschat (2014) shows that the intake of 3 50 ml bottles containing 72 mg/ml nicotine each, and a body weight of 72.5 kg is lethal. This corresponds with an oral dose of 149 mg/kg. For a child of 10 kg bw, this would mean that 9 ml of the highest concentration without protective closure could be fatal. Probably even lower dose levels can be fatal as this may have been a clear overdose. Therefore, a more

stringent classification for acute oral toxicity at the community level resulting in the requirement of child resistant fastenings at lower concentrations is considered justified.

RAC's response

In our view there is a need for action at the community level since nicotine is used in all European Union member countries, and there are differences in the opinion on its classification and labelling, therefore harmonisation of its classification is justified.

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2015	Italy	University of Catania	BehalfOfAnOrganisation	8

Comment received

The Dutch National Institute for Public Health and the Environment, RIVM has proposed a reclassification of nicotine toxicity. What is this based on?
Oral toxicity data, LD50 from tests in rats and mice. The issue has been raised because of cases of children dying from swallowing e-juice and results in mice (which are more sensitive than rats). Inhalation studies are not available and are more complicated to run hence the recommendation is based on related studies. The suggested reclassification to category 1 is very conservative.
CLP is only for labelling of pure products, so why is this relevant?
Labelling is relevant both for ingredients and the final product, has implications for occupational health and the supply chain, and for public relations. Labelling is concentration dependent in the final product; anything than less than a certain level remains unclassified while other warnings may range from "harmful if swallowed" to "toxic if swallowed". It would be incorrect to label an inhalation product "fatal if inhaled". It is essential to obtain the inhalation data; test pure nicotine on a batch of animals to find the LD50.

The statement "fatal if inhaled" is associated with the LD50 for lab animals and does not say that people always die if they inhale it.
The statement "fatal if" is nonsense without a dose since anything is toxic above a certain level. This is not a scientific question but a PR and legal question. "Fatal if swallowed/inhaled" refers to pure nicotine, different statements are made for the different concentrations. For 12mg/ml products the labelling will go from harmful to toxic if swallowed.

Dossier Submitter's Response

Thank you for your comment. CLP is for labelling of both substances and mixtures. Classification is based on animal test data and, if available, human data, as described in the guidelines. Hazard statements for acture toxicity depend on the classification category, which in turn is based on LD50 studies. Which hazard statement comes with which catergory, is described in the CLP guidelines. It is important to note that labelling is not only meant to warn against lethality, but also to other/milder forms of acute toxicity.

RAC's response

Thank you for the general considerations and reflections on classification and labelling.

It also important to note that hazard classification is identification of hazardous properties of the substance and should not be mistaken with the risk assessment, which determines the probability of the definite effect e.g. death to occur, which is a dose-dependent phenomenon.
So even for highly acutely toxic substance classified to category 1 or 2 the ingestion or inhalation or dermal exposure to very small amount of this substance will not cause death or poisoning, as is a case with nicotine.

Classification of hazards and subsequent labelling are aimed at providing information to the users on the need to limit the amount of the substance available for absorption to human organism. It is also important to note that labelling of the product which depends on the classification of containing substances, is dependent on the concentration of this substances in this product (mixture of substances).

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2015	Italy	LIAF Lega Italiana Anti Fumo	BehalfOfAnOrganisation	9

Comment received

Challenge of pure nicotine reclassification by RIVM

Background

The Dutch National Institute for Public Health and the Environment, RIVM has proposed a reclassification of nicotine toxicity for the purpose of nicotine labelling (CLH report).
They propose to reclassify pure (i.e. 99.9%) nicotine as follows:
• From oral acute toxicity category 3 (Toxic if swallowed) into oral acute toxicity category 1 (i.e. Fatal if swallowed),
• To add inhalation acute toxicity category 2 (i.e. Fatal if inhaled)
• Retain the dermal acute toxicity category 1 (i.e. Fatal in contact with skin).

Implications for vaping industry

• Potential effects on manufacturing practices where pure nicotine is handled
• Adverse publicity for e-liquids, i.e. an inhalation from e-products "fatal if inhaled"
• Additional labelling requirements.

Grounds for challenge
1. Community need

CLH Report's justification for why action is needed is summarised below:

1. "…the current policy discussions on the use of the e-cigarette, the increase in accidents with e-cigarette refills and its increasing popularity"
2. Current acute oral toxicity classification for nicotine is a "minimum classification". That means manufacturers and importers should investigate if a more severe classification applies.

Grounds for challenge:
Although incidence is increasing with increased use with millions consumers worldwide, the absence of symptoms or minimal toxicity experienced as a result of the accidental nicotine exposures indicates the current labelling of both nicotine and the 'mixtures of nicotine' (i.e. e-liquids) is effective enough and no reclassification is required.

Grounds for challenge
2a. Toxicological classification details – Acute Oral Tox

CLH Report in brief

• Metabolism differences between rats and humans means the rat is not the appropriate species to base oral tox classification on.
• Lowest available LD50 is from mice: 3.34 mg/kg hence warranting category 1 classification (most restrictive).

Grounds for challenge:
CLP guidelines are to use rat as preferred species, not the most sensitive species.
Based on total nicotine hepatocyte metabolism, the metabolism differences are bigger between mice and humans than between rats and humans. Therefore, the default ECHA CLP guidance that rat is the preferred species, still stands. Last but not least, all rat oral acute toxicity studies indicate category 3 oral toxicity (less restrictive).

Grounds for challenge
2b. Toxicological classification details – Acute Inhalation Tox

CLH Report in brief:

No standard LC50 study with nicotine inhalation exposure exists in the public domain. The proposed classification is based on a single 20 minute acute toxicity study with conventional tobacco cigarettes, where the most conservative value was used and a factor of 4 was applied to account for the accumulation of dose that might have taken place had the exposure been over the required 4 hours.

Grounds for challenge:

In the absence of appropriate animal LC50 data (as in this case), ECHA recommends a weight of evidence approach that includes human experience. Decades of occupational, smoking and NRT experience, as well as the more recent vaping, does not indicate lethality concerns via inhalation.
Recommended NRT dosages equate up to 0.027 mg/L and it is recognised as a safe and well tolerated treatment. Similar nicotine levels are measured with current e-vapour products. Moreover, peak arterial nicotine levels from tobacco smoking are typically around five times higher than those from NRT and current e-vapour products. Hence, ECHA recommendation cannot be extended from human experience with the vastly more efficient tobacco cigarette to other less efficient nicotine containing products.

Grounds for challenge
2c. Toxicological classification details – Acute Dermal Tox

CLH Report in brief

• Nine acute dermal exposure studies in rats, rabbits and one is cats, are referenced. Dermal LD50's reported are 50 - >360 mg/kg bw.
• Classification is effectively based on the most sensitive rabbit study (50 mg/kg bw)

Grounds for challenge

First, the most sensitive rabbit study only reported an estimated LD50, which is not accurate. Therefore, the other studies should be considered as well. The only two the CLH report indicates as acceptable are LD50 = 285 mg/kg in rats (nicotine sulfate) and 66-100

mg/kg in cats (40% dilution). The range of all the reported LD50's is 50 - >360 mg/kg bw. Second, the cat dermal study used a 40% aqueous solution, whereas classification is based on the toxicity of the pure compound. Nicotine dermal penetration shows a parabolic dependence on nicotine concentration where 100% nicotine had similar flux as 1% w/w nicotine aqueous solution. It is thus likely the pure nicotine is less dermally toxic than what was tested on the cats. Category 2 rather than category 1 would be more appropriate.

[ECHA note: The following attachment was provided with the comment above:]
Pure nicotine reclassification by RIVM

Dossier Submitter's Response

Thank you for your comments regarding the classification of nicotine. Several points are disputed: (1) the need for action at the community level, (2) the relevant species for determining the oral LD50, (3) the estimation of the LC50 for inhalation, (4) classification for acute dermal toxicity.

Regarding (1), we would like to refer to the answer we gave on comment number 7.
Regarding (2) and (3), we would like to refer to the answer we gave on comment number 3.
Regarding (4), classification of acute dermal toxicity.
The current harmonised classification with Acute Tox. 1 is based on a dermal study in rabbits with an LD50 value of 50 mg/kg bw which is only available to us as a reference. As such this study would not be acceptable to propose a new harmonised classification. A new dermal study in rabbits has been performed. This study is assessed and the results taken into account for the revised proposed classification in our response to comment 41.

RAC's response

Thank you for the considerations and reflections on classification of nicotine. The ECHA guidelines recommend rats for testing of acute oral, inhalation and dermal toxicity, however data on acute toxicity in other species can be used for classification. In fact as it is written in: Annex I: 3.1.2.2. Specific considerations for classification of substances as acutely toxic: "When experimental data for acute toxicity are available in several animal species, scientific judgement shall be used in selecting the most appropriate LD50 value from among valid, well-performed tests." "In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested."

It also important to note that hazard classification is identification of hazardous properties of the substance and should not be mistaken with the risk assessment, which determines the probability of the definite effect e.g. death to occur, which is a dose-dependent phenomenon. So even for highly acutely toxic substance classified to category 1 or 2 the ingestion or inhalation or dermal exposure to very small amount of this substance will not cause death or poisoning, as is the case with nicotine.

So even for highly acutely toxic substance classified to category 1 or 2 the ingestion or inhalation or dermal exposure to very small amount of this substance will not cause death or poisoning, as is a case with nicotine.

Classification of hazards and subsequent labelling are aimed at providing information to the users on the need to limit the amount of the substance available for absorption to human organism. It is also important to note that labelling of the product which depends on the classification of containing substances, is dependent on the concentration of this substances in this product (mixture of substances).

Date	Country	Organisation	Type of Organisation	Comment number

03.06.2015	United Kingdom	ECITA	BehalfOfAnOrganisation	10

Comment received

ECITA is a trade association with membership based mainly in the UK, although its members trade across the whole of the European Union, and indeed globally. Although ECITA has a significant focus on consumer protection, we have concerns about the nature of the changes contained in this CLH proposal, and the poor level of scientific justification for them. Changes to the harmonised classification have the potential to have significant effects on the transport and supply of these products, with the potential for significant increases in the costs to business. (The vast majority of the businesses operating in this sector are small to medium enterprises (SMEs).) This would inevitably have an effect on the price consumers pay for the products potentially damaging their value proposition as an alternative to cigarettes. Recent Eurobarometer survey data (http://ec.europa.eu/public_opinion/archives/ebs/ebs_429_en.pdf)has identified price as a significant factor in selection of electronic cigarette products, making this a cause for significant concern .

The survey also highlighted that the number of people who consider electronic cigarettes harmful has increased, an aspect covered in more detail in the UK by Action on Smoking and Health, ASH. ASH data http://www.ash.org.uk/files/documents/ASH_891.pdf) indicate that the number of people who incorrectly believe that electronic cigarettes are as harmful as smoking has increased dramatically, leading ASH CEO Deborah Arnott to comment:

"The number of ex-smokers who are staying off tobacco by using electronic cigarettes is growing, showing just what value they can have. But the number of people who wrongly believe that vaping is as harmful as smoking is worrying. The growth of this false perception risks discouraging many smokers from using electronic cigarettes to quit and keep them smoking instead which would be bad for their health and the health of those around them."

This is echoed by Dr Leonie Brose, who was involved in the statistical analysis of the data:

"We must clearly communicate the relative safety of electronic cigarettes to smokers. The proven harm of tobacco is currently getting less coverage than the much smaller and far less certain harm from electronic cigarettes. We owe it to smokers to provide them with accurate information."

An increase in the perceived danger of nicotine that is not justified, by robust scientific methodology and data, runs a very real risk of causing harm through unintended consequences.

At 4.2.2 RIVM cite that "[t]hey report 35 cases – 4 in 2010, 12 in 2011 and 19 in 2012. Age range 8 months to 60 years. Reported symptoms were mild and transient. Product concentrations ranged from 4 to 30 mg of nicotine per ml", Cantrell (2015).

The increase in the number of exposures can be expected to follow the increase in the availability of the products, something identified in the cited research. However, as the symptoms were categorised as "mild and transient" this does not seem to make a case for a change in classification. Indeed, the reference cited states that "[o]ur modest results suggest that adverse effects and accidental exposures to ECIG cartridges are unlikely to result in serious toxicity."

However, despite an increasing number of exposures to weak nicotine solutions, these have not resulted in serious toxicity. UK Poison Information Service data for 2013/14 indicated

204 reported exposures, of which 21 involved intentional overdose. Of these 204, 103 had no features of toxicity, and 94 had only mild toxicity. Only one case of severe toxicity was reported (although it is not clear if this was an accidental or deliberate exposure), and no deaths. No deaths have been reported as a result of either intentional inhalation of nicotine vapours via electronic cigarettes, or through accidental exposure to the concentrations found in the European market.

This would seem to indicate that there is not a pressing need to address the issue of reclassification.

If a change to the current classification is to be justified, it should be supported by a robust justification for the choice of key species for any given exposure. Given the age of much of the available data, it should also ideally include new toxicological data for any given route and species. This would resolve the current ambiguity surrounding much of the literature data.

In the absence of robust data, a change to the classification would risk significant unintended consequences, particularly if such a change increased costs and/or reduced the availability of affected products, with the associated ramifications for SMEs and consumers.

[ECHA note: The following attachment was provided with the comment above:]
ECITA response to proposal submitted for harmonised classification and labelling of nicotine, CAS 54-11-5, EC 200-193-3.

Dossier Submitter's Response

Thank you for your comment; it is argued that there is no need to address the issue of reclassification. We disagree, and would like to refer to comment number 7 as to why we disagree.
Regarding the justification for the choice of species for the acute oral toxicity classification, we would like to refer to the answer we gave to comment number 3, point (1).
Pertaining to new data, we would like to refer to comment number 41; here new studies regarding the acute toxicity of nicotine are described. These studies are assessed and the results taken into account for the revised proposed classification in our response to comment 41.Downstream consequences such as costs due to a change in classification are not relevant to the CLH proposal. As such, we cannot comment on this issue.

RAC's response

The Dossier Submitter provided sufficient information to assess acute toxicity of nicotine. The data used for classification are evaluated by RAC based on their scientific value and according criteria of Regulation 1272/2008

It also important to note that hazard classification is identification of hazardous properties of the substance and should not be mistaken with the risk assessment, which determines the probability of the definite effect e.g. death to occur, which is a dose-dependent phenomenon.
So even for highly acutely toxic substance classified to category 1 or 2 the ingestion or inhalation or dermal exposure to very small amount of this substance will not cause death or poisoning, as is a case with nicotine.

Classification of hazards and subsequent labelling are aimed at providing information to the users on the need to limit the amount of the substance available for absorption to human organism. It is also important to note that labelling of the product which depends on the classification of containing substances, is dependent on the concentration of this substances in this product (mixture of substances).

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON NICOTINE (ISO); 3-[(2S)-1-METHYLPYRROLIDIN-2-YL]PYRIDINE

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2015	Germany		MemberState	11
Comment received				
DE supports the CLH proposal. Nevertheless we noticed that some clarification concerning the water solubility would be helpful. The stated water solubility of 1000 g/L at unknown temperature and pH should be elucidated. Different sources state diverse solubilities. Taken the given pKa values and the partition coefficient into account the water solubility seems to be pH dependent whereas the ionic form of nicotine should be more soluble in water than the non-protonated form.				
Dossier Submitter's Response				
Thank you for your support for the CLH proposal. Regarding the water solubility, this is based on information provided in the registration dossier for nicotine. Water solubility was recently determined (2014) according to OECD 105. Nicotine was found to be miscible with water in all proportions, and gives an alkaline solution when mixed with water.				
RAC's response				
Your support to the proposed classification is noted. The solubility of nicotine in water depending upon pH and temperature will be clarified by the Dossier Submitter.				

Date	Country	Organisation	Type of Organisation	Comment number
02.06.2015	United Kingdom	New Nicotine Alliance	BehalfOfAnOrganisation	12
Comment received				
NNA wants consumers to have accurate information about nicotine containing products, and for that information to be based on the basis of good science. Our disquiet about the proposed re-classification and labeling of nicotine is that (a) the justification for action now at a community level has not been established and (b) the weak evidential basis for the proposed changes and additions to the classifications. We believe that consumers of nicotine need accurate assessment of the hazards of the products that they consume, but that the proposed re-classification does not provide this. (a) The assessment used for justification for action at a community level (sec3) RIVM argues that re-classification is justified given policy discussion on the use of e-cigarettes, and the increase in accidents with e-cigarette refills and their increasing popularity (para 3). It should be noted that no e-cigarette users handle nor consume pure nicotine. Consumers buy strengths of 0 - 4% (0-40mg/ml) mixed ready for use. 7.5% is the maximum concentration that can be purchased in the UK without a poisons licence. With the transposition of the European Tobacco Products Directive in 2106 the maximum strength available will be 20mg/ml. The proposal provides no evidence of acute incidents or fatalities in supply side occupational exposures. Para 4.2.2 notes an increase in exposures trough e-cigarettes. However the epidemiological study of adverse events and fatalities needs to take into account not only the number of incidents (which is small), but also calculate these in terms of population at risk rates, and the consequences of exposure.				

Despite the extremely long history of the use of tobacco products, the three decades of use of nicotine replacement therapy products and coming up for a decade of the use of electronic cigarettes, fatalities linked to nicotine are extremely rare. The RIVM report mentions one fatality following the ingestion of an estimated 5000mg of nicotine – which appears to be a suicide.

E-cigarettes were introduced to the European market around 2007 and it is estimated that there are now between around 29 million e-cigarette users in the European Union (Vardavas et al 2014). There are no European wide data on reports to poison centres. UK Poisons reports for 2013/14 indicate 204 reported exposures (Public Health England 2014), Most cases were not associated with serious toxicity. This level if incidents has to be seen in the context of 2.1 million users of e-cigarettes that year (Action on Smoking and Health 2014). Given the widespread use of e-cigarettes, the rate per population at risk (consumers, and others exposed to potential risk such as infants) is extremely low.

Reports to US poison centres regarding exposure to e-cigarettes increased from 271 in 2011, 416 in 2012, 1553 in 2013 and to 3957 in 2014. To put these reports into context, there were 2.6 million calls to US poison centres in 2013 and 0.06% of those related to nicotine. The term 'exposure' means someone who has had contact with a substance in some way, for example by ingestion, inhalation, skin absorption – not all exposures are poisonings or overdoses, indeed many calls to poisons centres are for information. To put the e-cigarette exposure data into further context, in 2013 there were 298,633 calls to poison centres regarding analgesics and 199,838 with respect to cosmetics (American Association of Poison Control Centres, 2013).

Most acute incidents involving excessive nicotine exposure do not result in serious outcomes in humans. The most common symptoms include nausea, vomiting, diarrhoea, weakness or dizziness. Symptoms are transient and rarely require any therapeutic intervention.
The evidence shows that with increasing use of e-cigarettes there is an absence of symptoms or minimal toxicity experienced as a result of accidental nicotine exposures. Adverse effects to e-cigarettes are rare in normal use and include cough, dry mouth, and headache. No serious adverse effects have been reported in the Cochrane Review (McRobbie et al) and the level of adverse effects reporting was similar in electronic cigarette, NT and placebo groups.

There is extensive reporting of e-cigarette use on user forums which is consistent with adverse effects being rare and minor. We are unaware of any major serious adverse nicotine effects from the e-cigarette user forums. Indeed, with the widespread use of e-cigarettes, if there were serious acute effects these would have been well documented by now.

In conclusion, the current data do not appear to suggest justification for changes in classification at this time.

(b) Evidential basis for establishing LD50 and LC50

Acute oral toxicity.

The RIVM review indicates the dearth of information on which to establish an LD50. The study by Lazutka et al on mice (1969) is used to determine acute oral LD50. The RIVM proposal notes the difficulty of choosing the appropriate animal model for choosing a human LD50. There are large differences in metabolism of nicotine across species, and RIVM has chosen the most sensitive species (mice) without clear justification. The preponderance of

evidence from rat studies puts the LD50 for rats (all at 50mg/kg or more) much higher than for the Latuzka et al mouse study (3.3 mg/kl). It should also be considered that for humans, nicotine is emetic and it is extremely difficult to ingest large doses of nicotine. CLP guidelines are to use the rat as the preferred species, and not to select the most sensitive species (Regulation 1272/2008). The LD50 from rat studies would suggest Category 3. If one were to be more cautious, and taking into consideration the arguments put forward by Mayer (2014) then the data from the dog study (Franke and Thomas, 1932) would suggest Category 2.

Acute inhalation toxicity

The RIVM proposal notes that 'the available acute inhalation data do not allow determination of an LC50 value'. In such circumstances the Guidance on the Application of CLP criteria (ECHA 2013) state that it is necessary to take a 'weight of evidence approach' that includes human experience such as occupational data and data from accident databases, epidemiological and clinical studies and well-documented case reports and observations (para 1.1.1.3). As we argue above, decades of occupational, smoking and NRT experience, as well as the more recent widespread experience of vaping, does not indicate acute toxicity concerns via inhalation.

The committee may wish to check the proposed LC50 ATE of 0.25mg/l against the recommended NRT dose for the nicotine inhalator.

Whilst the increase in consumer use of nicotine via the inhalation route might suggest a need to examine inhalation toxicity, there is no current evidence to determine what the LC50 might be and we consider it premature and potentially misleading to consumers to determine an LC50 until adequate studies have been conducted.

(c) Damage to public confidence in e-cigarettes and nicotine replacement therapy products

The public need confidence that the scientific basis for the classification of nicotine is based on robust studies and robust evaluation of the evidence.

We see major negative effects of the proposed classifications for the public perception of nicotine, and in particular for current consumers of electronic cigarettes and for smokers considering switching from smoking cigarettes to using nicotine by other means (e-cigarettes, nicotine replacement therapy products). The suggestion that nicotine is fatal if inhaled or ingested will clearly raise concerns among consumers of nicotine products.

We reiterate that the public and consumers of nicotine products require accurate assessment of the risks of consuming these products, but that this is not provided in the RIVM proposal.

References

Action on Smoking and Health. (2014). Use of electronic cigarettes in Great Britain, (April 2014), 1–5.
American Association of Poison Control Centres (2013) Annual Report

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Franke FE, Thomas JE (1932). A note on the minimal fatal dose of nicotin for unanesthetized dogs. Proc Soc Exp Biol Med 29:1177-1179.

Mayer B (2014). How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century. Arch Toxicol 88, 5-7.

McRobbie, H., Bullen, C., Hartmann-Boyce, J., & Hajek, P. (2014). Electronic cigarettes for smoking cessation and reduction. The Cochrane Database of Systematic Reviews, 12, CD010216. doi:10.1002/14651858.CD010216.pub2

Public Health England 2014 National Poisons Information Service Report 2013/14

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.

Vardavas, C. et al (2014). Determinants and prevalence of e-cigarette use throughout the European Union: a secondary analysis of 26 566 youth and adults from 27 Countries. Tobacco Control, 1–7. doi:10.1136/tobaccocontrol-2013-051394

Dossier Submitter's Response

Thank you for your comment.
Regarding point (a), action at community level, we would like to refer to the answer we gave on comment number 7.
Regarding point (b) establishing LD50, we would like to refer to the answer we gave on comment number 3.
In addition, we agree that the criteria state that the rat is the preferred species to evaluate the acute oral toxicity. However, the criteria also state that when experimental data is available for other species, scientific judgement shall be used in selecting the most appropriate LD50 value from among valid, well performed tests. The CLP guidance states that "In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested." Therefore in general, the use of the results of other species than the rat is considered justified.
Regarding point (c), establishing an LC50, we would like to refer to the answer we gave on comment number 8.
In addition, a comparison between the NRT of 0.027 mg/L and the proposed LC50 of 0.25 mg/L is not possible based on only these external concentratioin because an LC50 value is based on continous exposure whereas an NRT is based on intermittent exposure as smokers inhale fresh air in between puffs and in between cigarettes. The difference in internal eposure is therefore expected to be much larger. As such, this is no justification that the proposed LC50 is too high.

RAC's response

In our view there is a need for action at the community level since nicotine is used in all European Union member coutries, and there are differences in the opinion on its classification, therefore harmonisation of classification is justified.

The Dossier Submitter provided sufficient information to assess acute toxicity of nicotine. The data used for classification are evaluated by RAC based on their scientific value and

according criteria of Regulation 1272/2008

It also important to note that hazard classification is identification of hazardous properties of the substance and should not be mistaken with the risk assessment, which determines the probability of the definite effect e.g. death to occur, which is a dose-dependent phenomenon. So even for highly acutely toxic substance classified to category 1 or 2 the ingestion or inhalation or dermal exposure to very small amount of this substance will not cause death or poisoning, as is the case with nicotine.

Date	Country	Organisation	Type of Organisation	Comment number
02.06.2015	Switzerland	Philip Morris International	BehalfOfAnOrganisation	13

Comment received

PMI disagrees with RIVM's recommendation and believes that the current classification of nicotine as "acute toxic 3" for oral exposure is appropriate and should be maintained.
We disagree with the RIVM's view that the mouse data from the selected study is more appropriate than rat data. The metabolic pathways of nicotine in mouse and human have more similarities to each other compared to pathways in rat and human, which is given as one reason by RIVM to prefer mouse studies (RIVM, 2015). However, the toxicity of nicotine is receptor specific and driven by the parent compound and not its metabolites. Compared to rats and mice the metabolic rate of nicotine is much slower in humans, nevertheless closer to rat than in mice. Considering that metabolism of nicotine results in a detoxification, we believe that the rat LD50 data are more relevant than the mouse data. More details are provided in chapter 3 of the attached document. Furthermore, the mouse acute toxicity data is less reliable than the rat data based on the Klimisch rating, as has been published by the French Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES, 2015). A comparison of three exposure scenarios based on elevated but not fatal reports from literature against the LD50 from mice or rats did not indicate that the use of the rat LD50 underestimates the human toxicity. The exposure scenarios are given in chapter 4 of the attached document.

[ECHA note: The following attachment was provided with the comment above:]
PMI COMMENTS AND ASSOCIATED BIBLIOGRAPHY RELATING TO THE APRIL 2015 CLH REPORT "PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELING" FOR NICOTINE BY RIVM

Dossier Submitter's Response

Thank you for your comment. We would like to refer to the answer we gave on comment number 3, regarding the choice on species.
The provided human data indicates that the acute toxicity in humans is above approximately 1 mg/kg bw and the absence of severe effects suggests that the lethal dose should be clearly higher. However, the available data on suicidal cases (for example: Bratschat, 2014) indicates that the lethal dose in humans is below 149 mg/kg bw. Therefore, the available data do not provide clear indications of a minimal lethal dose in humans. The minimal dose suggested by Mayer (2014) based on kinetic calculations of human cases is somewhat in the middle of the range. This would suggest that classification in category 2 would be more appropriate.

RAC's response

Your disagreement with the classification proposed by Dossier Submitter has been noted as well as your justification of this disagreement.

As for many other chemicals at present it is not possible to provide sufficient proof that

toxicity data generated on mice or rats are more relevant for human hazard assessment, therefore the rule of using the most sensitive species will be followed.

Date	Country	Organisation	Type of Organisation	Comment number
01.06.2015	United Kingdom		Individual	14
Comment received				
I believe this action is motivated by trying to ban electronic cigarette liquids, politically and financially motivated.				
Dossier Submitter's Response				
Thank you for your comment. We can only comment on issues relevant to the CLH proposal for nicotine.				
RAC's response				
Your opinion is noted, although not agreed.				

Date	Country	Organisation	Type of Organisation	Comment number
30.05.2015	Netherlands		Individual	15
Comment received				
This proposal is ridicules.It is based on assumptions in the studies they submit which have not scientifically been reproduced.This proposal is made because they have no idea how they can tax e-liquids , which they wish to do because they are loosing income out of the tobacco sales.We as a vape store provide our customers with fair warnings as well as labeling the bottles containing e-liquid.The lawmakers refuse to talk or listen to those in the e-liquid/ e-smoke business and just make things up as they go.This is yet another attempt by the State to keep people addicted to tobacco which is known to cause cancer and kills. Key facts Tobacco kills up to half of its users. Tobacco kills nearly 6 million people each year. More than five million of those deaths are the result of direct tobacco use while more than 600 000 are the result of non-smokers being exposed to second-hand smoke. Unless urgent action is taken, the annual death toll could rise to more than eight million by 2030. Nearly 80% of the world's one billion smokers live in low- and middle-income countries. (source: http://www.who.int/mediacentre/factsheets/fs339/en/) E-liquids provide a safer alternative for those unable to quit their nicotine addiction. Where second hand smoke from a regular cigarette is harmful is this, as of yet, not been proven with current generation of e-liquids and closed system e-cigarettes. What this proposal in reality means is that the State puts more effort in keeping its people on tobacco and making it harder to use and find alternative nicotine delivery system that could save lives, cut State health costs and prevention of second hand victims by tobacco smoke.				
Dossier Submitter's Response				
Thank you for your comment. Regarding the scientific justification for the proposal and the need for action at the community level, we would like to refer to the responses on comment number 3 and 7. With regard to the other comments, we can only comment on issues relevant to the CLH				

proposal for nicotine.
RAC's response
Your opinion is noted, although not agreed. It does not refer to the justification of classification of nicotine.

Date	Country	Organisation	Type of Organisation	Comment number
29.05.2015	United Kingdom		Individual	16
Comment received				
I am a vaper and I use nicotine containing eliquids. For safety it is essential that the hazard labelling on these products reflect the reality of the hazards according to modern scientific testing. I use liquids of 12mg/ml for consumption and produce liquids for my self using base which is 72mg/ml. To have the same labelling on both products is a severe safety hazard as it will lead people to under estimate the risk of handling the purer product.				
Dossier Submitter's Response				
Thank you for your comment. The CLP regulation requires labelling of products depending on the toxicity of the products. In the case of mixtures containing different concentrations of nicotine the toxicity is estimated using a formula based on the concentration and ATE of nicotine (see CLP regulation Annex I chapter 3.1.3.6).				
RAC's response				
Your opinion is noted, although it does not refer directly to the justification of classification of nicotine as a substance according to CLP regulation criteria.				

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2015	Germany	Eliquidlounge	BehalfOfAnOrganisation	17
Comment received				
Par. 3 An "increase in accidents with e-cigarette refills" is mentioned. There are no evidences given for this statement. There are also other nicotine containing products mentioned. References to any accident related to nicotine intoxication are simply not given. So this argumemnt does not fulfill scientific standards.				
Dossier Submitter's Response				
Thank you for your comment. Please refer to the response on comment number 7.				
RAC's response				
Your opinion is noted, although it does not refer directly to the justification of classification of nicotine as a substance.				

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2015	United Kingdom		MemberState	18
Comment received				
We note that some of the comments already submitted to ECHA, as part of the public consultation period, have argued that community wide action is not required for this substance and that the NL's justification for community wide action is not based on facts. Therefore we thought that you may find the following information useful: The UK National Poisons Information Service Annual Report for 2013-2014 states that 204				

enquiries regarding the consumption of e-cigarettes were received during this period. This was greater than the total number of enquiries about these products in the previous six years. The majority of exposures were accidental, however, twenty-one enquiries concerned intentional overdoses. Where the clinical features were known at the time of the enquiry, 103 patients had no features of toxicity and 94 had features of only mild toxicity (four unknown). Two patients had moderate toxicity (one aged 13 months), while another had severe toxicity and was sent to an intensive care unit. Features of toxicity included conjunctivitis, irritation of the oral cavity, anxiety, vomiting, hyperventilation and changes in heart rate. For more information, please see the NPIS Annual report at: http://www.npis.org/NPISAnnualReport2013-14.pdf
Dossier Submitter's Response
Thank you for providing us with additional information on incidents with e-cigarettes. The conclusion of the NPIS stating "Urgent consideration needs to be given to the safe storage and packaging of these products."confirms the need for reclassification resulting in better warnings and safer storage.
RAC's response
Thank you for information.

Date	Country	Organisation	Type of Organisation	Comment number
27.05.2015	Portugal		Individual	19
Comment received				
Dear Sirs, your persecutions of aerossol (popularly named electronic cigarettes) and e-liquids is a danger to public health as it will keep most of the current smokers smoking unless you totally ban tobacco. I used to consider myself a European citizen, it's a shame that you've been acting on behalf of the big corporations (namely the tobacco corporations and the pharmaceutical corporations in the current case)- today, I, as so many Europeans who formerly embraced the idea of Europe, see the EU as a vírus that is happily destroying small businesses and individual freedoms with total disregard for the citiezns. I clearly understand what you're aiming at with the proposed changes, as I'm not na ignorant or naive. Having said that, I'm ashamed that we are ruled by people who, behind their lovely speeches, could not care less for anybody's well being. I will accept this decision (as well as the decisions in the TPD, article 20) when you: a) Ban all tobacco products; b) Ban all pharmaceutical products destined to aid in quitting smoking and including substances which are generally regarded as dangerous for the same end, namely Champix.				
Dossier Submitter's Response				
Thank you for your comment. This proposal entails the classification of nicotine, other issues are not commented on.				
RAC's response				
Your opinion is noted, although it does not refer directly to the justification of classification of nicotine according to CLP regulation criteria.				

Date	Country	Organisation	Type of Organisation	Comment number
27.05.2015	Germany	Interessengemeinschaft E-Dampfen e.V.	BehalfOfAnOrganisation	20
Comment received				

Die Interessengemeinschaft E-Dampfen (IG-ED e.V.) spricht sich GEGEN eine Änderung in der Klassifizierung von Nikotin aus.

Nach unserem Kenntnisstand gibt es keinen vernünftigen Grund und schon gar keinen wissenschaftlichen Beleg dafür, der es nötig erscheinen lässt, die Einstufung von Nikotin neu zu klassifizieren. Seit Jahren wird dieses Produkt in der bestehenden Klasse gehandelt, und das mit Recht, denn es stellt keinerlei stärkere Sicherheitsbedrohung für die Allgemeinheit dar, als die im Handel üblicherweise erhältlichen Haushaltsreiniger!

Es ist vollkommen ausreichend, dieses Produkt analog zu den oben genannten Haushaltsreinigern kindersicher zu verpacken, und zusätzlich wie geplant den Verkauf an Kinder und Jugendliche zu untersagen. Erwachsenen muss nur das Risiko bekannt sein und kenntlich gemacht werden. Wie sie schlussendlich damit umgehen, ist allein ihre Sache.

Zwar sind wir keine Wissenschaftler, haben aber ständig Zugang zu den neuesten wissenschaftlichen Studien und stehen darüber hinaus mit einigen renommierten Forschern auf diesem Gebiet in -teilweise sogar persönlichem- Kontakt.

Wir sehen in diesem Antrag der niederländischen RIVM eher den Versuch, hier "Politik durch die Hintertür" zu betreiben und die bereits genügend strengen Vorschriften des Artikels 20 in der TPD2 zu verschärfen und somit stärker an die ursprünglichen Vorschläge der EU-Kommission anzunähern, die ja darauf abzielten, die tabaklose E-Zigarette sowie die zu ihrem Betrieb notwendigen Flüssigkeiten dem Arzneimittelgesetz zu unterstellen.

Diesen Bestrebungen hat das EU-Parlament im Herbst 2013 eine klare Absage erteilt. Es darf nicht sein, dass hier im Namen einer Wissenschaft, die im Fall von Nikotin ohnehin keinerlei Basis hergibt, jetzt der Beschluss unseres obersten demokratischen Gremiums ausgehebelt werden soll.

Als Verbrauchervertreter werden wir Ihre Bestrebungen sehr genau beobachten und nötigenfalls auch alle Hebel in Bewegung setzen, um diese Machenschaften einer breiten Öffentlichkeit bekannt zu machen.

ECHA unofficial translation:
The Interest group E-Dampfen (IG-ED e.V.) disagrees to a change in the classification of nicotine.
To our knowledge, there is no sensible reason and certainly no scientific evidence, which makes it necessary to reclassify the current classification of nicotine. For years, this product is traded with the existing classification, and deservedly, since it does not provide any greater threat to the security of the general public as any household cleaner, generally available on the market!
It is completely sufficient to use childproof package for this product analogue to household cleaners, and additionally prohibit the sale to children and teenagers, as planned. To adults the risk must only be known and [the product] labelled accordingly. How they finally deal with it, is solely their responsibility.
Although we are no scientists, we have permanent access to the latest scientific studies and furthermore have partly personnel contact to some renowned researchers in this field.
We see here, in this proposal from the Dutch RIVM rather an attempt to perform "politics through the back door" and to tighten the already sufficiently strict provisions of Article 20 in the TPD2 and hence to further approximate to the original proposals of the EU Commission, which aimed for the tobacco-free e-cigarette as well as the for liquids required for its operation to be regulated by the drug law.
In autumn 2013 the EU Parliament has clearly rejected this intention. It may not be, that

here in the name of science, which bears no basis in case of nicotine, now the decision of our supreme democratic body shall be undermined.
As representatives of the consumer, we will closely observe your efforts, and, if necessary, also pull out all the stops in order to inform the wider public of these machinations.

Dossier Submitter's Response

Thank you for your comment. Regarding the justification for our proposal, we would like to refer to the response to comment number 7.

RAC's response

Your opinion is noted, although it does not refer directly to the data used for justification of classification of nicotine as a substance.

Date	Country	Organisation	Type of Organisation	Comment number
26.05.2015	Austria		Individual	21

Comment received

Toxicity classification according to CLP is based on LD50 values determined with rats. It is essential to stick to this convention in order to ensure proper adjustment to the international GHS.

In the case of nicotine the generally accepted oral LD50 is 50 mg/kg, although values of up to 188 mg/kg were reported [1]. LD50 values between 3.3 mg/kg [2] and 60 mg/kg [3] were reported for mice. These studies and values obtained with other species including dogs, cats, and rabbits demonstrate unusually high variability in the letal dose of nicotine, presumably due to uncontrolled differences in the fraction of orally applied nicotine getting into the systemic circulation. Based on cases of fatal and non-fatal cases with documented nicotine plasma levels I have proposed an oral LD50 of around 10 mg/kg for humans. However, this was a careful estimate not taking into account that severe vomitting and diarrhoea will significantly reduce bioavailability of orally applied nicotine [4].

The lowest LD50 ever reported is being proposed for the re-classification of nicotine toxicity without providing a single convincing argument to justify this kind of cherry picking.

Dermal LD50 values are even higher (up to 360 mg/kg for rats), reflecting the very slow permeation of nicotine through skin that precludes poisoning by dermal contact with nicotine containing solutions.

Finally, inhalative toxicity is irrelevant, because it is impossible to achieve sufficiently high nicotine plasma levels by inhaling the vapor generated from commercially available nicotine containing E-liquids.

Despite extensive use of electronic cigarettes with nicotine containing liquids, no serious cases of poisoning upon normal use or accidental exposure have been reported. Considering the aim to adjust european toxicity classification to the international GHS and the lack of considerable toxicity of commercially available e-liquids, I strongly suggest to stick with the current classification that is based on an oral LD50 of 50 mg/kg.

References

[1] DECOS Nicotine. Health-based Reassessment of Administrative Occupational Exposure Limits. Health Council of the Netherlands: Committee on Updating of Occupational Exposure Limits, The Hague. 2000/15OSH/105. March 30 2004.

[2] Latzutka, F.A., Vasilyauskene, A.D. and Gefen, S.G.: Toxicological evaluation of the insecticide nicotine sulfate. Gig. Sanit. 34, 30-33 (1969)

[3] Trochimowicz, H.J., Kennedy, Jr. G.L., and Krivanek, N.D.: Heterocyclic and miscellaneous nitrogen compounds. In: Clayton GD and Clayton FE, ed. Patty's Industrial Hygiene and Toxicology, 4th ed. John Wiley & Sons, Inc. New York, p. 3374-3379, 3489-3491 (1994)

[4] Mayer, B.: How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century. Arch. Toxicol. 88, 5-7 (2014)

[5] Zorin, S., Kuylenstierna, F. nad Thulin, H.: In vitro test of nicotine's permeability through human skin. Risk evaluation and safety aspects. Ann. Occup. Hyg. 43, 405-413 (1999).

Dossier Submitter's Response

Thank you for your comments. We would like to refer to the answers we provided on comment number 3 and 7.

RAC's response

Thank you for the opinion.

As for many other chemicals at present it is not possible to provide sufficient proof that toxicity data generated on rats are more relevant for human hazard assessment than data on mice, therefore the rule of using the most sensitive species will be followed. In the opinion of RAC not only data from studies on rats , but also other data generated on mice and dogs have to be considered, and the principle to use the LD_{50} of the most sensitive species should be followed.

The data used for classification are evaluated based on their scientific value and according to the criteria of Regulation 1272/2008.

Date	Country	Organisation	Type of Organisation	Comment number
22.05.2015	Poland		MemberState	22

Comment received

PL CA for REACH and CLP agrees with NL proposal to change nicotine (CAS: 67-56-1) classification for oral acute toxicity into category 1 and for additional classification of nicotine for acute inhalation route (category 2).
There are several acute oral toxicity studies performed on different species including rat, mouse and dog. The metabolism of nicotine is complex and differs between species. The available information indicates that the rat may be less relevant to humans due to differences in the main type of P450 responsible for metabolism between rats and humans. Taking into account these information we agree with NL to select for classification for oral route - as a key study - the study performed on mouse – Lazutka et al (1969). In this study an acute oral LD50 in the mouse of 3.3 mg/kg bw was determined. Based on this value the nicotine should be classified, according to CLP, in category 1 for acute oral toxicity ($0 < LD50 \leq 5.0$ mg/kg bw).

Dossier Submitter's Response

Thank you for your comment and your support regarding the classification of nicotine for acute oral toxicity.

RAC's response

Your support to the proposed classification is noted.

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2015	Netherlands	Esigbond	BehalfOfAnOrganisation	23
Comment received				
On page 10 last section the submitter justifies that action is needed because of an increase of accidents with nicotine containing e-cigarette refills. But besides an increase in consultations on this matter, there is hardly, if any, intoxication reported. so the justification is not based on facts, but on emotion and unfounded fear. No intoxication, neither in prevalence nor in severity, justifies a more strict classification then we now know. Furthermore the proposed classification of nicotine is not, in any way, supported by the known human data over the last decades. If the suggested LD50 of nicotine in mice would be relevant for humans, we should have seen a lot more severity and prevalence of nicotine intoxications in humans. Therefore the suggested classifications are utterly misplaced. *[ECHA note: The following attachment was provided with the comment above]* How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century				
Dossier Submitter's Response				
Thank you for your comment. We would like to refer to the response we gave to comment number 7.				
RAC's response				
In our view there is a need for action at the community level since nicotine is used in all European Union member countries, and there are differences in opinion on its classification and labelling, therefore harmonisation of classification is justified. The human data alone in case of nicotine are not sufficient for classification. The data used for classification will be evaluated based on their scientific value and according to the criteria of Regulation 1272/2008.				

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2015	Netherlands		Individual	24
Comment received				
In Nederland moet men eliquid labellen met tekst als: ZEER GIFTG en ZEER VERSLAVEND, terwijl de nicotine in eliquid niet meer dan het minimale bevat. Het is belachelijk dat de Nederlandse overheid zelfs probeert te rechtvaardigen dat men de berekeningen om tot deze conclusie te komen basseert op proeven met muizen.. Waar dan? in de hele wereld wordt de rat als kleinste waarde genomen. *ECHA unofficial translation:* In the Netherlands eliquids have to be labelled with texts such as "highly poisonous" and "very addictive", while the eliquid contains no more than a minimum concentration. It is ridiculous that the Netherlands Government tries to justify that the calculations used to come to this conclusion are based on tests with mice. Where then? In the whole world the rat is taken as the smallest value?				
Dossier Submitter's Response				
Thank you for your comment. We would like to refer to the response we gave to comment				

number 3 and 7.
RAC's response
As for many other chemicals at present it is not possible to provide sufficient proof that toxicity data generated on rats are more relevant for human hazard assessment than data generated on mice, therefore the rule of using the most sensitive species will be followed. In the opinion of RAC not only data from studies on rats , but also other data generated on mice and dogs have to be considered, and the principle to use the LD_{50} of the most sensitive species should be followed. The data used for classification are evaluated based on their scientific value and according to the criteria of Regulation 1272/2008.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
27.05.2015	Portugal		Individual	25
Comment received				
Nicotine has been studied for a long time and there is no evidence whatsoever of its carcinogenity.				
Dossier Submitter's Response				
Thank you for your comment. The CLH proposal for nicotine is limited to classification for acute toxicity and does not entail carcinogenicity.				
RAC's response				
The Dossier Submitter did not propose to classify nicotine as carcinogen, therefore this hazard class is not considered in this RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
30.05.2015	Netherlands		Individual	26
Comment received				
An e-liquid and it's use still has less carcinogenics in them then regular cigarettes produce.				
Dossier Submitter's Response				
Thank you for your comment. The CLH proposal for nicotine is limited to classification for acute toxicity and does not entail carcinogenicity.				
RAC's response				
The Dossier Submitter did not propose to classify nicotine as carcinogen, therefore this hazard class is not considered in this RAC opinion.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
27.05.2015	Portugal		Individual	27
Comment received				
There are no serious studies that point to mutagenicity or, at least, harmful mutagenicity as a result of nicotine use.				
Dossier Submitter's Response				

Thank you for your comment. The CLH proposal for nicotine is limited to classification for acute toxicity and does not entail carcinogenicity.
RAC's response
The Dossier Submitter did not propose to classify nicotine as mutagen, therefore this hazard class is not considered in this RAC opinion.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
27.05.2015	Portugal		Individual	28
Comment received				
Liquid nicotine is only toxic in very high amounts. And it's obvious that only a fool will drink nicotine, even diluted as it is in e-liquids. In fact, it's so diluted that it becomes foolish and biased to classify it as toxic.				
Dossier Submitter's Response				
Thank you for your comment. We would like to refer to the response we gave to comment number 7.				
RAC's response				
Your opinion is noted, but it does not refer to the justification of hazard classification of nicotine.				

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
27.05.2015	Portugal		Individual	29
Comment received				
It has no affect on the respiratory system, save for the fact that the health of ex-smokers improves geeatly and quickly by using aerossols				
Dossier Submitter's Response				
Thank you for your comment. We would like to refer to the response we gave to comment number 3 and 7.				
RAC's response				
The Dossier Submitter did not propose to classify nicotine as specific target organ toxicity nor as respiratory sensitiser, therefore these hazard classes are not considered in the RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
26.05.2015	Austria		Individual	30
Comment received				
Airway sensation due to stimulation of nicotinergic acetylcholine receptors expressed on				

sensory C-fibers in the throat and bronchi. This airway sensation is thought to cause the typical "throat hit" that is desired by smokers and vapers as well and was reported to essentially contribute to tobacco addicition. Naqvi, N. H. & Bechara, A.: The airway sensory impact of nicotine contributes to the conditioned reinforcing effects of individual puffs from cigarettes. Pharmacol. Biochem. Behav. 81, 821-829 (2005)
Dossier Submitter's Response
Thank you for your comment, however, it is considered not specific to the CLH proposal on nicotine.
RAC's response
The Dossier Submitter did not propose to classify nicotine as respiratory sensitiser, therefore this class of hazard is not considered in this RAC opinion.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2015	Netherlands	Esigbond	BehalfOfAnOrganisation	31
Comment received				
Oral: The suggested change in the oral toxicity classification of nicotine is based on a Russian study, performed in 1969 (so 46 years old!) by Lazutka et al. The outcome of this study is never reproduced, other studies not even approached their outcome. The submitter states that this study is acceptable, but in this study the number of tested mice is not known. So, it is possible that only 1 mouse was tested. For sure we cannot determine if the number of tested mice was significant or reliable enough. Other studies performed with mice, although not accepted by the submitter, show a huge spread in outcome, but always with a substantially higher LD50. In conclusion, the study that is used as a key study by the submitter is 46 years old, unreliable, not reproduced and not consistent with other studies performed on mice. To use this study as key study is scientifically unacceptable. The current classification of nicotine, based on numerous reproduced studies in rats, is adequate and, in relation to known human data, strict enough to ensure the safety of the public. I would like to recommend the attached article which was published in the Archives of toxicology in 2014. this article clearly sets out the presumed (limited) nicotine toxicity in human. Dermal: No comment, The conclusions in the proposal seem correct. Inhalation: The proposed ATE for the acute inhalation toxicity is not properly argument. The submitter notes only two studies, acknowledges that these studies do not allow a determination of an LC50 value (page 32) but does not stop him of justifying an LC50 value of 0.25 mg/L because this is "in the middle?" Seriously, doe we make EU laws based on assumptions or guesses, or based on facts? With all do respect, the proposed classification of the oral and inhalation toxicity of nicotine argumented in this report, is ridicule. the submitter should be ashamed to propose this and try to "sell" it to you on a scientific basis.				
Dossier Submitter's Response				
Thank you for your comment. We would like to refer to the answers we gave in response to				

comment number 3 and 7.
In addition the publication by Lazutka (1969) states that in total 332 rats and mice were used for 25 experiments. This indicates that on average 13 animals were used for each experiment. This is probably more than what is used in the currently applicable OECD TGs for acute oral toxicity.

RAC's response

Thank you for your opinion.

Oral: In the oral acute toxicity study of Lazutka *et al.* (1969), a single dose of nicotine or nicotine sulphate in an aqueous solution was given by gavage to mice or rats. Mice were given the test substance in a dose range of 0.25 – 16 mg/kg, and rats in a dose range of 1 – 90 mg/kg bw. 25 dose groups of animals were used in this oral acute toxicity study.The number of animals per dose group was not stated, however it was reported that in total 332 animals were used in the various experiments reported in this paper (Lazutka *et al.*, 1969), thus in acute oral toxicity testing the number of animals was probably at least 5 per group, i.e. 125 animals. The other animals (207 out of the 332) were used for testing dermal absorption (6 rats and 6 rabbits), for short –term oral toxicity (8 weeks) on four groups of rats and for a sub-chronic inhalation toxicity study (4-months) on three groups of animals.

As for many other chemicals at present it is not possible to provide sufficient proof that the toxicity data generated on rats are more relevant for human hazard assessment than data generated on mice, therefore the rule of using the most sensitive species will be followed. In the opinion of RAC not only data from studies on rats, but also other data generated on mice and dogs have to be considered, and the principle to use the LD_{50} of the most sensitive species should be followed.

Taking into account all data on oral acute toxicity for mice (Heubner and Papierkowski, 1938; Lazutka *et al.*, 1969, CONTRAFT-NICOTEX-TABACCO study, 2015 submitted during public consultation), and considering uncertainties linked with determination of LD_{50} in each of these studies the LD_{50} which could be derived for this species based on all three studies is in a range of >5 mg/kg and ≤ 50 mg/kg.

Taking into account that the estimated oral LD_{50} of nicotine in mice (being in a range of >5 mg/kg and ≤ 50 mg/kg) and LD_{50} of nicotine in dogs (9.2 mg/kg) are within the range of >5 mg/kg and ≤ 50 mg/kg, RAC is of the opinion that nicotine warrants classification as Acute Tox. 2 (oral) with the hazard statement H300: *Fatal if swallowed*. The rat is the least sensitive species to acute oral toxicity of nicotine among the animal species tested with the lowest reported LD_{50} – 52.5 mg/kg. This value is just slightly above the upper bound of the criterion range of >5 mg/kg and ≤ 50 mg/kg for category Acute Tox. 2 (oral).
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Dermal acute toxicity –
RAC is of the opinion that there were no acceptable acute dermal toxicity studies in rats or rabbits for nicotine presented in CLH report.
Only the acute dermal toxicity study on rabbits recently submitted by lead registrant is acceptable to be used for classification purposes.

Taking into account that dermal LD_{50} of nicotine for rabbits in this study equals 70.4 mg/kg bw, which is within the range of 50mg/kg - ≤200 mg/kg, RAC is of the opinion that nicotine warrants classification Acute Tox. 2 (dermal) with the hazard statement H310 *Fatal in contact with skin*.

The study of Travell (1960) in cats may be taken as supportive for assessment of acute

dermal toxicity, although due to specific experimental design deficiencies the calculation of an exact LD_{50} value is not possible. The dose of 66 mg/kg is taken as LD_{50} of nicotine for cats, because this dose is at the lower end of the range of doses (66 – 100 mg/kg) at which 81% of mortality was observed. It is probable that cats administered a lower dose i.e. 66 mg/kg had a higher probability for survival than those receiving higher dose close to 100 mg/kg. Thus a dose of 66 mg/kg is a rather conservative estimate of the LD_{50} based on data from Travell study (1960).

Inhalation acute toxicity –

According to point 3.1.2.1. (c) of the CLP Regulation, the conversion of existing inhalation toxicity data which have been generated using a 1-hour exposure can be carried out by dividing by a factor of 4 for dusts and mists. In this case, knowing that time of exposure to nicotine mist (20 minutes) was 12-fold shorter than 4 hours (240 minutes), in line with Haber's law(C xt=constant), for direct comparison with the criteria even larger factor of 12 instead of 4 could be used. In this case the LC_{50} (4hours) of nicotine derived from Shao *et al.* study (2012) would be: 2.3 mg/L divided by 12= 0.19mg/L.

It is in line with Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7a: Endpoint specific guidance Version 3.0 August 2014) which states: "*Nowadays a modification of Haber's Law is used ($C^n.t = k$) as for many substances it has been shown that n is not equal to 1 (Haber's Law). In case extrapolation of exposure duration is required, the n value should be considered. If this n value is not available from literature, a default value may be used. It is recommended to set n = 3 for extrapolation to shorter duration than the duration for which the LC50 or EC50 was observed and to set n = 1 for extrapolation to longer duration (ACUTEX TGD, 2006), also taking the range of approximately 30 minutes to 8 hours into account.]".*

Considering the experimental animal welfare and avoidance of additional, unnecessary animal experiments, it is assumed that 20 minutes exposure in testing acute inhalation toxicity is not substantially different from 30 minutes exposure and extrapolation can be made to 4 hour LC_{50} value.

Date	Country	Organisation	Type of Organisation	Comment number
22.05.2015	Poland		MemberState	32
Comment received				
There are several acute oral toxicity studies performed on different species including rat, mouse and dog. The metabolism of nicotine is complex and differs between species. The available information indicates that the rat may be less relevant to humans due to differences in the main type of P450 responsible for metabolism between rats and humans. Taking into account these information we agree with NL to select for classification for oral route - as a key study - the study performed on mouse – Lazutka et al (1969). In this study an acute oral LD50 in the mouse of 3.3 mg/kg bw was determined. Based on this value the nicotine should be classified, according to CLP, in category 1 for acute oral toxicity (0 < LD50 ≤ 5.0 mg/kg bw).				
Dossier Submitter's Response				
Thank you for your comment and support regarding the classification of nicotine for acute oral toxicity.				

RAC's response
Your support to the proposal of classification is noted.

Date	Country	Organisation	Type of Organisation	Comment number
02.06.2015	Switzerland	Philip Morris International	BehalfOfAnOrganisation	33
Comment received				
We disagree with the proposed classification for oral toxicity as Acute Tox 1 based on LD50 from mice. We consider that the relevant LD50 data for acute oral toxicity are the ones from rat and we believe that the current classification Acute Tox 3 (oral) is correct.				
Dossier Submitter's Response				
Thank you for your comment. Please refer to the answer on comment number 3.				
RAC's response				
As for many other chemicals at present it is not possible to provide sufficient proof that toxicity data generated on rats are more relevant for the human hazard assessment than data generated on mice, therefore the rule of using the most sensitive species should be followed. Taking into account all data on oral acute toxicity for mice (Heubner and Papierkowski, 1938; Lazutka *et al.*, 1969, CONTRAFT-NICOTEX-TABACCO study, 2015- submited during public consultation), and considering uncertainties linked with determination of LD_{50} in each of these studies the LD_{50} which could be derived for this species based on all three studies is in a range of >5 mg/kg and ≤ 50 mg/kg. Taking into account that the estimated oral LD_{50} of nicotine in mice (being in a range of >5 mg/kg and ≤ 50 mg/kg) and LD_{50} of nicotine in dogs (9.2 mg/kg) are within the range of >5 mg/kg and ≤ 50 mg/kg, RAC is of the opinion that nicotine warrants classification as Acute Tox. 2 (oral) with the hazard statement H300: *Fatal if swallowed*.				

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2015	Germany	Eliquidlounge	BehalfOfAnOrganisation	34
Comment received				
Par. 4.2.2.1/ Table 11 The synopsis of different studies for LD50 Values gives a good impression about how reproducable the results are. The chosen value of 3.34 mg/ kg cannot be reproduced in any way. The high difference (one dimension!) to other values (24, 24, 50-60 mg/ kg) implifies a fundamental error or systematic error. Why is this Value chosen and why ist it chosen now (2015) and not earlier? What criteria are used to define a study as acceptable? For better comparebility acute oral toxicologie is measured from studies taken preferebly by rats. Why are mice taken into account? Are there any indications, that mice can be compared with human in a better way than rats?				
Dossier Submitter's Response				
Thank you for your comment. We would like to refer to the answer we provided on comment number 3.				
RAC's response				
It is known that intra-species sensitivity as well as inter-species sensitivity to toxicity of				

chemical substances may differ 10 times, thus the set of data presented by the Dossier Submitter are consistent with this knowledge and sufficiently reliable.

As for many other chemicals at present it is not possible to provide sufficient proof that toxicity data generated on rats are more relevant for human hazard assessment than data generated on mice, therefore the rule of using the most sensitive species should be followed.

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2015	United Kingdom		MemberState	35

Comment received

Classification for acute toxicity via the oral route:

We note that the assessment of acute oral toxicity is made complicated by the variety of studies available and the apparent difference in sensitivity between species(i.e. the rat appears to be the least sensitive species tested. Vomiting is a common characteristic of human nicotine poisoning, which may provide humans with added protection compared to non-emetic rodent species. However, an LD50 of 9.2 mg/kg bw was reported in an old study conducted with dogs, which is an emetic species.

As there is limited information available to explain the observed species difference and the relevance to humans, we agree with the Netherlands approach to base the classification on the most sensitive species (i.e. the mouse). However, we note that there is a range of LD50 values (3.35-24 mg/kg bw) reported in studies with this species, therefore, further comparison of the mouse studies may be beneficial.

In addition, is it possible that some of the studies reporting the same LD50 values are repeats of the same study, taken from secondary literature sources? It would be useful if this could be noted in the CLH report if this may be the case as it could be misleading for the reader.

Classification for acute toxicity via the inhalation route:

We consider that the proposed classification for inhalation toxicity is dubious as both available studies have significant limitations and a LC50 could not be derived from either study.

Dossier Submitter's Response

Thank you for your comment. Please refer to the answer we gave to comment number 3, regarding the LD50 and LC50. We agree that both inhalation studies have limitations, but according to article 9.3, in such cases a weight of evidence evaluation is needed. Underneath table 11 in the CLH report, we have indeed noted that it is likely that the same LD50 values are repeats of the same study.

RAC's response

Thank you for the opinions on acute oral and inhalation toxicity classification of nicotine. Please see RAC replies to comment 31. RAC agrees with a new proposal of the Dossier Submitter, taking into account results of acute toxicity studies provided during public consultation. See revised RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	United Kingdom	Totally Wicked Ltd	BehalfOfAnOrganisation	36

Comment received

RIVM have justified the proposed reclassification of nicotine to Acute Tox. 1 based upon current policy discussions on the use of the e-cigarette, the increase in accidents with e-cigarette refills and on their increasing popularity. Whilst there is literature evidence to support the claim that reported incidents of accidental nicotine exposure have increased with the growth of its use in e-cigarettes, the reported exposures have resulted in minimal toxicity1,2,3, indicating that the current labelling of both nicotine and mixtures of nicotine (e-liquids) is effective and no reclassification is justified.

The submitter also refers to the current acute oral toxicity classification for nicotine being the minimum classification, meaning manufactures and importers should investigate if a more severe classification applies for their specific product. The current classification of nicotine acute oral toxicity is category 3 (rat acute oral LD50 of between 50-300 mg/kg bw) and literature values range from 50-188 mg/kg bw, with most between 50-83 mg/kg bw.4-9 The proposal claims that the lowest available LD50 from mice of 3.34 mg/kg bw (Lazutka et al. 1969) warrants a category 1 classification. Section 3.1.2.2.1 of EC regulation 1272/2008 clearly states that the preferred test species for evaluation of acute toxicity by the oral and inhalation routes is the rat. When experimental data for acute toxicity are available in several animal species, scientific judgement shall be used in selecting the most appropriate LD50 value from valid and well performed tests. The Lazutka study is 46 years old and not validated as subsequent studies have not been able to reproduce the reported LD50 = 3.34 mg/kg bw acute oral toxicity value in mice.4,7,8 As stated above, the CLP guidelines are to use the rat as the preferred species, and not to use the most sensitive species. Also, based on total nicotine hepatocyte metabolism, the metabolism differences are greater between mice and humans than between rats and humans. 10 This reproducible scientific data justifies the CLP guidelines that the rat acute oral toxicity data is preferred and all of these studies indicate category 3 acute oral toxicity for nicotine.

The current acute dermal toxicity classification of nicotine is category 1 (ATE < 50 mg/kg bw), and based upon an estimated LD50 = 50 mg/kg bw in rabbits (FDA, 1952). This study is not acceptable by modern standards as there is no access to the original study. The submitter supports this classification with reference to an estimated LD50 in cats of "probably below 80 mg/kg bw" due to 81 % mortality being observed at approximately 80 mg/kg bw (Travell, 1960). The other acceptable study reports an LD50 = 285 mg/kg bw in rats for nicotine sulfate, and this must also be considered (Gaines, 1960). The range of all the reported LD50's is from 50 (estimated) to >360 mg/kg bw, and this data would indicate that category 2 would be more appropriate.

The proposal to add nicotine to category 2 (fatal if inhaled) for inhalation acute toxicity has been made by RIVM due to a lack of standardised LC50 (4 hour) nicotine inhalation data. The submitter acknowledges that of the two studies referenced in the proposal (with nicotine as an aerosol), both have limitations and the available acute inhalation data does not allow determination of an LC50 value. The estimated ATE (acute toxicity estimate) of 0.25 mg/L is based upon a series of assumptions and guess work due to a lack of validated inhalation acute toxicity data. The proposed inhalation acute toxicity classification of category 2 is not justified with this poor quality data and classification should be based upon valid data and well performed test (CLP regulation). Without appropriate animal LD50 data, ECHA recommends that human experience evidence should also be considered. Years of occupational, smoking, nicotine replacement therapies (NRT's) and e-cigarette usage does not indicate lethality concerns via inhalation. NRT's are recognised as a safe and well tolerated treatment and we can estimate (as the submitter has done) that a 15 mg nicotine

replacement device delivers a dose of up to 0.027 mg/L. 11 This is only tenfold below the proposed ATE of 0.25 mg/L. The current classification of category 3 (0.5<ATE<1.0 mg/L) s adequately conservative and protective.
To summarise, the proposed reclassification of the oral and inhalation toxicity of nicotine is not supported by the overwhelming majority of the published scientific data and CLP guidelines for valid and well performed tests. There is also valid data indicating that the acute dermal toxicity of nicotine should be reclassified from category 1 to category 2.Indeed, a recent peer reviewed study performed by a toxicology consultancy (Bibra) has concluded that nicotine containing mixtures (2.5 < 16.6 %) exhibit an acute oral toxicity of 300-2000 mg/kg bw (CLP category 4). 12 This peer reviewed data warrants further investigation.

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12 EU Classification of nicotine mixture under CLP Regulation 1272/2008. Bibra (toxicology advice and consultancy) proposal, June 2014.

[ECHA note: The following attachment was provided with the comment above]
EU Classification of nicotine mixtures under CLP Regulation 1272/2008 (as amended and corrected) Bibra Proposal

Dossier Submitter's Response
Thank you for your comment. Please refer to the answers provided on comment number 3 (species selection), 7 (need for reclassification), 9 (acute dermal toxicity) and 12 (comparison of the LC50 with the NRT). The study by BIBRA was available to us before submission of the proposal and does not contain additional information.
RAC's response
Thank you for the opinion. Your disagreement with classification proposed by Dossier Submitter has been noted as well as your justification of this disagreement. Please read a RAC response to comment No. 31 which referes also to your comments.

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	Netherlands	Fontem Ventures	BehalfOfAnOrganisation	37
Comment received				
Please see attached document for Fontem Ventures' specific comments on the oral toxicity of nicotine, which are supported by a number of references. *[ECHA note: The following attachment was provided with the comment above:]* Submission challenging nicotine CLP reclassification proposal				
Dossier Submitter's Response				
This is the same comment as comment number 4, please refer to the response to comment number 4.				
RAC's response				
Thank you for the opinion. Your disagreement with classification proposed by Dossier Submitter has been noted as well as your justification of this disagreement. Please read a RAC response to comment No. 31 which referes also to your comments. It is also important to note that hazard classification is the identification of hazardous properties of the substance and should not be mistaken with the risk assessment, which determins the probability of the definite effect e.g. death to occur, which is a dose-dependent phenomenon. So even for highly acutely toxic substance classified to category 1 or 2 the ingestion or inhalation or dermal exposure to very small amount of this substance will not cause death or poisoning, as is a case of nicotine.				

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	Finland		MemberState	38
Comment received				
Oral The current classification Acute Tox. 3; H301 is based on rat LD50 values ranging 50-80 mg/kg and criteria laid down in Directive 67/548/EEC. The CLH report lists oral toxicity studies in mouse, rat and dog. Most of these studies have not been conducted according to validated test guidelines or other recognized test methods. The studies accepted by the dossier submitter (DS) have shortcomings. However, when LD50 values of all the accepted studies by the DS are weighed together the impression is that the current classification may underestimate acute oral toxicity of nicotine according to the CLP criteria. LD50 values of the accepted studies in mice range 3.34-24 mg/kg (for nicotine) and 8.55-87 mg/kg				

(nicotine salts), and LD50 value of one accepted study in dog (9.2 mg/kg) would also lead to more severe classification.

According to the CLP criteria, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. If there is information available on species relevance, then the studies conducted in the species most relevant for humans should normally be given precedence over the studies in other species. Based upon the reported data, it appears to be unclear which species is the most relevant. Therefore, the data from the most sensitive species (mouse) have been used. Thus, it is important to further clarify the toxicokinetics in various species to evaluate whether mouse is the most relevant species for nicotine toxicity to humans. In conclusion, weight of evidence approach should be used and RAC should carefully consider whether the data presented in the dossier warrants a classification in Acute Tox. 1; H300.

Inhalation

The proposed classification, Acute Tox. 2; H330, is based on two studies accepted by the DS. The LC50 value of 0.25 mg/L as ATE was determined from the results of these studies. The calculation of the ATE is difficult to understand and should be better justified. Since studies on mixtures are generally not acceptable for classification, the study on tobacco extract should not be used. Therefore, the classification would only be based on the 20 min study using the "up and down method" with LC50 value 2.3 mg/L (20 min). The reasoning and the derivation of the final ATE for classification from this LC50 value is questionable. The Finnish CA is of the opinion that to enable judgement whether the proposed classification is warranted, more emphasis should have been laid down on this derivation in the CLH report.

Dermal

The current classification Acute Tox, 1; H310 is based on an acute dermal study in rabbits resulting in a LD50 value of 50 mg/kg. Although the study does not fulfil the current test method requirements it is considered to be justified (with supporting evidence from one study on cat) to retain the classification.

Dossier Submitter's Response

Thank you for your comment and support regarding the classification of nicotine for acute dermal toxicity. For further clarification regarding the acute oral LD50 and LC50 for inhalation, please see our response to comment number 3.

We agree that estimation of the LC50 should preferably not be based on tests with mixtures. However,as the ingredients of the mixture are known and all ingredients either are present at low concentration (tobacco essential oils) or at high concentrations but known to have limited acute inhalation toxicity (others), and are only used to determine a concentration that does not induce mortality, this is considered acceptable as it is a worst case approach.
We agree that the reasoning of the final ATE is questionable. However, when no ATE is determined and the classification is category 2, this would result in the ATE of 0.05 mg/L according to table 3.1.1 of Annex I of CLP, which is also not justified.

RAC's response

Thank you for the suggestions which has been taken into accout in our opinion.

Date	Country	Organisation	Type of Organisation	Comment

				number
04.06.2015	France		MemberState	39
Comment received				
P32 France agrees with the conclusions on classification for oral and dermal exposure. France agrees with the conclusions on classification for inhalation, but the methodology employed to derive the ATE seems questionable. First, we disagree to say that the value of 0.1 mg nicotine/L is close to a LC50. This value can be more related to a LClo. Consequently, we should not suggest a LC50 value of 0.25 mg nicotine/L as this is in the middle of the results of the two studies available, as stated in the CLH report. We propose to divide the LC50 observed after a 20-minute exposure by 12 (extrapolation from 20 minutes to 4 hours), to obtain a LC50 of 0.20 mg nicotine/L. It seems to us that this methodology is subject to less bias.				
Dossier Submitter's Response				
Thank you for your comment and support for the classification of nicotine for acute oral and dermal toxicity. One female out of 12 in group 1 of the study by Werley (2014) died. This dose is therefore considered to be close to the LC50. The suggestion to apply a factor of 12 to extrapolate from 20 minutes to 4 hours (240 minutes) means using Haber's law. This is in line with the CLP guidance (3.1.2.2 page 262) and the REACH guidance (R7a). The proposed method is used to calculate the LC50 as described in the reponse to comment 41.				
RAC's response				
RAC is of similar opinion as France MSCA on classification of acute toxicity of Nicotine. Your suggestions have been considered.				

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2015	United Kingdom	Nicoventures Ltd.	BehalfOfAnOrganisation	40
Comment received				
Acute ORAL toxicity. Category 1 not warranted, the data interpreted via the CLP guidelines support a category 3 classification. CLP guidelines (ECHA (European Chemicals Agency), 2013) confirm the rat as preferred species for oral acute toxicity testing. Where data are available in several animal species, the guidelines do not advocate basing classification on the most sensitive species, but on the most scientifically appropriate species. Classification is then based on the lowest ATE of the acceptable studies from within that most appropriate species. The CLH report argue that rat is not an appropriate species based on metabolism differences between rats and humans. Instead they use a mouse study. Based on total nicotine hepatocyte metabolism, the metabolism differences between mice and humans are actually bigger than between rats and humans (Kyerematen, Morgan et al., 1990), making mice a less appropriate species than rats. This is further confirmed by plasma nicotine half-life, in mice this is even further removed from the half-life in humans than in rats, viz. 6-7 minutes in mice and 45 minutes in the rat versus 2 hours in humans (Matta, Balfour et al., 2007). Therefore, the default ECHA CLP guidance that rat is the preferred species, still stands. Most appropriate are the oral the studies using nicotine, rather than nicotine salts because the oral uptake is expected to be different. All 11 rat oral acute toxicity studies on nicotine				

in the CLH report indicate category 3 oral toxicity. The lowest ATE of the rat studies indicated as 'acceptable' by the CLH report, is 52.5 mg/kg bw. This is from the same Lazutka et al 1969 reference as the mouse study used for classification by the CLH report.

However, the Latzuka et al 1969 study is not of an acceptable standard. It is incompletely reported, the study design unclear, the strain, sex and number of animals not specified. Although the total number of animals mentioned (332) sounds high, only very few of these were used for the rat acute oral toxicity study. The 332 animals refers to the total of all the studies reported in that paper and is distributed over rats, mice and rabbits, with peroral, inhalation and dermal exposures, with test durations varying between single acute exposures up to two month repeated tests. In contrast, by far the most reliable study of all the studies reported in table 11 of the CLH report is Van den Heuvel et al, 1990. This is a well-reported, cross-laboratory study using 31 laboratories, where the classical versus fixed dose procedure to establish acute oral toxicity of nicotine were compared. The study complied with OECD guidelines and used a total of 355 rats across both genders and three rat strains.

This resulted in an LD50 of 70 mg/kg as established by the classical method, and results from the fixed dose procedure were consistent with this LD50. Based on the available evidence, an oral ATE of 70 mg/kg is proposed.

Acute INHALATION toxicity

Category 2 not warranted, the data support a category 3 classification.

The CLH report considers the LC50 (20 min.) that is reported for nicotine of 2.3 mg/L (95%CI: 1.24-4.07)(Shao, Xu et al., 2013) as the most appropriate for determining the inhalation toxicity potency. They refer to the ECHA guidelines that recommend converting 1 hour to 4 hour exposures by dividing with a factor four, resulting in 0.58 mg/L. The CLH report postulates that with only 20 minute exposure, that probably means the LC50 is in reality less than 0.58 mg/L. In fact, application of Haber's law to extrapolate from exposures of less than 30 minutes is not scientifically recommended, as recognised by ECHA in REACh registration CSA guidance, Section R.7.4.4.1. If one were to extrapolate, the default factor 4 is very conservative for compounds with a short half-life, such as nicotine. Nicotine uptake via inhalation is a matter of minutes and plasma half-life in rats is reported as approximately 45 minutes (Matta, Balfour et al., 2007). Even though plasma half-life in humans is longer than in rats (approx. 2 hours), even in humans, time to peak plasma concentration was reported as only 6.7 minutes for 20 minute nicotine exposure via an inhaler in humans (Schneider, Olmstead et al., 2001). This suggests that the 20 minute rat inhalation exposure may well have come close to peak plasma concentration. In the study, all the rats that died did so either within the 20 minute exposure period or within 1-3 minutes after the end of the exposure period. This suggests that the nicotine plasma concentration is a reasonable proxy for dose for this toxic end point. Overall this indicates that a factor 4 to extrapolate to 4 hour exposures, and therewith the derived LC50 of 0.58 mg/L, is likely to be conservative. An LC50 of 0.58 mg/L would indicate acute inhalation category 3.

In the absence of clear, appropriate animal LC50 data, ECHA recommends a weight of evidence approach that includes human experience. This should thus take into account the human inhalation exposure to nicotine via decades of occupational, smoking and NRT experience, as well as the more recent vaping, which does not indicate any acute inhalation toxicity issues.

A world-wide survey of over 19,000 e-cigarette consumers indicate the majority use between 2 to 5 ml of e-liquid per day and for most the e-liquid contains 0 to 18 mg/ml nicotine, i.e. normal daily usage range is 0 to 90 mg nicotine/day. Assuming this is vaped over 8 hours, with a breathing rate of 0.83 m^3/hour this would equate to 13 mg/m^3 or 0.013 mg/mL. Yet the vast majority of such exposures for 8 hours each day only resulted in local and transient effects (Farsalinos, Romagna et al., 2014).

A review of clinical trials performed with nicotine inhalers reported exposures to aerosol concentrations up to 130 mg/ml, i.e. 130,000 mg/L (!), for 5 minutes, repeated over 3 subsequent days (Caldwell, Sumner et al., 2012). Even at those very high acute exposures, the only reported side effects were cough, burning throat and excess salivation.

Recommended inhaled NRT dosages (Johnson & Johnson Pacific Pty Limited, 2015) equate up to 0.027 mg/L (details provided in the attachment). This is less than tenfold below the proposed LC50 of 0.25 mg/L as ATE (acute toxicity estimate) even though NRT is recognised as a safe and well tolerated treatment (RCP - Tobacco Advisory Group of the Royal College of Physicians, 2007).

Peak arterial nicotine levels from smoking have been reported as 8 times higher than those from an inhaler (Lunell, Molander et al., 2000), serum nicotine from the Nicorette inhalator as 6 times lower than from a cigarette (Bullen, McRobbie et al., 2010). Blood levels from smoking will thus be very close to the blood levels that might be anticipated from exposure to the proposed "LC50".

Indeed, nicotine inhalation exposures during smoking are close to the proposed LC50. Based on the smoking profiles of 133 smokers, it was reported smokers are exposed to 1.54 to 2.60 mg nicotine from a typical conventional cigarette, smoked over 4 minutes on average (Djordjevic, Stellman et al., 2000). Mouth level exposure (MLE) values measured via used filter tips from thousands of smokers all over the world are generally slightly lower but still within the same range with averages per country up to 1.77 ±0.69 mg nicotine/cigarette (Mariner, Ashley et al., 2011) (Cunningham, Sommarstrom et al., 2015). Assuming a breathing rate of 0.83 m^3/hour, 1.50 to 2.60 mg nicotine over the 4 minutes of smoking a cigarette, would equate to 0.03 to 0.05 mg/L. Conventional smoking are thus only 5-fold below the proposed LC50. Clearly the variation around normal smoking patterns means that smokers will therefore routinely be exposed to levels comparable to the proposed LC50 as well. Nicotine exposure from smoking is in many ways a conservative comparator due to the significant co-exposure with a variety of smoke toxicants.

Although nicotine users will have developed a certain nicotine tolerance due to their smoking exposure, it can still be assumed the LC50 relevant to humans will be more than 5-fold higher than daily acute smoking exposures. Similarly it would be expected to be significantly more than tenfold above the well tolerated therapeutic dose.

Human experience, combined with taking the short half life of nicotine into account when interpreting the reported LC50(20 min) of 2.3 mg/L, indicate category 3 (0.5<ATE<1.0 mg/L) would be adequately conservative and protective.

Acute DERMAL toxicity

Category 1 not warranted, the data support a category 3 classification.

Classification is effectively based on the most sensitive rabbit study (50 mg/kg bw) for which no study details were available to the authors of the CLH report. This may be because the FDA (1952) reference details given in the CLH report, refer to a report that does not discuss dermal acute toxicity studies (Lehman, 1952b). The dermal acute toxicity study performed by those authors was actually reported in an earlier report in the series (Lehman, 1952a). The CLH report lists it twice by also referencing a secondary source (Trochimowicz et al, 1994) that actually refers to the same 1952 study. The original reference lists the outcome as an estimated LD50. Rabbits were exposed for 24 hours. It is unclear whether the exposure was to neat nicotine or a solution of unspecified concentration in dimethyl phthalate. Strain, sex, number of animals are not specified. No study protocol details are provided on matters such as potential pretreatment of the application site or observation period, and no results other than the estimated LD50 are described. This report is not acceptable for hazard classification setting.

Six other dermal toxicity studies are listed in the CLH report (one rat study is listed twice by again referencing the original report and a secondary source). Dermal LD50's reported are 66 to >360 mg/kg bw. The CLH report deems two reports "acceptable": the cat study and a rat LD50. Rodents are the recommended species for dermal LD50 studies (OECD 402).

In fact the cat study does not meet acceptability criteria. It is a single dose study, which is not suitable for deriving an LD50. Dose application was to the groin, an area where skin penetration is generally recognised to be higher than at more conventional application sites such as the back. The recommendation for liquid test substances, such as nicotine, is to use them undiluted. This study used a 40% aqueous solution. Nicotine dermal penetration shows a parabolic dependence on nicotine concentration where 100% nicotine had similar flux as 1% w/w nicotine aqueous solution (Kuswahyuning & Roberts, 2014). It is thus likely pure nicotine would have been significantly less bioavailable and therewith resulted in less deaths at the tested dose than the results from this study.

The single remaining acceptable dermal acute toxicity study reported a LD50 in 70 rats of 285 mg/kg bw which meets the criteria for acute dermal toxicity category 3 classification. The proposed dermal ATE is 285 mg/kg bw.

Dossier Submitter's Response

Thank you for your comment. Please refer to our responses to comments number 3 (species choice), 7 (need for classification) , 9 (acute dermal classification) and 12 (comparison LC50 with NRT).
The suggestion to apply a factor of 1to extrapolate from the 20 minute LC50 towards a 4-hour LC50 is disputed. The description of the time of death does not allow a conclusion that all rats died within 20+3 minutes or not. Rats that did not die within 20 minutes may have died if exposure would have been longer.
Comparison of the estimated average daily exposure of e-cigarette users (0.013 mg/l daily) and smokers (0.03 – 0.05 mg/l for 4 minutes) that show no clear toxic effects with the proposed LC50 of 0.23 mg/L may indicate a steep dose response curve. Also for smokers the exposure time of 4 minutes followed by inhalation of clean air may reduce the possible effects. In addition, smokers may have adapted to the exposure of nicotine resulting in higher resistance.
The inhalation of an aerosol concentration of 130000 mg/L does not seem realistic as this converts to inhaling almost 1 kg of dust in 5 minutes (70 l inhaled air). This would be toxic irrespective of which substance would be inhaled.

We agree that the results by (Kuswahyuning & Roberts, 2014) show that the study in cats using 40% nicotine solution in water may overestimate the acute dermal toxicity. However,

the study by Gaines (1960), which Nicoventures suggests for determining the dermal LD50 used nicotine sulphate that may have a lower dermal intake as well due to the ionisation of nicotine. The new study in rabbits is considered more relevant (see comment 41).

Underneath table 11 in the CLH report, we have indeed noted that it is likely that the same LD50 values are repeats of the same study.

RAC's response

Thank you for the opinion.
Your disagreement with classification proposed by Dossier Submitter has been noted as well as your justification of this disagreement.

Please read RAC response to comment No. 31 which referes also to your comments.

It is also important to note that hazard classification is identification of hazardous properties of the substance and should not be mistaken with the risk assessment, which determins the probability of the definite effect e.g. death to occur, which is a dose-dependent phenomenon. So even for a highly acutely toxic substance classified to category 1 or 2 the ingestion or inhalation or dermal exposure to very small amount of this substance will not cause death or poisoning, as is a case with nicotine.

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2015	Germany	CONTRAF-NICOTEX-TOBACCO GmbH (CNT) Lead registrant for Nicotine CAS 54-11-5	BehalfOfAnOrganisation	41

Comment received

Comment on the proposal for harmonised classification and labelling of nicotine

The Guidance on the preparation of CLH dossiers, Version 2.0, August 2014 states in section 5.3 that all available information should be reviewed in order to determine if they are relevant, adequate and reliable. For detailed guidance on how this information shall be used it refers to the Guidance on the application of the CLP criteria.

The registrants of nicotine came to the conclusion (submitted in January 2015) that reliable data on nicotine were very poor and only two valid studies on acute oral toxicity (van den Heuvel et al., 1990 and Yam et al., 1991) were available. The classification was therefore based on these two studies in rat which both resulted in an LD50 value of 70 mg/kg bw leading to a classification as Acute Tox. 3, H301 for nicotine, which was in accordance with the current harmonised classification.

The Netherlands National Institute for Public Health and the Environment (RIVM) considered the mouse to be more sensitive than rat when assessing the acute toxicity and chose the results of Lazutka et al., 1969, for their proposed new harmonised classification, i.e. LD50 value of 3,34 mg/kg bw for mice. RIVM was aware of the shortcomings of the data and wrote: "Acceptability: Limited description but acceptable given the period in which it was performed."

The procedure how to evaluate available information is described in ECHA's Guidance on information requirements and chemical safety assessment, Chapter R.4: Evaluation of

available information. The evaluation includes the relevance, reliability and adequacy of available information. Reliability is specified according to the Klimisch codes (Klimisch et al., 1997) which allows information to be rated from "reliable without restrictions" (Score 1) to "not assignable" (Score 4). The Guidance on the Application of the CLP Criteria, Version 4.0, November 2013 states in chapter 3.1.2.4. that classification for acute toxicity "[…] has to be performed with respect to all routes of exposure (oral, dermal, inhalation) on the basis of all adequate and reliable available information." This indicates that information rated with Klimisch score 3 (not reliable) is not sufficient for classification purposes. For aquatic toxicity this is further specified in chapter 4.1.3.2.1. of the same guidance document "[…] Regarding the use of test data, in general, only reliable information (i.e. with a Klimisch reliability score of 1 (reliable without restrictions) or 2 (reliable with restrictions)) should be used for classification purposes. […]."

Recently, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) evaluated the potential human health effects of nicotine "Évaluation des dangers de la nicotine" (2015). They came to the conclusion that with the exception of the above mentioned two studies in rat (van den Heuvel et al., 1990 and Yam et al., 1991) all other listed studies on nicotine had to be rated with Klimisch score 3 (not reliable).

Concerning the study in mice (Lazutka et al., 1969) which was the basis of the RIVM proposal, ANSES came to the same conclusion as the registrants because there was substantial information missing in the publication of the test results, i.e. RIVM considered the study as "acceptable" whereas ANSES and the registrants considered it as "not reliable" according to the Klimisch criteria.

With respect to the dermal toxicity and the classification as Acute Tox. 1, H310, there were no valid studies in rabbit available. The existing harmonised classification was based on an LD50 value of 50 mg/kg bw in rabbit (FDA, 1952 and Trochimowicz et al., 1994), which was only available as a value but not as a report. Another LD50 value of 140 mg/kg bw in rabbit was also only available as a value (UK PSD, 2008). Listed values in rat were > 140 mg/kg bw. RIVM proposed to maintain the existing harmonised classification referring to a study in cat (Travell, 1960) which did not list an LD50 value but a range of doses (66-100 mg/kg bw) which caused death in 81% of the animals. RIVM assumed therefore the LD50 value to be below 80 mg/kg bw in cats. A value of 80 mg/kg bw corresponds to a classification of Acute Tox. 2 (not 1). Furthermore, RIVM was aware of the deficiencies of the study. "Acceptability: Limited description but acceptable given the period in which it was performed." Nonetheless, RIVM proposed to maintain the harmonised acute dermal toxicity at Acute Tox. 1.

The registrants understand the intention of RIVM to clarify the acute toxicity of nicotine. However, they consider the quality of information used by RIVM to prepare its proposal as not to be sufficient to justify a change of harmonised classification.
The registrants agree that valid and reliable information on nicotine toxicity is necessary to achieve a high level of protection for human health. Due to the fact that reliable information to classify nicotine was not available, and in line with the recommendation of ANSES in their report, the registrants initiated a new test for acute oral toxicity in mice (OECD 425) to gain reliable and valid information in the most sensitive species as predicted by RIVM.
The preliminary results of the oral toxicity in mice are already available. The initial dose was 17,5 mg/kg bw and was sequentially increased by a factor of 3,2. Three of three animals survived the dose of 17,5 mg/kg bw, one of three animals survived the dose of 55 mg/kg bw, one of two animals even survived the dose of 175 mg/kg bw and the one animal tested with 550 mg/kg bw died. Provided that all animals survive the post-treatment observation period, the calculated LD50 would be 77,8 mg/kg bw. This result exceeds even the available

LD50 values in rat of 70 mg/kg bw, demonstrating no difference in sensitivity, whereas RIVM assumed mice to be significantly more sensitive than rats based on the results of Lazutka et al., 1969.

Provided the preliminary LD50 to be confirmed, the current harmonised classification of Acute Tox. 3, H301 is to be maintained and the asterisk to be removed.

Due to the lack of valid values for dermal toxicity, the acute dermal toxicity is furthermore currently being tested in rabbit (OECD 402) which is considered to be more relevant for humans than rat which was taken into account in the proposal of RIVM.

The results of the preliminary study for acute dermal toxicity are already available. The doses tested were 5; 50; 200 and 1000 mg/kg bw. None of the rabbits died at 5 mg/kg bw and 50 mg/kg bw and all rabbits died at 200 mg/kg bw and 1000 mg/kg bw. The doses for the main study are therefore 50; 100 and 200 mg/kg bw. These first results indicate that the LD50 for acute dermal toxicity in rabbit will most probably be > 50 mg/kg bw leading to a classification of Acute Tox. 2, H310 instead of currently Acute Tox. 1, H310. If these results are confirmed by the main test, it requires the harmonised classification of nicotine to be adapted accordingly.

Both results are supported by the Local lymph node assay conducted for the REACH registration. The tested concentrations were 0,5; 1 and 2% which were approximately 12,2; 24,4 and 48,8 mg/kg bw. Four mice per dose were treated by topical application for three consecutive days. None of the treated animals showed severe signs of systemic toxicity or died.

Taken together, none of the tests conducted with a high purity of nicotine and according to current guidelines did confirm the proposals of RIVM. Existing valid studies of acute oral toxicity in rat gave LD50 values of 70 mg/kg bw with nicotine. For mice the new LD50 value would be 77,8 mg/kg bw, assuming no deaths in the post-treatment observation period. The preliminary results of the dermal study in rat indicate an LD50 value of probably > 50 mg/kg bw in rabbit.

The registrants propose to postpone any decision on classification and labelling of nicotine until the studies on oral and dermal acute toxicity are finalized. Draft reports are expected for July 2015.

Regarding the acute inhalation toxicity, RIVM listed two studies: Shao et al., 2012 and Werley et al., 2014. RIVM considered both studies as "Acceptable with limitations" due to "20 minutes exposure only" and "mixture tested, testing not up to the limit dose", respectively. RIVM rounded the calculated LC50 values of > 0,114 mg/L and 0,58 mg/L down to 0,1 mg/L and 0,5 mg/L and suggested a "middle" of 0,25 mg/L. This procedure does not seem to be scientifically sound enough to justify a harmonised classification. RIVM's proposal for a new classification of nicotine demonstrated that there was a need to reassess the acute toxicity of nicotine but also triggered a debate how unreliable several old studies were. The preliminary results of studies recently initiated by the registrants to clarify the acute toxicity of nicotine indicate that nicotine is less hazardous than so far considered.

Provided that the final outcome of the recent studies is in line with the preliminary results the registrants of nicotine propose a harmonised classification as outlined in the right column of the table (see also attachment).

Current entry in	Proposed future entry in	Proposed future entry in

	Annex VI of CLP Regulation (proposal of RIVM)	Annex VI of CLP Regulation (proposal of the registrants of nicotine)	Annex VI of CLP Regulation
Acute (without asterisk) oral toxicity	Acute Tox. 3 *, H301	Acute Tox. 1, H300	Acute Tox. 3, H301
Acute dermal toxicity	Acute Tox. 1, H310	Acute Tox. 1, H310	Acute Tox. 2, H310
Acute toxicity inhalation	---	Acute Tox. 2, H330	---
Aquatic toxicity	Aquatic Chronic 2, H411	Aquatic Chronic 2, H411	

[ECHA note: The following attachment was provided with the comment above:]
Attachment to the comment on the proposal for harmonised classification and labelling of nicotine

Dossier Submitter's Response

Thank you for your comment. The draft reports of the new studies are summarised below followed by an adapted proposal for classification for acute toxicity via the oral, dermal and inhalation route based on the new studies and comments received during the public consultation.

Acute oral toxicity study, up-and-down procedure, OECD TG 425.

Table 1. Study summary

Method	Dilution	LD_{50} (mg/kg bw)	Animal	Remarks	Acceptability	Reference
Single dose, oral, gavage	In water*, a treatment volume of 10 ml/kg bw was maintained.	77.83	Mouse	Crl:NMRI BR, female, 9 in total, see table below for further details. Default dose progression factor: 3.2	Unacceptable	Registrant, 2015

* Aqua purificata Ph. Hg. VIII
Purity nicotine: 99.8%

Brief study description
The acute oral toxicity of nicotine was determined by the up-and-down method (OECD TG 425), using oral gavage. This test is intended for use with rodents (rat female preferably). For this study, young female mice were chosen as they were considered the most sensitive species.
Mice were fasted the day prior to treatment, food was given back 1 hour after treatment. The first animal was dosed a step below the best preliminary estimate of the LD50, resulting in a dose of 17.5 mg/kg bw. The next animal was dosed after the first animal survived for 48 hours after dosing. In case of survival, the next dose was increased by a factor of 3.2, in

case of death the next dose was decreased by a factor of 3.2. In total, 9 animals were treated with 4 different doses, as can be seen in Table 2.
In the study report, 2 reasons are given to stop dosing:

1. "Dosing was stopped, because the stopping criteria according to Guideline was met: LR criterion (if the likelihood-ratios calculated exceed the critical likelihood-ratio, the LR stopping criterion is satisfied and testing stops)." However, no information on the likelyhood ratio is provided.
2. "Dosing was stopped, because the maximum number of animals tested". However, according to paragraph 33, the maximum number of tested animals is 15.

Gross pathological examination was carried out on treatment day and 15th day after treatment.

Results:

Table 2. Animals, in order of testing

Animal	Dose (mg/kg bw)	Outcome
1	17.5	Survived
2	55	Survived
3	175	Survived
4	550	Died (10 sec after treatment)
5	175	Died (10 sec after treatment)
6	55	Died (20 sec after treatment)
7	17.5	Survived
8	55	Died (30 min after treatment)
9	17.5	Survived

Table 3. Lethality and clinical symptoms per dosage group

Group	# of animals	Dose (mg/kg bw)	# Dead	Clinical symptoms
1	3	17.5	0 (0%)	decreased activity, tremor, clonic convulsion, abnormal gait, dyspnoea, between 30 min and 3 hrs after treatment.
2	3	55	2 (66%)	decreased activity, tremor, clonic and tonic convulsion, closed eyes, abnormal gait, dyspnoea, between 30 min and 4 hrs after treatment.
3	2	175	1 (50%)	decreased activity, tremor, clonic convulsion, abnormal gait, decreased respiration rate, dyspnoea, between 30 min and 4 hrs after treatment. Clonic convulsion; 10 seconds after treatment
4	1	550	1 (100%)	Tonic and clonic convulsion, 10 seconds after treatment

All surviving animals survived until the end of the 14-day observation period. The body weight development in all surviving animals was undisturbed. No pathological changes were found during macroscopic examination of the animals.

Conclusion:
The LD50 for acute oral toxicity of nicotine was 77.83 mg/kg bw in mice. This was determined by statistical test (Probit analysis by SPSS+software). The very quick occurrence of clinical symptoms and lethality indicates that uptake via mucous membranes is very relevant for this substance. This may be caused by uptake in the forestomach or by

residues of nicotine on the gavage needle coming into contact with other parts of the upper gastro-intestinal tract for example the mouth. The use of gavage exposure may limit the uptake via the mucous membranes and therefore underestimate the actual lethality. The 95% confidence limits were not calculated because their range is too wide. Therefore, the reliability of the determined LD50 is considered to be low.

Acceptability
Survival and lethalities occurred at several exposure levels indicating a shallow dose effect curve. Also, the 95% confidence limits could not be calculated, indicating that the reliability of the obtained results is poor. Confidence intervals indicate how much the average value is likely to predict the true LD50. If these cannot be calculated, this indicates a low reliability.

According to OECD 425 guidelines (paragraph 33), testing stops when one of the following stopping criteria is first met:
(a) 3 consecutive animals survive at the upper bound;
(b) 5 reversals occur in any 6 consecutive animals tested;
(c) at least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value. (See paragraph 44 and Annex 3. Calculations are made at each dosing, following the fourth animal after the first reversal).

Criteria a or b were not met. No values are given for c. See also page 18, Annex 3 of the OECD guideline. Stopping was not yet warranted.

In addition, it is unclear from the available information whether the LD50 was calculated using the method described in OECD 425.

Taken together, this draft study report is currently not acceptable. However, the results do suggest that the oral LD50 of nicotine in this study is above 17.5 mg/kg bw.

Acute dermal toxicity, OECD TG 402
Table 4

Method	Dilution	LD_{50} (mg/kg bw)	Animal	Remarks	Acceptability	Reference
Single dose, dermal, applied undiluted for 24 hrs	Nicotine was applied undiluted	70.4 (females)	Rabbit	NZW rabbit, 5 females/dose (3 doses; 50, 100, and 200 mg/kg bw), 5 males (50 mg/kg bw)	Acceptable	Registrant, 2015

Purity nicotine: 99.8%

Brief study description
Nicotine was applied undiluted to the skin of rabbits (10% of the total surface area). It was left in contact with the skin for 24 hours. Any residual test item was removed using water at the end of this period. The rabbits were observed for 14 days after application. A preliminary study with 2 animals per group (1000, 200, 50 and 5 mg/kg bw) resulted in death of the animals in the 2 highest dose groups. The main study consisted of five animals per group, 3 doses in female rabbits, *i.e.* 50, 100 and 200 mg/kg bw, and 1 dose in male rabbits, *i.e.* 50 mg/kg bw.

Results
Table 5

Dose (mg/kg bw)	# of animals	# Dead	Clinical symptoms	Dermal observations
50 (only dose tested in male and female)	5	1 female (2 hrs after treatment) (20%) 0 male	Decreased activity, tremor, incoordination; lateral position, dyspnoea. 1 hr after treatment. In males; no systemic clinical symptoms were observed.	Females; very slight to severe erythema (between treatment day and day 2). Males; very slight to severe erythema (day 1 – 7), desquamation, crusting and wound (day 6-14).
100	5	4 (between treatment and day 1) (80%)	Decreased activity, tremor, closed eyes, clonic convulsion, abnormal gait, salivation. Between 30 min and 5 hrs after treatment.	Very slight severe erythema and slight oedema (between treatment day and day 1)
200	5	5 (4 after 1 hour, 1 after 2 hrs) (100%)	Decreased activity, tremor, closed eyes, clonic convulsion, abnormal gait, salivation. Between 30 min and 1 hr after treatment.	Moderate to severe erythema and slight to moderate oedema (on treatment day).

In total, 10 female rabbits died during the study. All male rabbits and 5 female rabbits survived until the end of the 14-day observation period after treatment. The male rabbits were not found to be more sensitive to nicotine that the females.

Conclusion
The LD50 value is 70.4 mg/kg bw in female rabbits. This was on the basis of statistical test (Probit analysis by SPSS+software). 95% confidence limits: lower: 28.3 and upper 131.2).

Acceptability and classification
This study should be used for classification purposes, and is currently the only acute dermal toxicity study that has been performed according to OECD guidelines. The LD50 of 70.4 mg/kg bw falls within the range of 50 < and ≤200 mg/kg bw, which leads to classification of nicotine for acute dermal toxicity in Category 2.

Adapated proposal for harmised classification for acute toxicity.

<u>Oral route</u>

The original proposal for category 1 was based on the lowest LD50 in the most sensitive species (mice) although this study was only limitedly reported. A new draft study report was provided for a study in mice. The study was summarised and assessed above. The resulting LD50 of this study of 77.8 mg/kg bw is considered of limited reliability given the low number of tested animals and the variability in responses resulting in the absence of a confidence interval. In addition, several comments were provided regarding the use of old studies which were limitedly reported. We agree that several of the older studies have limitations in their reporting as already stated in the proposal. However, many older studies used higher number of animals resulting in a more accurate estimate of the LD50.

Although the new study has limited reliability, it suggests that the oral LD50 of nicotine in this study is above 17.5 mg/kg bw. Due to this new data in mice, we now conclude that the

proposed classification in category 1 based on a LD50 of 3.3 mg/kg bw in mice, is too conservative. Therefore , we now propose classification in category 2 and an ATE of 9 mg/kg bw based on the most sensitive species in the assessment below.

Rats
Several acute oral studies in rats are available with LD50 values in the range of 52.5 to 188 mg/kg bw. Three gavage studies are considered acceptable with LD50 values in the range of 52.5, to 70 mg/kg bw. The results in rats would justify classification in category 2 (ATE between 50 and 300 mg/kg bw).

Mice
Three acute oral studies in mice are available which are limitedly reported or have limitations. The LD50 values were 3.34, 28 and 77.8 mg/kg bw. Although the new study has limitations making the derived LD50 of 77.8 mg/kg bw of limited reliability, this study shows that the LD50 in mice is most likely above 17.5 mg/kg bw as this dose level was tested three times and did not result in mortality. The differences between the three mice studies may be caused by the differences in individual susceptibility between mice but could also be caused by difference in susceptibility between mice strains. However, a comparison of differences in sensitivity to the induction of seizures by IP injection of nicotine in 19 imbred strains of mice resulted in a difference of LD50 of only a factor of 3. This does not explain the observed differences in LD50 between the three studies. The differences in LD50 values that were obtained in the three limited studies would result in three different classification categories. Therefore, no classification category can be determined based on the studies in mice only.

Dogs
A single study in dogs is available with an LD50 of 9.2 mg/kg bw. It should be noted that nicotine was applied within the oral cavity and resulted in mortality within 2 – 4 minutes. This indicates that absorption via the mucous membranes is the most likely route of absorption. Although there are some limitations in reporting, this data are considered acceptable and would result in classification in category 2.

Humans
Some information on human lethal dose levels is available from a suicide case (see comment and repsonse number 7) indicating that the lethal dose in humans is less than 150 mg/kg bw. Also an estimate of the LD50 in humans was made by Mayer (2015) based on the lower limit of lethal nicotine blood concentration and pharmacokinetic data. The suggested oral LD50 was 6.5 to 13 mg/kg bw. This result would suggest classification in category 2.

Choice of the most relevant species
The CLP criteria (3.1.2.2.1) require a scientific judgement of the most appropriate species from among valide, well performed tests when information on several animal species is available. Because in several studies clinical effects and mortality occurred within seconds to a few minutes, the uptake of nicotine after oral administration is considered very relevant and probably even more relevant than the half-live of nicotine because the time to death is much shorter than the half-live in the species for which such data is available. No quantitative information on the uptake via mucous membranes and the intestinal tract in any species, including humans, is available. Consequently, no relevant species can be selected. In line with the CLP guidance the most sensitive species should therefore be selected. The occurance of clinical effects and lethality within minutes also indicates that uptake via mucous membranes is very relevant and studies using gavage treatment that prevent uptake via the mucous membranes in the mouth may underestimate the acute oral

toxicity. Therefore, some preference is given to studies applying nicotine directly into the mouth.

Conclusion
Classifiction in category 2 is proposed because this is in line with the results in several species including the most senstive species and strains with the exception of one study in mice. This study (Lazutka, 1969) was the only study out of a large range of studies indicating an LD50 value below 5 mg/kg bw (cut-off value for category 1). Using this study for determining the classification is considered to be too conservative. Classification in category 3 was also considered as many studies, including more recent studies performed according to OECD, showed LD50 values above 50 mg/kg bw. However, all these studies used gavage treatment, which may underpredict the toxicity of nicotine. In addition, this would not be in line with the guidance that states that the most sensitive species and strains should be used. Classification in category 1 was also be considered based on a strict interpretation of the guidance using the most sensitive study in the most sensitive species. However, seen the range of available studies including the new study in mice and the limited description of the Lazutka study, this is now considered too conservative. An ATE of 9 mg/kg bw is proposed based on the results of the study in dogs being the most sensitive species tested without gavage. This ATE is supported by the estimate for a human LD50 made by Mayer (2013).

<u>Dermal route</u>

The original proposal to retain the current classification in category 1 was based on the fact that the current classification is based on a study for which only the dermal LD50 value is known supported by a study in cats with limitations (i.e. the absence of a clear LD50 and the use of a dilution of nicotine in water). A new dermal study in rabbits was provided as summarised above and considered acceptable. The new study resulted in a dermal LD50 value of 70.4 mg/kg bw. This value is close to the other available incompletely reported dermal study in rabbits (LD50 = 50 mg/kg bw) and the mortality observed in cats (81% at 80 mg/kg bw). All these LD50 values are within the range or on the border of the range for category 2 (50 – 200 mg/kg bw). Therefore, classification in category 2 is now proposed based on the assumption that the draft study report on the acute dermal toxicity in rabbits is representative for the final study report. The proposed ATE of 70 mg/kg bw is also based on the new study.

However, it should be noted that for calculating the acute dermal classification of mixtures containing nicotine in water, the increased dermal uptake of nicotine in water (Kuswahyuning & Roberts, 2014) should be taken into account according to article 12c of CLP.

<u>Inhalation route</u>

The original proposal for Acute Tox 2; H330 was based on two acute inhalation studies with limitations in the study design. No new studies via this route were provided. There were several comments challenging these studies. However, these comments are not considered to limit the acceptability of these studies as explained in response to these comments. Other comments were on the time extrapolation method to estimate the ATE. The approach proposed by France (comment 39) is considered the best way to determine an ATE based on the available database. The use of n=1 is also in line with the REACH guidance on time extrapolation (R7a). Consequently, classification in category 2 is still considered justified based on the acute inhalation toxicity in both available inhalation studies in the range of 0.05 – 0.5 mg/l for dusts and mists. An ATE of 0.19 mg/L is proposed based on

extrapolation of the LC50 of 2.3 mg/L in the 20 minutes inhalation study using Haber's law ((2.3 mg/L * 20 minutes) / 240 minutes = 0.19 mg/L).

RAC's response

Thank you for the opinion.
Your disagreement with classification proposed by Dossier Submitter has been noted as well as your justification of this disagreement.

Please read a RAC response to comment No. 31 which referes also to your comments.

The results of the currently performed studies by the registrant the acute oral toxicity study on mice (LD_{50} of approx. 77.8 mg/kg), acute dermal toxicity study on rabbits (LD_{50} being in a range of doses 50 to 200 mg/kg) contributes to a total body of evidence of acute toxicity of nicotine, therefore RAC supports the new classification of nicotine proposed by the Dossier Submitter in the comments above which take into account the results of new studies.

It also important to note that hazard classification is identification of hazardous properties of the substance and should not be mistaken with the risk assessment, which determins the probability of the definite effect e.g. death to occur, which is a dose-dependent phenomenon. So even for highly acutely toxic substances classified to category 1 or 2 the ingestion or inhalation or dermal exposure to very small amount of this substance will not cause death or poisoning, as is a case with nicotine.

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2015	United Kingdom	ECITA	BehalfOfAnOrganisation	42

Comment received

Acute oral toxicity
The RIVM CLH report discusses in some detail the metabolism of nicotine in section 4.1.3 and the extent to which it varies between species. While this may question the applicability of the rat data for humans, it does not make a case for the mouse data being any more representative of human metabolism.
This is particularly concerning when the rat data (particularly van den Heuvel et al., 1990) is comparatively recent and of good methodology, whereas the mouse data is older and of more uncertain method. There is also considerable variation in the measured LD50 for mice, with the study identified as key by RIVM appearing to be uncharacteristically low compared with the remaining data. Examination of alternative sources also suggests that this LD50 may be unrepresentatively low, for example the United States Department of Health and Human Services National Toxicology Program data on Nicotine (http://tools.niehs.nih.gov/cebs3/ntpviews/index.cfm?action=testarticle.toxicity&cas_number=54-11-5#Non-Human%20Toxicity%20Values)reports an oral mouse LD50 of 24mg/kg (although the reference is to a book, and the exact origin of the data is unclear) and additionally refers to a study in which mice were administered i.v. triterated (3H) nicotine, causing only 5% deaths at a dose of 5mg/kg, although 'most' died at an i.v. dose of 10mg/kg. An LD5 of 5mg/kg delivered intravenously is not supportive of a lower oral LD50 value.
The choice of this study is also concerning in light of RIVM's expressed opposition to the applicability of the gavage method in rats, to human oral exposure, since this study also used a gavage method.
RIVM reported that they were unable to retrieve a more recent set of mouse data

(Trochimowicz et al., 1994), but a fairly trivial search indicates the likely source of this as "Trochimowicz HJ, Kennedy Jr GL, Krivanek ND. Heterocyclic and miscellaneous nitrogen compounds. In: Clayton FE, ed. Patty's Industrial hygiene and toxicology. 4th ed. New York, John Wiley & Sons, Inc. 1994; IIE:3374-9, 3489-91.". It would seem that this reference would benefit from further evaluation.
Section 4.1.3 also cites Tutka, 2005 as identifying that "rabbits seem to be a good model for studying human NIC metabolism"
Taken as a whole, the RIVM proposal identifies that there are some issues with the current classification that it would be of benefit to address. However it does not make a robust case for the selection of mouse data over rat data for determining acute oral toxicity, nor for the selection of the specific mouse data selected. Before making such a sweeping change, a more thorough literature search should be conducted. A robust justification for the selection of the key species for classification should also be made, since section 4.1.3 contains nothing to suggest that the mouse data is more representative of human toxicology than other species, and as currently written actually proposes that rabbit data would be the most suitable. Any decision-making would be much more robust if, after selection of a key species is justified, robust data using modern methodology could then be obtained for this exposure route (if it is not already available).

Acute inhalation toxicity
There seems very little justification for adopting the proposed LC50, when the proposal itself identifies that "The available acute inhalation data do not allow determination of an LC50 value". A comparison with criteria (4.2.4) that applies "can be estimated", "probably even lower" and "this is in the middle" to the calculation of an LC50, following this statement, does not seem sufficiently robust.
While there may be some merit in the identification of an LC50 for nicotine as a pure substance, this is not a likely route of consumer exposure, and there have been no reports of serious acute toxicity involving inhalation of either nicotine-containing smoke from tobacco products or vapour/aerosol from electronic cigarettes. Since there are already extremely conservative workplace exposure limits in place to address occupational exposure to nicotine-containing mists or dusts (US NIOSH 0.5mg/m3 8 hour TWA; UK HSE 0.5mg/m3 8 hour TWA, 1.5mg/m3 15 minute STEL) there does not seem any pressing need to address this issue.
It would not seem either appropriate or necessary to adopt an LC50 value unless better data on acute inhalation toxicity can be obtained on which to base a decision.

Dossier Submitter's Response

Thank you for your comment. We would like to refer to our response to comment number 3 for further clarification. Regarding the reference Trochimowicz et al, 1994; data contained in this reference is all secondary data; no original studies are included, and thus are considered less relevant to the CLH proposal.

RAC's response

Thank you for the opinion.

Your disagreement with classification proposed by Dossier Submitter has been noted as well as your justification of this disagreement.

We agree that more thorough description of studies would be appreciated, however some of them are not accessible, therefore RAC makes its opinion based on available data taking into account original studies used in CLH report when available

Please read a RAC response to comments No. 31 and No. 41 which refers also to your

comments.

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	Spain		Ministry of Justice	43

Comment received

El Instituto Nacional de Toxicología y Ciencias Forenses (INTCF) se articula según la Ley Orgánica del Poder Judicial (LOPJ) como órgano consultor de la Administración de Justicia. Su finalidad es asesorar a Jueces, Magistrados, Fiscales y Tribunales acerca de materias de orden toxicológico. Por ello, emite informes a instancia de los mismos cuando así es requerido por la Administración de Justicia.

En él se integra el Servicio de Información Toxicológica (SIT) que asume, entre otras, la función de emitir tanto informes toxicológicos judiciales como clínicos a petición de un organismo oficial y de particulares en caso de intoxicación por ser un servicio médico integrado por Médicos Forenses y Facultativos que actúan como peritos oficiales judiciales.

El INTCF contribuye a la unidad de criterio científico y a la calidad de la pericia analítica, así como al desarrollo de las ciencias forenses, tal y como se establece por ley.

En relación a la propuesta de cambiar la clasificación, nuestras reflexiones son las siguientes:

- Es favorable y pertinente el añadir en el CLH la nueva categoría 2 respecto a a inhalación de nicotina, puesto que el nuevo uso para tratamiento de dependencias a dicha sustancia es a través de la inhalación de nicotina mediante los nuevos dispositivos de cigarrillos electrónicos, los cuales y como ya hemos comentado en otras ocasiones, no están exentos de toxicidad, aparte de que en nuestro Servicio seguimos recogiendo nuevos casos de intoxicados ante un uso erróneo, accidental o por desconocimiento de dichos dispositivos.
- El cambiar de categoría 3 (tóxico al ser ingerido) por la categoría 1 (fatal al ser ingerido), nos parece acertado siempre y cuando se interprete la potencialidad de ingerir una dosis tóxica, según cantidad y peso del sujeto afectado. Es preciso resaltarlo así, puesto que un trago casual de dicha sustancia no siempre resultará fatal, sino que pudiera resultar con ese calificativo en caso de ingesta potencialmente tóxica, que no fatal, puesto que esta última expresión pudiera conllevar el resultado de muerte, y como sabemos no siempre será así. Es decir, lo catalogado como tóxico no conlleva necesariamente a ser catalogado como fatal.
- Por último, en relación a la clasificación actual armonizada de Toxicidad Aguda 1 (fatal al contacto por la piel), discrepamos al ser catalogado con tal calificativo, puesto que, como anteriormente ha sido expresado, dicha expresión puede conllevar al resultado de muerte, y no es el caso realmente con dicha vía de entrada al organismo.

Un cordial saludo.

a 5 de junio de 2015

SERVICIO DE INFORMACION TOXICOLOGICA

ECHA unofficial translation:

The National Institute of Toxicology and Forensic Sciences (INTCF) is defined under the Organic Law of the Judicial Power (LOPJ) as a consulting organism of the Administration of Justice. Its aim is to assist Judges, Magistrates, Prosecutors and Tribunals of Justice on matters related to toxicology. Thus, it submits reports to them when required to do so by the Administration of Justice.

The Service of Toxicological Information (SIT) is integrated within the INTCF and has the task among others to submit both judicial and clinical toxicological reports when required by an official organism or by private individuals in case of intoxication since it is a medical service integrated by forensic doctors and facultatives that act as official judicial experts.

The INTCF contributes to the scientific criteria and to the quality of the analytical expertise as well as to the development of the forensic sciences as defined by the law.
In relation to the proposal to change the classification, we have the following comments:

- It is adequate and pertinent to add to the CLH the new category 2 related to the inhalation of nicotine, since the new use for the treatment of dependence on this substance is through the inhalation of nicotine with electronic cigarettes, which, as we have already pointed out in other occasions, are not exempted from toxicity; in our service we continue to identify new cases of intoxication due to misuse of these dispositives either by accident or by lack of knowledge.
- The change of category 3 (toxic by ingestion) for category 1 (lethal by ingestion) is in our view correct as far as the potentiality to ingest a toxic dose, taking into account the quantity and weight of the affected person, is considered. It has to be specified in this way as a casual ingestion of the substance will not always be fatal, but it might be considered as such in case of an ingestion potentially toxic since this situation may lead to death, and as we know, it will not always be the case. That is, what is classified as toxic does not necessarily imply to be classified as fatal.
- Finally in relation to the harmonised classification of Acute Toxicity 1 (lethal in contact with the skin) we disagree on the classification since, as previously discussed, this expression may result in death and it is not really the case through this route of entrance to the organism.

Best regards

[ECHA note: the comment above was provided in the following attachment:]
NOTIFICATION

Dossier Submitter's Response

Thank you for your comment and support for our CLH proposal.

RAC's response

Your support to the classification proposed by the Dossier Submitter for acute oral and inhalation toxicity of nicotine is noted as well as your disagreement to the proposed classification for dermal toxicity.

RAC is of the opinion that there were no acceptable acute dermal toxicity studies in rats or rabbits for nicotine presented in CLH report. Only the acute dermal toxicity study on rabbits recently submitted by the lead registrant is acceptable to be used for classification purposes.

Taking into account that dermal LD_{50} of nicotine for rabbits in this study equals 70.4 mg/kg bw, which is within the range of 50mg/kg - ≤200 mg/kg, RAC is of the opinion that nicotine warrants classification Acute Tox. 2 (dermal) with the hazard statement H310 *Fatal in contact with skin*.

The study of Travell (1960) in cats may be taken as supportive for assessment of the acute dermal toxicity, although due to specific experimental design deficiencies the calculation of an exact LD_{50} value is not possible. The dose of 66 mg/kg is taken as LD_{50} of nicotine for cats, because this dose is at the lower end of the range of doses (66 – 100 mg/kg)at which 81% of mortality was observed. It is probable that cats administered a lower dose i.e. 66 mg/kg had a higher probability for survival than those receiving higher dose close to 100 mg/kg. Thus a dose of 66 mg/kg is a rather conservative estimate of the LD_{50} based on data from Travell study (1960).

OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2015	Netherlands	Fontem Ventures	BehalfOfAnOrganisation	44
Comment received				
Please see attached document for Fontem Ventures' specific comments on the inhalation toxicity of nicotine, which are supported by a number of references. *[ECHA note: The following attachment was provided with the comment above:]* Submission challenging nicotine CLP reclassification proposal				
Dossier Submitter's Response				
This is the same comment as comment number 4, please refer to this comment for our response.				
RAC's response				
The classification of aspiration hazard of nicotine was not proposed by the Dossier Submitter and therefore it is not considered by RAC.				

Date	Country	Organisation	Type of Organisation	Comment number
29.05.2015	United Kingdom		Individual	45
Comment received				
To introduce a CLP classification of acute inhalation toxicity based on an estimate based on data that is deemed insufficient makes a mockery of the system that people rely on to provide sound scientific fact. Classification in this area should be delayed until sound evidence is available and it should be made a matter of priority to gain that evidence.				
Dossier Submitter's Response				
Thank you for your comment. Please refer to our response to comment number 3.				
RAC's response				
The classification of aspiration hazard of nicotine was not proposed by the Dossier Submitter and therefore it is not considered by RAC. Please see also response to comments No.31.				

NON-CONFIDENTIAL ATTACHMENTS RECEIVED

1. **EU Classification of nicotine mixtures under CLP Regulation 1272/2008 (as amended and corrected) Bibra Proposal** – submitted by Totally Wicked on 05/06/2015 [Please refer to Comment number 36]
2. **JTI comment on the RIVM proposal for a new CLP classification of nicotine** – submitted by JT International SA on 05/06/2015 [Please refer to Comment number 3]
3. **Submission challenging nicotine CLP reclassification proposal** – submitted by Fontem Ventures on 05/06/2015 [Please refer to Comment number 4, 37, 44]
4. **Supporting information for Nicoventures' response to RIVM's nicotine CLH report** – submitted by Nicoventures Ltd. On 03/06/2015 [Please refer to Comment number 7]
5. **Attachment to the comment on the proposal for harmonised classification and labelling of nicotine** – submitted by CONTRAF-NICOTEX-TOBACCO GmbH (CNT) on 03/06/2015 [Please refer to Comment number 41]
6. **Pure nicotine reclassification by RIVM** – submitted by LIAF Lega Italiana Anti Fumo on 03/06/2015 [Please refer to Comment number 9]

7. **ECITA response to proposal submitted for harmonised classification and labelling of nicotine, CAS 54-11-5, EC 200-193-3** – submitted by ECITA on 03/06/2015 [Please refer to Comment number 10]
8. **PMI COMMENTS AND ASSOCIATED BIBLIOGRAPHY RELATING TO THE APRIL 2015 CLH REPORT "PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELING" FOR NICOTINE BY RIVM –** submitted by Philip Morris International on 02/06/2015 [Please refer to comment number 13]
9. **How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century** – Article submitted by Esigbond on 08/05/2015 [Please refer to comment 23]– Not published on the ECHA website
10. **NOTIFICACION** – comment submitted by INSTITUTO NACIONAL DE TOXICOLOGÍA Y CIENCIAS FORENSES on 05/06/2015 [Please refer to comment number 43]