CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: nicotine (ISO); 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine

EC Number: 200-193-3

CAS Number: 54-11-5

Index Number: 614-001-00-4

Contact details for dossier submitter:

RIVM, Bureau REACH

PO Box 1, 3720 BA Bilthoven. The Netherlands

bureau-reach@rivm.nl

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**nicotine (ISO); 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine**
EC number:	***200-193-3***
CAS number:	***54-11-5***
Annex VI Index number:	***614-001-00-4***
Degree of purity:	***Minimum purity >99%***
Impurities:	***cotinine <= 0.15%,*** ***myosmine <= 0.15%,*** ***FAB (N-(4-oxo-4-pyridin-3-yl-butyl)-formamide) <= 0.10%,*** ***nicotine N-oxide <= 0.15%,*** ***nornicotine <= 0.15%,*** ***anatabine <= 0.15%,*** ***beta-nicotyrine <= 0.10%,*** ***anabasine <= 0.10%.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox. 3* (H301) Acute Tox. 1 (H310) Aquatic Chronic 2 (H411)
Current proposal for consideration by RAC	Changing Acute Tox. 3* (oral) into Acute Tox. 1 (oral)

	Adding Acute Tox 2 (inhalation)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 1 (H300) Acute Tox. 1 (H310) Acute Tox. 2 (H330) Aquatic Chronic 2 (H411)

*Minimum classification

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Not assessed
2.2.	Flammable gases				Not assessed
2.3.	Flammable aerosols				Not assessed
2.4.	Oxidising gases				Not assessed
2.5.	Gases under pressure				Not assessed
2.6.	Flammable liquids				Not assessed
2.7.	Flammable solids				Not assessed
2.8.	Self-reactive substances and mixtures				Not assessed
2.9.	Pyrophoric liquids				Not assessed
2.10.	Pyrophoric solids				Not assessed
2.11.	Self-heating substances and mixtures				Not assessed
2.12.	Substances and mixtures which in contact with water emit flammable gases				Not assessed
2.13.	Oxidising liquids				Not assessed
2.14.	Oxidising solids				Not assessed
2.15.	Organic peroxides				Not assessed
2.16.	Substance and mixtures corrosive to metals				Not assessed
3.1.	Acute toxicity - oral	Acute Tox. 1 (H300)		Acute Tox. 3 (H301)	
	Acute toxicity - dermal			Acute Tox. 1 (H310)	
	Acute toxicity - inhalation	Acute Tox. 2 (H330)			
3.2.	Skin corrosion / irritation				Not assessed
3.3.	Serious eye damage / eye irritation				Not assessed
3.4.	Respiratory sensitisation				Not assessed
3.4.	Skin sensitisation				Not assessed
3.5.	Germ cell mutagenicity				Not assessed
3.6.	Carcinogenicity				Not assessed
3.7.	Reproductive toxicity				Not assessed
3.8.	Specific target organ toxicity –single exposure				Not assessed
3.9.	Specific target organ toxicity – repeated exposure				Not assessed

3.10.	Aspiration hazard				Not assessed
4.1.	Hazardous to the aquatic environment			Aquatic Chronic 2 (H411)	
5.1.	Hazardous to the ozone layer				Not assessed

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Pictogram: GHS06, GHS09

Signal word: Danger

Hazard statements: H300 "Fatal if swallowed",
H310 "Fatal in contact with skin",
H330 " Fatal if inhaled",
H411 "Toxic to aquatic life with long lasting effects"

Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry:

: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The current harmonised classification of nicotine for acute toxicity is the translation of the DSD classification with T+; R27 and T; R25. The old proposal for harmonised classification (available in the IUCLID file) shows that the proposal for acute oral toxicity was based on a list of LD50 values with references. The list for acute oral toxicity included several species including rat, mouse and dog. However, the DSD criteria were based on rats. The rat LD50 values in the range of 50-80 mg/kg bw resulted in a classification with R25 (criterion: LD50 oral, rat: 25 < LD50 ≥ 200 mg/kg). Translation of R25 resulted in Acute Tox. 3*; H310 because the DSD and the CLP criteria differ and a lower classification could not be excluded without going back to the original proposal. For acute dermal toxicity, LD50 values were available for rat (140-280 mg/kg bw) and rabbit (50 mg/kg bw/day). The classification with R27 was based on the rabbit LD50 (criterion: LD50 dermal, rat or rabbit: ≤ 50 mg/kg) using the lower value of both species. Translation of R27 resulted in Acute Tox. 1; H310 because the DSD and the CLP criteria both relate to an LD50 below 50 mg/kg bw.

2.2 Short summary of the scientific justification for the CLH proposal

This proposal is based on the information available in the REACH-registration (accessed January 2015), the DAR of nicotine (1), EFSA 2009 (2) and other information available in literature.

The proposed classification for acute oral toxicity with Acute Tox. 1; H300 is based on the lowest LD50 of 3.34 mg/kg bw for the available LD50 values for different species and strains. This LD50 value fulfils the requirement for Acute Tox. 1; H300 being an ATE (LD50) below 5 mg/kg bw.

The available acute dermal toxicity studies are very limitedly described. The current classification is based on a study in rabbits with an LD_{50} of 50 mg/kg bw. This LD_{50} value fulfils the requirement for Acute Tox. 1; H301 although there is no access to the original study. However, one acceptable study with nicotine in cats is available that supports the current harmonised classification. As such, it is considered justified to keep the current classification.

There are two acute toxicity inhalation studies with limitations in tested concentration or exposure duration. However, combined these two studies indicate an LC50 in the range between 0.1 and 0.5 mg/L (aerosol), justifying classification in category 2.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Acute Tox. 3* (H301), Acute Tox. 1 (H310), Aquatic Chronic 2 (H411).

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Table 4. Self-classification by the registrant (on 23 December 2014)

Hazard Class	Statement Code	# of notifiers
Acute Tox. 3	H301	174
Acute Tox. 1	H310	177
Aquatic Chronic 2	H411	178
Acute Tox. 2	H300	4
Aquatic Acute 1	H400	4

Total number of notifiers; 178. Number of aggregated notifications; 6.

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The current classification of nicotine for acute oral toxcitiy, Acute Tox. 3*, has led to confusion in several European countries, including the Netherlands. The * indicates that this is a minimum classification and requires manufacturers and importers of nicotine to investigate whether he has access to data or other information that lead to a more severe category and apply this more severe category (CLP Annex VI, 1.2.1). Whereas industry bases its classification on an $LD50_{oral}$ of 50 mg/kg bw in rats, the RIVM came to an $LD50_{oral}$ of 5 mg/kg bw based on the possible translations of R25 into Cat 2 or Cat 3 and the much lower LD50 values for mouse and dogs compared to rats. The lowest available LD_{50} value of 3.34 mg/kg bw (for mice) warrants a harmonized classification of Acute Tox. 1, instead of 3.

In addition for the classification of mixtures containing nicotine for acute toxicity, the determination of the ATE of nicotine for the calculation of the ATE of the mixture is very relevant as there is a difference in opinion between inspectorates and industry. An advice of RAC on the LD50 value that was determinative for the classification and that should be used in the ATE calculation of the mixture would therefore be very helpful.

Given the current policy discussions on the use of the e-cigarette, the increase in accidents with e-cigarette refills and its increasing popularity, the Netherlands deems it important to submit a CLH dossier on nicotine to propose a classification change from Acute Tox. 3 to Acute Tox. 1 and if possible have an advice on the ATE for acute oral toxicity.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

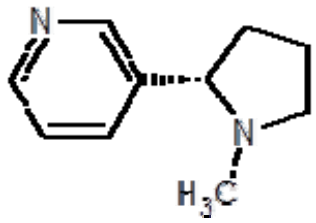
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	200-193-3
EC name:	nicotine (ISO); 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine
CAS number (EC inventory):	54-11-5
CAS number:	54-11-5
CAS name:	Pyridine, 3-[(2S)-1-methyl-2-pyrrolidinyl]-
IUPAC name:	3-[(2S)-1-methylpyrrolidin-2-yl]pyridine
CLP Annex VI Index number:	614-001-00-4
Molecular formula:	$C_{10}H_{14}N_2$
Molecular weight range:	162.23

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Nicotine	99%	99-100%	According to European Pharmacopoeia 8.0

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
cotinine myosmine FAB (N-(4-oxo-4-pyridin-3-yl-butyl)-formamide) nicotine N-oxide nornicotine anatabine beta-nicotyrine anabasine	<= 0.15% <= 0.15% <= 0.10% <= 0.15%, <= 0.15% <= 0.15% <= 0.10% <= 0.10%		Impurity profile derived from European Pharmacopoeia 8.0

Current Annex VI entry: Not relevant

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry: Not relevant

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Colourless liquid with brown tint and fishy smell	DAR	Visual and olfactory assessment Print; DAR3 B1-B5
Melting/freezing point	-79 °C	DAR	
Boiling point	247 °C	DAR	
Relative density	1.010	DAR	
Vapour pressure	5.62 Pa at 25 °C	DAR	
Surface tension	No data provided	DAR	
Water solubility	1000 g/L at unknown temperature and pH	DAR	
Partition coefficient n-octanol/water	Log K_{ow} = 1.17 at unknown temperature and pH, Log K_{ow} = 0.93	DAR	
Flash point	Not relevant	DAR	
Flammability	101 °C, auto flammability 243 °C, auto-ignition 244 °C	DAR	
Explosive properties	Based on molecular structure, nicotine is unlikely to be explosive as it does not possess any of the chemical groups expected to impart explosive properties on a molecule, with only carbon, hydrogen and nitrogen present.	DAR	
Self-ignition temperature			
Oxidising properties	No data or case provided, stated by notifier as non-oxidising	DAR	
Granulometry			
Stability in organic solvents and identity of relevant degradation products	Soluble in chloroform, diethyl ether, ethanol and petroleum ether.	DAR	
Dissociation constant	pKa1= 3.1; pKa2 = 8.2	DAR	
Viscosity			

2 MANUFACTURE AND USES

2.1 Manufacture

Nicotine is a naturally occurring alkaloid obtained from the leaves of the tobacco plant.

2.2 Identified uses

Nicotine is the main constituent in tobacco smoke. In recent years there has been an increased interest in the development of nicotine replacement therapies based on alternative exposure routes. As such, the primary therapeutic use of nicotine is in treating nicotine dependence in order to eliminate smoking. Controlled levels of nicotine are given to patients through gums, dermal patches, lozenges, electronic/substitute cigarettes or nasal sprays in an effort to wean them off their dependence. Nicotine is also used in e-cigarettes. Nicotine is also present in mushrooms (2) possibly due to the use as insecticide. Nicotine was not included in Annex I of 91/414 because the existing evidence did not demonstrate safe use.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not relevant as this proposal is limited to classification for acute toxicity.

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion of

3.1.2 Comparison with criteria

3.1.3 Conclusions on classification and labelling

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

4.1.2 Human information

4.1.3 Summary and discussion on toxicokinetics

As summarized by EFSA, 2009 (2):

"Nicotine is rapidly absorbed through the oral cavity, lung, and gastrointestinal tract. Absorption of nicotine across biological membranes depends on pH. In its ionised state, such as in acidic environments, nicotine does not rapidly cross membranes. The respiratory absorption of nicotine was found to be 60% to 80%. Nicotine base can be absorbed through the skin, and there have been cases of poisoning after skin contact with pesticides containing nicotine. Nicotine is poorly absorbed from the stomach because it is protonated (ionized) in the acidic gastric fluid, but is well absorbed in the small intestine, which has a more alkaline pH and a large surface area. Following the administration of nicotine capsules or nicotine in solution, peak concentrations in blood are reached in about 1 h (Benowitz et al., 1991; Zins et al., 1997; Dempsey et al., 2004). The oral bioavailability of nicotine is incomplete because of the hepatic first-pass metabolism and ranges between 20% to about 45% (Andersson et al., 2003; Benowitz et al., 1991; Compton et al., 1997; Zins et al., 1997; Hukkanen et al., 2005). After intravenous administration, the highest levels of nicotine were found in spleen, liver, lungs and brain (UK DAR, 2007).

The metabolism of nicotine is mediated mostly through the hepatic cytochrome P450 CYP2A6 with the C-oxidation of nicotine to cotinine as the major detoxication reaction, followed by the hydroxylation of cotinine to 3-hydroxycotinine (Dorne et al., 2004; Hukkanen et al., 2005). The lungs and the kidneys are also partially involved in the metabolism of nicotine. Variants in the CYP2A6 gene have been associated with altered nicotine metabolism and with effects on smoking behaviour. A number of genotypes of CYP2A6 have been determined and a recent intravenous study (Benowitz et al, 2006b) classified subjects in three phenotypes according to CYP2A6 activity (fractional clearance of nicotine to cotinine and on plasma ratio of 3-hydroxycotinine to cotinine) with respective CYP2A6 activities and mean total plasma clearances of 100%, 80% and 50%, and 18.5, 15.5 and 11.7 ml/min/kg. Elimination half-lives ranged from 1.8 to 2.9 hours between the three phenotypes (Benowitz et al., 2006b). Considering the short biological half-live of nicotine in humans, no accumulation of nicotine is foreseen.
Nicotine readily crosses the placenta. Nicotine is mainly excreted through urine, and faeces. The rate of nicotine excretion is influenced by the pH of the urine. When the pH of the urine is made alkaline, the proportion of uncharged nicotine increases and re-absorption of nicotine and as a result, less nicotine is excreted (UK DAR, 2007).

Recently, a mechanistic population model for the pharmacokinetics of nicotine, its primary (CYP2A6-generated) metabolite cotinine and 3-hydroxycotinine has been developed from sixty-six subjects receiving orally 2 mg of deuterium-labelled nicotine and 10 mg deuterium-labelled cotinine simultaneously. The model showed high correlation between nicotine clearance to cotinine and the 3-hydroxycotinine to cotinine concentration ratio in saliva supporting the idea that the 3-hydroxycotinine: cotinine ratio can be used as a predictor of CYP2A6 activity and nicotine clearance. The model-based analysis extends and further justifies this conclusion (Levi et al., 2007a). This model has been applied to predict nicotine clearance using cotinine and 3-hydroxy-cotinine spot saliva samples (Levi et al., 2007b).
A recent study (Yun et al., 2008) in subjects exposed to transdermal nicotine patches administered as single and multiple doses, demonstrated that nicotine clearance in smokers is slower than in non-smokers: in smoking individuals nicotine induces glucuronidation, and higher plasma concentrations are thus maintained."

Species differences in nicotine metabolism as summarised by Hukkanen, 2005 (25):

"Nicotine metabolism in various species has been reviewed previously (Gorrod and Jenner, 1975; Scheline, 1978; Seaton and Vesell, 1993). Cotinine and 3-hydroxycotinine are major urinary nicotine metabolites in all mammalian species studied (Jenner et al., 1973; Nwosu and Crooks, 1988; Kyerematen et al., 1990a); however, about as much nicotine *N*-oxide as cotinine and 3-hydroxycotinine is formed by guinea pigs and rats. Guinea pig and hamster hepatocytes show the highest total metabolism of nicotine, followed by mouse, rat, and human hepatocytes (Kyerematen et al., 1990a). In general, there is considerable variation between rodent species in the activity of nicotine metabolism, as well as in the stereospecificity and relative amounts of nicotine metabolites produced. Also, P450 enzymes responsible for nicotine metabolism vary in species. For example, CYP2B1/2 is the P450 enzyme metabolizing nicotine in rats, whereas rat CYP2A is inactive in nicotine metabolism (Hammond et al., 1991; Nakayama et al., 1993).
Nicotine metabolism in nonhuman primates resembles human metabolism. In macaque monkeys, nicotine and cotinine half-lives are similar to humans (Seaton et al., 1991). Like humans, African green monkeys metabolize 80 to 90% of nicotine via a CYP2A6-like enzyme, but hepatic protein levels are about 4 times higher in green monkeys than humans resulting in 2-fold higher *V*max for cotinine formation (Schoedel et al., 2003). Rhesus monkey hepatocytes metabolize about 80% of nicotine to cotinine (Poole and Urwin, 1976).

Nicotine *N*-glucuronidation activity is highest in human liver microsomes followed by rhesus and cynomolgus monkey microsomes, although the activity in monkey microsomes is only about 7 to 11% of human glucuronidation activity (Ghosheh and Hawes, 2002a). Low-level nicotine glucuronidation activity was also detected in minipig and guinea pig microsomes, whereas activity was not measurable in rats, mice, dogs, and rabbits. Cotinine glucuronidation was below limit of quantification for all the animal species, including rhesus, cynomolgus, and marmoset monkeys (Tsai and Gorrod, 1999; Ghosheh and Hawes, 2002a)."

In addition, according to Tutka, 2005 (26) this indicates that the rat may not be the most relevant species for humans:
"In a recent study of Tutka et al. [unpublished data], the significant differences in NIC metabolism were found among human, rabbit, and rat, confirming species variability in NIC metabolism. The study showed that a profile of NIC metabolism in rabbit was different from that of the rat. In contrast to rats, rabbits seem to be a good model for studying human NIC metabolism."

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Table 11: Summary of acute oral toxicity studies using nicotine

Method	**Dilution**	**LD_{50} (mg/kg bw)**	**Animal**	**Remarks**	**Acceptability**	**Reference**
Single dose administration, peroral administration	0.15-0.3% aqueous solutions	52.5	Rat	Strain, sex and number not specified, nicotine base	Acceptable	Lazutka *et al.*, 1969
Single dose administration, oral	Not described	70	Rat	Strain, sex and number not specified	Not acceptable	Ben-Dyke *et al.*, 1970
Not described, oral	Not described	50 - 60	Rat	Strain, sex and number not specified	Not acceptable	Farm Chemicals Handbook, 1991
Up and down method; gavage	Not described	70	Rat	Sprague-Dawley, 5 male & 5 female	Acceptable	Yam *et al.*, 1991
OECD 1981, acute oral toxicity; gavage Fixed dose procedure	Not described	70 LD50 between 25 and 200 mg/kg bw	Rat	Sprague-Dawley, 15 male, 15 female On average 14 rats per test	Acceptable	Van den Heuvel *et al.*, 1990
Unkown, oral	Not described	50 – 60	Rat	Strain, sex and number not specified	Not acceptable	Trochimowicz et al., 1994*
Unkown, oral	Not described	188	Rat	Strain, sex and number not specified	Not acceptable	DECOS, 2004 (cited as Ray91)*
Unknown, oral	Alkaloid dissolved in water, pH adjusted to 7.0, dilution not described	188	Rat	35 animals, strain and sex not described	Acceptable	Ambrose and DeEds, 1946
Unknown, oral	Not described	24	Mouse	Strain, sex and number not specified	Not acceptable	DECOS 2004; Trochimowicz et al., 1994*
Unknown, oral	Not described	50 – 60	Mouse	Strain, sex and number not specified	Not acceptable	Trochimowicz et al., 1994*
Single dose administration, oral, peroral administration	0.15-0.3% aqueous solutions	3.34	Mouse	Strain, sex and number not specified, nicotine base	Acceptable	Lazutka et al., 1969
Single dose, gavage	Aqueous solution,	24	White	36-55 mice; 5 mice/group. Strain and	Acceptable	Heubner and

	dilution unknown, nicotine base		mouse	sex not specified.		Papierkowski, 1938
Single dose administration, oral; dropped on tongue or between lips and gums	Undiluted alkaloid	9.2	Dog	# of animals; 19, strain and sex not specified	Acceptable	Franke and Thomas, 1932

*These studies could not be retrieved. The above description was derived from the Bibra proposal, 2014 (3). They will not be further described below. As other studies showed the same LD50 values as for some studies which could not be retrieved, it is considered likely that these are the same studies.

Table 12 Summary of oral toxicity studies using nicotine salts

Method	**Dilution**	**LD_{50} (mg/kg bw)**	**Animal**	**Remarks**	**Acceptability**	**Reference**
Single dose, oral, gavage	Not described	75	Rat	Sprague-Dawley, male, # not specified (5/dose), nicotine sulfate	Acceptable	Vernot et al., 1977
Single dose administration, oral, gavage	Suspension in water-lead arsenate and calcium arsenate	83	Rat	Sherman, female, 80/group, nicotine sulfate	Acceptable	Gaines, 1960
Single dose administration, oral, peroral administration	0.15-0.3% aqueous solutions	56.7	Rat	Strain, sex and number not specified, nicotine sulfate*	Acceptable	Lazutka *et al.*, 1969
Single dose administration, oral, peroral administration	0.15-0.3% aqueous solutions	8.55	Mouse	Strain, sex and number not specified, nicotine sulfate*	Acceptable	Lazutka *et al.*, 1969
Single dose, oral, gavage	Not described	16	Mouse	CF-1, male, # not specified (5/dose), nicotine sulfate	Acceptable	Vernot et al., 1977
Single dose, gavage	Aqueous solution, dilution unknown,	87	White mouse	36-55 mice; 5 mice/group. Strain and sex not specified. Nicotine tartrate	Acceptable	Heubner and Papierkowski, 1938

*Nicotine sulfate is an aqueous solution containing 40% nicotine equivalent.

Reference: Lazutka et al., 1969 (4)

Study design:

Short-term toxicity studies included 25 series of experiments using a single peroral administration (gavage) of aqueous solution of nicotine base and nicotine sulfate in doses of 1 -90 mg/kg for albino rats and 0.25 – 16 mg/kg for white mice. Mouse and rat strains, sex and number are not specified. Rats and rabbit were used for skin absorption and conjunctiva studies. Only the total number of animals used is mentioned, which is 332.

Results:

The peroral administration of lethal doses caused irritation of the respiratory tract and motor restlessness, followed by marked hyperemia of the ears and extremities. After 30-40 min, there

were tonic contractions of various groups of muscles, often with transition to clonic spasms. Traube's symptom was positive in the majority of cases. After 40-50 min the spasms were superseded by relaxation of the muscles, and the animals assumed a one-sided position. There was marked dyspnea and tremor of the entire body. In animals surviving the lethal dose the symptoms of poisoning gradually disappeared after 3-7 hr from the beginning. In other animals, their condition became worse, and they ceased to react to outside stimuli. They developed asphyxia and died within 1-3 days. The severity of poisoning and the rate of its development, as well as the interval before death, were directly related to the dose.

Table 13

Parameter	Mice		Rats	
	dose, mg/kg			
	Nicotine	Nicotine sulfate*	Nicotine	Nicotine sulfate
LD_{16}	0.25	1	20	30
LD_{50}	3.34	8.55	52.5	56.7
LD_{100}	10	16	80	90

*Nicotine sulfate is an aqueous solution containing 40% nicotine equivalent.

Acceptability:
Limited description but acceptable given the period in which it was performed.
Conclusions:
The mice and rats differed in their susceptibility, the mice proving more sensitive to nicotine than rats, the LD_{50} for mice being 3.34 mg/kg, and for rats 52.5 mg/kg.

Reference: Ben-Dyke et al., 1970 (5)
This paper lists acute toxicity data for a number of pesticides, including nicotine. This data has been prepared from experimental results of the Toxicology Laboratory, Chesterford Park Research Station, or from published literature and manufacturer's bulletins. However, there are no actual references, the study design is not described, nor the number of animals used; only the oral LD_{50} of 70 mg/kg bw is mentioned.
Acceptability:
Not acceptable

Reference: Farm Chemicals Handbook, 1991 (6)
Only the value for the rat oral LD_{50} is listed; 50-60 mg/kg bw. No mention of study design, number of animals used, etc.
Acceptability:
Not acceptable

Reference: Ambrose and DeEds, 1946 (27)
The acute toxicity of nicotine was determined orally in 35 rats. The alkaloid was dissolved in distilled water and the pH was adjusted to 7.0 with concentrated hydrochloric acid. There is no further mention of study design, strain or sex of rats used.
In this study nicotine was also intraperitoneally injected into 12 rats.

Results: Oral LD_{50} rat 188 mg/kg bw, LD_{50} after intraperitoneal injection rats 30 mg/kg bw. Convulsions were observed after administration.
Acceptability: Acceptable

Reference: Heubner and Papierkowski, 1938 (28).
Between 36 and 55 white mice (17-26 grams) whose strain was not specified were used to assess the acute oral toxicity of nicotine. Nicotine was administered in aqueous solution by gavage at 5 animals per group. The total number of animals are not mentioned. The doses follow a geometric progression , with a range of 20 %, but the actual dosaging is not described. The LD50 was estimated by the method of Spearman_Kärber (Kärber, 1931).
A comparable study was performed using nicotine tartrate.

Results: Oral LD_{50} mouse nicotine base 24 mg/kg bw. Mortality occurred within 25 minutes.

Oral LD_{50} mouse nicotine salt 87 mg/kg bw equivalent with 28 mg/kg bw based on nicotine fraction.

Acceptability: Acceptable

Reference: Franke and Thomas, 1932 (7)
Nicotine was administered orally to 19 dogs. Nicotine was dropped on the tongue or between the lips and gums in the form of the undiluted alkaloid.

Table 14 Results:

Dose (mg/kg bw)	# of dogs	# fatal	# non-fatal	% fatal	Average time till death (minutes)
20	2	2	0	100	2.5
12	1	1	0	100	3.5
9.2 – 10.3	14	8	6	57.1	3.77
4.6 – 5.0	2	0	2	0	-

Acceptability:
Limited description but acceptable given the period in which it was performed.
Conclusions:
Oral LD_{50} dogs 9.2 mg/kg bw.

Reference: Yam et al., 1991 (8)
Study design:
Two different methods were used, the fixed-dose procedure and the up-and-down method, which were compared to the classical method of obtaining an LD_{50}. The fixed-dose procedure was conducted according to the method described by van den Heuvel *et al.*, 1990 (9). It involves dosing 10 rats (5 males and 5 females) with one of four predetermined dose levels, selected on the basis of a sighting study (3-4 animals) so that only evident toxicity and no deaths were observed. Depending on the outcome of the first dose, a second dose group was used. As the fixed-dose procedure does not use death as an endpoint, no LD_{50} can be determined.
The up-and-down method was conducted according to the method described by Bruce (1985 and 1987). Female rats were dosed, one at a time, starting the first animal at the best estimate of the

LD_{50}. If the first animal was alive at the end of 24hr, the next animal was given a higher dose. If the first animal died, the next received a lower dose. The dose was either increased or decreased by a factor of 1.3. The dosing options were repeated until 4 animals had been treated after reversal of the initial outcome.
Classical LD_{50} data were generated by another laboratory (van den Heuvel *et al.*, 1990), described below.

Results:

In all 3 methods, nicotine produced the first sign of toxicity within 1 day. The duration of signs of toxicity was 3 days in the classical LD_{50} and fixed-dose study, but 5 days in the up-and-down study. There were no autopsy findings in the classical LD_{50} and up-and-down-method, but in the fixed-dose method lungs appeared red and slightly congested.

Table 15 Rat LD_{50} values

LD50 values (mg/kg bw)		
Classical method		**Up-and-down method**
Females only	**Combined sex**	**Females only**
71 (42-128)	70 (49-109)	70 (51-96)

Acceptability:

Acceptable

Conclusions:

For the fixed-dose procedure, death is not an endpoint and thus an LD_{50} can't be determined. The conclusion based on the results of the classic method was classification as toxic meaning an expected LD50 between 50 and 500 mg/kg bw. The up-and-down method resulted in a similar LD_{50} as when using the classical LD_{50} method (70-71 mg/kg bw).

Reference: van den Heuvel et al., 1990 (9)

Study design:

The classical LD_{50} method is being compared to the fixed-dose procedure (described above). The classical LD_{50} study is performed according to OECD 1981, using 15 male and 15 female rats. For the fixed-dose procedure, nicotine is tested in 26 different laboratories. In total 355 rats are used, half of which male and the other half female. In total, 31 labs are involved, 21 of those used Sprague-Dawley rats, 9 used Wistar and 1 used Fischer 344 rats; this is not further specified. Mortality occurred in both methods within a day. Observed effects were none for the classical method and oedema of the stomach and pale kidney for the fixed dose procedure.

Table 16 Results:

LD50 values (mg/kg bw)		
Classical method, OECD 1981		
Males only	**Females only**	**Combined sex**
68 (41-129)	71 (42-128)	70 (49-109)

Table 17

Classification*

Classical LD_{50}	Fixed-dose tests - #of labs classifying compound as:			
	Very toxic	Toxic	Harmful	Classified
Toxic	-	23	3	-

*(see van den Heuvel et al., 1990 for criteria) (toxic relates to the DSD criteria meaning an LD50 between 25 and 200 mg/kg bw)

Acceptability:
Acceptable
Conclusions:
Oral LD_{50} rat: 70 mg/kg bw

Reference: Vernot et al., 1977 (10)
Study design:
The single oral LD_{50} of nicotine sulfate in mouse and rat was determined by the method of Smyth et al. 1962 (24), which is not further specified in this paper. The paper from Smyth et al (24) was retrieved for the method use, summarized here: single oral toxicity is estimated by gastric intubation of groups of 5 non-fasted male rats. The dosages are arranged in a logarithmic series differing by a factor of 2. Whenever possible, the chemical is administered undiluted. Based upon mortalities during a 14-day observation period, the most probable LD50 value and its fiducial range are estimated by the method of Thompson (1947) using the Tables of Weil (1952).

Table 18. Results:

LD_{50} values (mg/kg bw)	
Sprague-Dawley rat, male	CF-1 mouse, male
75 (44-127)	16 (12-21)

Acceptability:
Acceptable

Reference: Gaines, 1960 (11)
Study design:
Eighty female rats, at least 90 days old were used. They were not fasted prior to dosage. The survivors were held for daily observation until they appeared to have recovered completely or for a minimum of 14 days. The poisoned rats were observed at least once each hour during the first day after dosage, and twice a day thereafter, for symptoms of poisoning and time of death. The compounds were given orally by means of a stomach tube. Dosing was done with a syringe with 0.1-cc graduations and a blunt-pointed 17-gauge spinal needle which served as the stomach tube. The tube did not actually reach the stomach of the rats, but extended far enough into the esophagus to prevent regurgitation. The poison formulations were given at the rate of 0.005 ml per gram of body weight. Nicotine sulphate was suspended in water-lead arsenate and calcium arsenate (concentrations unknown) at dosage rates as high as 0.00096 ml/g. The LD_{50} values were determined by the method of Litchfield and Wilcoxon (1949).The oral LD_{50} values for lead arsenate and calcium arsenate were determined to be 1050 mg/kg bw and 298 mg/kg bw, respectively.

Table 19 Results:

Acute oral toxicity, female rats

Compound	Survival time		LD 50 mg/kg bw
Nicotine sulphate	Min. (hr)	Max. (days)	
	1	4	83 (75-91)

Acceptability:
Limited description but acceptable given the period in which it was performed.
Conclusion:
Oral LD_{50} rat 83 mg/kg bw

4.2.1.2 Acute toxicity: inhalation

Table 20 Summary of acute inhalation studies

Method	Dilution	LC_{50} (mg/L)	Animal	Remarks	Acceptability	Reference
Long term exposure (4 months)	Liquid nicotine sulfate aerosol	> 0.33 mg/m^3	Not described	Type and # of animals not specified	Not acceptable	Lazutka et al., 1969
Up and down method	Nicotine (freebase), in water or NaCl solution	2.3 (20 minutes)	Rat	Sprague-Dawley, 7 males	Acceptable	Shao et al., 2012
OECD 403, 1981	Tobacco extract with 4.1% nicotine	> 2	Rat	Sprague-Dawley, 6 male, 6 female	Acceptable	Werley et al., 2014

Reference: Lazutka et al., 1969 (4)
Study design:
Only long-term (4-months) exposure to liquid nicotine sulfate aerosol was investigated, in concentrations of 0.33 and 0.2 mg/m^3. These are the maximum and minimum concentrations determined under industrial conditions in the respiration zone of personnel working with nicotine sulphate. Type and number of animals are not specified. No information is available on the duration of the exposure per day and the particle size.

Results:
The animals did not exhibit any visible phenomena after long-term (4-month) exposure at either concentration. Exposure to 0.33 mg/m^3 inhibited the inculcation of the conditioned reflex to bell with alimentary reinforcement, throughout the entire four-month period of poisoning, whereas there was no difference with controls in the lower concentration.
Acceptability:
Unacceptable due to absence of information on many essential parameters.
Conclusion:
Due to the limitations of the reporting no conclusion can be drawn.

Reference: Shao *et al.*, 2012 (12)

Nicotine in water can be in three forms: freebase (Nic), monoprotonated (NicH+), and diprotonated (NicH2 2+). Nic and NicH+ are predominant, with pKa = 8.06 at 20 °C (Pankow, Tavakoli, Luo, & Isabelle, 2003). Therefore, ~50% of nicotine is as Nic at pH 8.0. For inhalation route, the pH of the particles of tobacco smoke or testing aerosol affects nicotine absorption in the lung and its bioavailability (Burch et al., 1993; Pankow et al., 2003).

Study design:

Male Sprague-Dawley rats of 8–11-week-old (body weight 250–400 g) were used in this study, and nicotine used was (s)-(-)-nicotine freebase (liquid, 99%) ordered from Alfa Aesar Co. The rats were housed in the vivarium under a 12-hr light/dark cycle and had *ad libitum* access to food and water. Rats were exposed to nicotine aerosol by inserting rat holders into a nose-only chamber. The MMAD was between 1.69 and 3.55 um with a GSD of 1.8 to 2.48 depending on the nicotine concentration. Nicotine (freebase) was dissolved in water or NaCl solution for an osmolality ~300 mOsm/kg. pH was adjusted with HCl to pH 8.0 except when indicated otherwise.

Nicotine LC50 in rats was examined using the up and down procedure (UDP) recommended by EPA Health Effects Test Guidelines (EPA, 2002). With this method, 6–9 animals could be used to obtain LC50 and its confidence interval (*CI*). Rats were exposed to nicotine aerosol for a fixed time (20 min) and with a fixed air pressure (40 psi) to the nebulizer. To determine the inhalation LC50 of nicotine for rats using the UDP, the nicotine concentrations in the nebulizer solution container were varied. An ordered concentration progression in a range of 5%–56% nicotine was defined. Since the nicotine dose–response curve is quite steep, a concentration progression factor of antilog 0.25 = 1.78 was chosen. pH was 8.0 in the first experiment. Starting with a nicotine concentration of 10% in the nebulizer container, the first rat survived. A concentration of 18% (increase of one progression factor) was used for the next rat. According to the UDP, if the animal survives, the concentration for the next animal is increased by one step. The post exposure observation period was limited to 24 hours.

Results:

Table 21

pH	Nicotine concentration in nebulizer (%)	95% CI	LC_{50} in air (20 min) (mg/L)[a]	95% CI of LC_{50} (mg/L)[a]	n
6.8	>56[b]		>4.1		6
7.4	32	20.4-69.2	2.3	1.46-4.96	7
8.0	32	12.3-56.7	2.3	1.24-4.07	7

Note. *CI* represents confidence interval. Air pressure for generating nicotine aerosol was 40 psi in LC_{50} experiments.
aLC_{50} in air and its *CI* are calculated as nicotine concentration in solution × aerosol mass concentration in air.
bThe value is at least 56%. Only one rat died at 56% nicotine solution during the up and down procedure. No other rats died at 32%, 56%, or 68% in multiple trials.

LC50 values were not significantly different between experimental groups of nicotine solutions at pH 7.4 and at pH 8 (Table 21). Note that the *CI* values of LC50 at pH 8 were slightly lower than those at pH 7.4. However, the LC50 of nicotine solution at pH 6.8 was >4 mg/L (>56% nicotine concentration in the nebulizer). Higher nicotine concentrations could not be used, since pure nicotine freebase is liquid and very alkaline (pH ~10). The amount of HCl required to adjust pH to 6.8 significantly diluted the solution; therefore, 68% was the maximum concentration we could achieve. Although the exact value of LC50 cannot be determined, the (see legend of Table 21) experiment with pH 6.8 suggests that the LC50 at pH 6.8 is much higher than those at pH 7.4 and pH 8. These results suggest that the method of delivering nicotine through aerosol inhalation is very efficient. Exposure to 2.3 mg/L nicotine in air for 20 min causes death in 50% of rats. In addition,

we showed that pH affects nicotine actions. Acidification, but not basification, of the nicotine solution in the nebulizer minimizes the effects of nicotine, probably due to a reduction in nicotine absorption and/or bioavailability in the lungs.

Acceptability:

Acceptable with limitations (20 minutes exposure only)

Conclusion:

The acute inhalation toxicity of nicotine: LC_{50} (20 minutes) = 2.3 mg/L.

Reference: Werley *et al.*, 2014 (13)

Acute inhalation exposure effects to increasing concentrations of propylene glycol and glycerol aerosols containing tobacco extract and nicotine in rats was studied. Tobacco extract formulation was composed of the USP grade ingredients in the following proportions: 37.3% glycerol, 28.6% propylene glycol (PG), 19.2% ethanol, 4.1% nicotine, 8.8% water and 2% tobacco essential oils by weight, derived using a patented extraction process. The nicotine formulation was 38.4% glycerol, 28.8% PG, 19.2% ethanol, 4% nicotine and 9.6% water by weight. A nose-only exposure chamber was used. A single capillary tube CAG (capillary aerosol generator) was used to attain the targeted exposure concentrations up to approximately 2 mg/L.

Study design:

The acute inhalation study was conducted in accordance with OECD Guideline for testing of Chemicals (OECD 403, 1981) entitled Acute Inhalation Toxicity. Twelve male and 12 female Sprague Dawley_ rats (Crl:CD(SD)IGS BR) were obtained from Charles River Laboratories, Inc. (Wilmington, MA). They were 7–8 weeks of age and weighed 168–237 g and 135–193 g, males and females, respectively. The rats were acclimated for approximately 2–3 weeks, double-housed in stainless steel hanging cages to determine suitability for use before assignment to the study. Two groups of six rats of each sex were used in the study; each group (Group 1 and Group 2) was exposed to different concentrations of tobacco extract formulation test material for four hours to estimate the LC50 (the inhaled concentration of test material which produces 50% mortality in the test animals). Group 1 was exposed to a target concentration of 2 mg/L and group 2 was exposed to a target concentration of 1 mg/L. Animals were observed for signs of toxicity during exposure and then daily for 14 d post-exposure. Body weights were determined immediately before exposure, and weekly thereafter. At necropsy on Day 14, the rats were euthanized using an overdose of sodium pentobarbital, and all tissues and organs were examined for signs of gross pathology.

Results:

The mean exposure concentrations in the LC50 determinations for Group 1 and Group 2 were 2.13 and 1.00 mg/L, respectively, and corresponding nicotine concentrations were 0.114 and 0.060 mg/L, respectively. Particle size distribution (MMAD and GSD) from the aerosol in Group 1 and Group 2 were 0.40 (2.61) mm and 0.81 (2.72) mm, respectively. One female in Group 1 died on Day 1. The remaining females in this group had hypoactivity, wet and discolored inguinal fur, weight loss, redness around eyes and nose, convulsions, lethargy, hunched posture, severe tremors, reduced body temperature and salivation over Days 1–4. Males in Group 1 had wet inguinal fur, redness around the eyes, slight tremors, reduced body temperature, and salivation, which resolved by Day 2. Males and females in Group 2 had wet inguinal fur, redness around the eyes and nose, and salivation which resolved by Day 2. Necropsy showed no abnormal gross observations except for darkened spleen and mottled lungs in the female from Group 1 that died. All animals, except one female, survived and gained weight during the 14-day recovery period, at which time all animals appeared healthy and active. The LC50 for the inhaled tobacco extract was considered to be greater than 2 mg/L and 1 mg/L was determined as the maximum exposure concentration for repeated inhalation exposure.

Acceptability:

Acceptable with limitations (mixture tested, testing not up to the limit dose)

Conclusions:
The rat LC_{50} for inhaled tobacco extract (containing 4.1% nicotine) is considered to be > 2mg/L corresponding to > 0.114 mg/L for nicotine.

4.2.1.3 Acute toxicity: dermal

Table 22: Summary table of relevant acute dermal toxicity studies

Method	Dilution	LD_{50} (mg/kg bw)	Animal	Remarks	Acceptability	Reference
Single dose application	Suspension in water-lead arsenate and calcium arsenate	285	Rat	Sherman, 70 females, nicotine sulfate	Acceptable	Gaines, 1960
Single dose application	Not described	140	Rat	Strain, sex and number not specified	Not acceptable	Ben-Dyke *et al.*, 1970
Not specified		140	Rat	Strain, sex and number not specified	Not acceptable	Trochimo wicz *et al.*, 1994*
OECD 402		>360 (no deaths were seen	Rat	Sprague-Dawley, 5 male, 5 female. A mixture of 18% nicotine and 82% of an ion-exchange resin applied at 2 g/kg to the covered skin, followed by rinsing with water	Not acceptable	Guerriero et al., 2001*
Not described, repeated exposure	0.15-0.3% aqueous solutions	-	Rabbit & Rat	Not described, 6 of each, nicotine sulfate	Not acceptable	Lazutka et al., 1969
Single dose application	Not described	50	Rabbit	Strain, sex and number not specified	Not acceptable	FDA, 1952
Not described		50	Rabbit	Strain, sex and number not specified	Not acceptable	Trochimo wicz *et al.*, 1994*
Not described		140	Rabbit	Strain, sex and number not specified	Not acceptable	UK PSD, 2008*
Single dose application	40% aqueous solution	66-100	Cat	21 cat received nicotine base, and 21 cats received nicotine sulfate.	Acceptable	Travell, 1960

*These studies could not be retrieved. The above description was derived from the Bibra proposal, 2014. They will not be further described below.

Reference: Gaines, 1960 (11)
Study design:
Seventy female rats, at least 90 days old, were used. They were not fasted prior to dosage. The survivors were held for daily observation until they appeared to have recovered completely or for a

minimum of 14 days. The poisoned rats were observed at least once each hour during the first day after dosage, and twice a day thereafter, for symptoms of poisoning and time of death. Nicotine was dissolved in water-lead arsenate and calcium arsenate at dosage rates as high as 0.00096 ml/g. It is unclear how the presence of arsenate affected the study but it could only reduce the LD_{50} value.

Table 23 Results:

Acute dermal toxicity, female rats			
Compound	**Survival time**		**LD_{50} mg/kg bw**
Nicotine sulphate	**Min. (hr)**	**Max. (days)**	
	7	5	285 (228-356)

Acceptability:
Limited description but acceptable given the period in which it was performed.
Conclusion:
Dermal LD_{50} rat 285 mg/kg bw for nicotine sulfate.

Reference: Lazutka et al., 1969 (4)
Study design:
The general effect of absorption of nicotine sulphate was studied by application of 1/5 LD_{50} to the skin of 6 rabbits and 6 rats. The experiment lasted 2 months. Further details are not described.
Results:
The experimental animals' behaviour did not differ from that of the controls after the application of nicotine sulphate. There were no local reactions or clinical manifestations of poisoning, with the exception of a lag in weight-growth. The reflex to bell was completely inhibited and was not recovered by the experimental rats throughout the poisoning period. Cutaneous application of nicotine sulphate increased the amount of potassium ions in blood serum by 41% and diminished that in the erythrocytes by 30%; ATP decreased by 80%.
Acceptability:
Unacceptable as no dose levels in mg/kg bw are stated.

Reference: Ben-Dyke et al., 1970 (5)
This paper lists acute toxicity data for a number of pesticides, including nicotine. This data has been prepared from experimental results of the Toxicology Laboratory, Chesterford Park Research Station, or from published literature and manufacturer's bulletins. However, there are no actual references, the study design is not described, nor the number of animals used; only the dermal LD_{50} of 140 mg/kg bw is mentioned.
Acceptability:
Not acceptable

Reference: FDA, 1952 (14)
No access to the original study.
LD_{50} rabbit, dermal: 50 mg/kg bw
Acceptability:
Not acceptable

Reference: Trochimowicz *et al.*, 1994 (15)

No access to the original study (but mentioned in the bibra report and the report of the Health Council of the Netherlands, 2004 (17)).
LD_{50} rat, dermal: 140 mg/kg bw.
Acceptability:
Not acceptable

Reference: Travell, 1960 (16)
Study design:
21 cats received a single dose (200 mg) dermal application of nicotine base, and 21 cats received nicotine sulfate; the concentration of nicotine in each instance was 40% with respect to the base. Solutions were prepared by rapidly weighing the fluid nicotine oil and diluting it to volume with either distilled water or a solution of sulphuric acid to provide a slight excess of acid above the theoretical neutralization equivalent. Application of 0.5 cc. was done after fur was clipped from about a 5x6 cm. area of skin over the groin. Weights of the cats were about 2 to 3 kg, and the percutaneous dose of nicotine was thus about 66 – 100 mg/kg.
Results:
When nicotine base was used, 81% of the animals died between 21 to 195 minutes. When nicotine sulfate was used, none of the animals died. The dermal LD50 of nicotine is probably below 80 mg/kg bw in cats.

Table 24 Results

Acute dermal toxicity, female & male cats				
Compound	**# of cats**	**Incidence of**		
		Nausea (%)	**Vomiting (%)**	**Death (%)**
Nicotine base	21	100	100	81
Nicotine sulfate	21	52	19	0

Acceptability:
Limited description but acceptable given the period in which it was performed.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

Nicotine poisoning produces nausea, vomiting, abdominal pain, diarrhea, headaches, sweating, and pallor. More severe poisoning results in dizziness, weakness, and confusion, progressing to convulsions, hypotension, and coma. Death is usually due to paralysis of respiratory muscles and/or central respiratory failure (Health council of the Netherlands (17), Karaconji, (18)).

Dermal exposure can also lead to poisoning. Such exposure has been reported after spilling or applying nicotine-containing insecticides on the skin or clothes and as a consequence of occupational contact with tobacco leaves (Health council of the Netherlands (17), Benowitz, 1987 (19)). Acute intoxication of children has been reported after ingestion of tobacco materials. Ingestions of tobacco are rather common, but deaths as a result are extremely rare, due to early vomiting and first pass metabolism of the nicotine that is absorbed (18 and ref therein).

Reviews of nicotine contain estimates of the lethal dose in human mostly in the range of 30-60 mg/person indicating a dose in the range of 1 mg/kg bw. However, most reviews refer to secondary

literature which does not contain actual case descriptions. A review of the available human data and a search to the origin of the value of 60 mg/person as performed by Mayer (20) shows that this value cannot be scientifically justified. Mayer estimates a lower limit value for fatal outcomes of 6.5 – 13 mg/kg bw. The often stated value of 1 mg/kg bw in humans is not reliable and cannot be used without additional justification.

In 1991, a report was published of a fatal nicotine ingestion. A 17-year-old male smoker had ingested an unknown amount of liquid nicotine base. The container was later assayed to contain 870 mg/ml of nicotine. Serum nicotine levels were shown to be 13,600 ng/ml, and he died 64 hours post ingestion. As such, this person was estimated to have ingested in excess of 5000 mg (or 71 mg/kg bw). (21). A more recent report describes a non-fatal nicotine poisoning of a 27-year-old man after ingestion of potentially 420 mg (i.e. 6 mg/kg bw) (22). Finally, another paper reports that nicotine exposure through e-cigarettes is increasing. They 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 (23).

As summarised by EFSA (2):

A report (Woolf et al., 1997) on a postmarketing surveillance study over a 24-month period, involving 34 United States poison centres, was published in 1997. Patients were represented by 36 children aged 0 to 15 years (mean: 3 years) exposed to a Transdermal Nicotine Patch (TNP). Eighteen exposures were dermal; 18 additional children had bitten, chewed, or swallowed part of a patch. Exposures were unintentional and transient (<20 minutes duration). Twenty-two children (64%) suffered no toxic effects from the TNP exposure: 13 of the 18 children (72%) with oral exposures and 9 of the 18 (50%) with dermal exposures remained asymptomatic. The 5 children who became symptomatic after an oral exposure to a TNP had only transient and local signs of toxicity; children with dermal exposures more often had systemic complaints. Seven of the nine children who were symptomatic after a dermal TNP exposure had nausea and/or vomiting. Five of the nine children were triaged to the emergency department and two were admitted. Fourteen children (39%) developed symptoms, including gastrointestinal distress (nausea, vomiting, diarrhea, abdominal pain), weakness, dizziness, or localized rashes. Occurrence of symptoms after a dermal exposure of children to a TNP was associated with an estimated nicotine dose of 100 µg (10 µg/kg b.w.). All children recovered fully (Woolf et al, 1997).

Lindgren et al. (1999) investigated the dose-response relationship for electroencephalographic parameters (EEG) and heart rate frequency over a wide range of intravenously infused nicotine doses in human volunteers. Fourteen regular smokers who had abstained from nicotine for at least 12 h were given intravenous infusions of 0, 3.5, 7, 14 and 28 µg/kg b.w. nicotine over 10 min in a single-blind randomised cross-over design and they were monitored for 120 minutes. Findings showed linear dose-related changes in EEG measures indicative of arousal, i.e., decrease in EEG delta and theta power, and increase in the alpha2 power, at all doses tested, markedly at 14 and 28 µg/kg b.w. Nicotine infusion caused heart rate acceleration (ranging from 8% to 20% of the baseline), with a highly significant linear trend contrast. The nicotine X time interaction was significant, with pronounced heart rate acceleration after infusion of the 14 and 28 µg/kg nicotine dose. Heart rate frequency returned back to a level comparable to the baseline within 2 hours from the end of the intravenous infusion. It is noted that changes in the heart rate frequency in the order of up to 50% of the baseline heart frequency are considered in a light physical exercise.
In a semi-blinded, within-subject, crossover study with inhaled nicotine, Benowitz et al. (2006a) examined plasma nicotine and cardiovascular responses in 12 healthy smokers receiving cigarettes with 5 graded nicotine contents (between 0.6 and 10.1 mg/cigarette). Non-abstinent smokers were asked to smoke on five subsequent occasions a research cigarette, each with a different nicotine

content. Systemic nicotine exposure (0.26-1.47 mg per cigarette) varied linearly with the nicotine content of the cigarette (average intake of 13- 43% of the cigarette's nicotine content). Cigarette smoking increased heart rate and decreased skin temperature, but the nicotine dose-response curve showed a flattening at higher doses, with a maximal response being observed from 8 mg of nicotine per cigarette. An increase in the heart rate was observed after a systemic dose of approximately 0.004 mg/kg b.w. equal to 0.26 mg in a 60 kg b.w. person (BfR, 2009). The effects on the blood pressure were not significant. The flat nicotine dose–cardiovascular response curve may be consistent with the tolerance of smokers to the cardiovascular effects of nicotine. In non-smokers stronger effects would possibly be observed (Benowitz et al., 2006a).

4.2.3 Summary and discussion of acute toxicity

Overall, no reliable human data are available.

For acute oral toxicity, the quality of the available studies and their reports vary significantly. Of all the available oral studies only the rat studies by Van den Heuvel et al (1990) and Yam et al (1991) would probably fulfil the OECD TG requirements although the reporting is incomplete. All the acute oral rat studies show a comparable range of LD50 values between 50 and 70 mg/kg bw/day with one exception. However, the available acute oral data for mouse and dog show a much lower LD50 value for nicotine. This is specifically shown in the study by Lazutka (1969) which used both rats and mice within the same experimental conditions. Although the quality of the reporting of these studies is limited some studies are considered acceptable seen the period (pre OECD and GLP) in which they were performed and seen the absence of more recent data from the same species. For these species the LD50 values are dog: 9.2 mg/kg and mouse: 3.34 and 24 mg/kg, respectively. The species differences may be due to toxicokinetic and or toxicodynamic differences. Limited information is available on toxicodynamic differences but some information is available for species differences in toxicokinetics. 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. 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 resulted in lethalities after at least 50 minutes (Lazutka, 1969) whereas the studies by Franke and Thomas in dogs (1932) using drops into the mouth resulted in lethalities within a few minutes. This is probably caused by direct uptake via the gums. This route is not possible when animals are exposed via gavage treatment. However, this route is considered relevant for human exposure to nicotine. Also, 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). Therefore, the oral LD50 values in the rat using gavage exposure seem to be less relevant to humans and may underestimate the human toxicity. The acceptable studies in other species than the rat are limited to mouse and dogs. As it is unknown which of these two species is more relevant to humans, it is suggested to take the lowest value in the most sensitive species in line with the CLP guidance. Therefore, it is proposed to use the acute oral LD50 in the mouse of 3.3 mg/kg bw as determined by Lazutka et al (1969) as the key study. Although this is also a gavage study, the LD50 after uptake via the gums is expected to be even lower. The value of this study is increased by the fact that in the same study rats were tested and showed an LD50 value in line with most other LD50 values in the rat. It is also proposed to assign this value of 3.3 mg/kg bw as the best ATE for calculation of the ATE of mixtures containing nicotine.

For acute dermal toxicity, also most studies are old and the reports are limited. The only acceptable study was in rats and performed using nicotine sulfate and in cats using both nicotine and nicotine sulfate. It shows a higher LD50 than the study in rabbits. For dermal toxicity, the study with nicotine sulphate is considered less relevant because dermal transport over the skin strongly

depends on the presence of nicotine as a neutral molecule as in nicotine or as an ion as in nicotine sulphate. The transport of neutral molecules over the skin is much better than for ions. This is confirmed in the cat study which at equal dose of approximately 80 mg/kg bw showed no mortalities for the sulfate and 81% mortalities for the base. The currently applied classification as Acute dermal 1; H310 was based on the acute dermal LD50 of 50 mg/kg bw in rabbits (FDA, 1952). However, this study is not acceptable according to the current requirements. However, the current classification is supported by the results of the cat study with nicotine which showed a dermal LD50 below approximately 80 mg/kg bw.

There are two acceptable acute inhalation studies available in which nicotine was tested as an aerosol. However, both have limitations. In the first study using the up and down method, the exposure duration was limited to 20 minutes and the post exposure observation period to 24 hours. The observed LC50 (20 minutes) was approximately 2.3 mg/L. In the second study a specified mixture was tested for four hours up to a limited concentration corresponding to 0.114 mg nicotine/L. A single lethality was observed and severe transient clinical effects. Overall this indicates that the tested concentration was close to the LC50 of nicotine.

4.2.4 Comparison with criteria

oral

Lazutka et al (1969) is selected as the key study, this study determined an acute oral LD50 in the mouse of 3.3 mg/kg bw. An acute oral LD50 of 3.34 mg/kg bw fulfils the requirement for classification in category 1 (LD50 below 5 mg/kg bw). An LD50 value of 3.3 mg/kg bw is suggested as ATE.

dermal

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. However, there are no acceptable acute dermal studies in rabbits or rats using nicotine and the only available acceptable study in cats indicates also that the dermal LD50 is in the range of 50 mg/kg bw as 81% mortality was observed at a dose of approximately 80 mg/kg bw. An dermal LD50 value of 50 mg/kg bw or lower warrants classification in category 1. Therefore, it is considered justified to keep the current classification as Acute Tox. 1 H310. The proposed ATE value is 50 mg/kg bw.

inhalation

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.

4.2.5 Conclusions on classification and labelling

According to Regulation (EC) No. 1272/2008 on Classification, Labelling and Packaging, nicotine should be classified as Acute Tox. 1, H300, thereby replacing the current classification of Acute Tox. 3, H301. It is proposed to assign an ATE of 3.3 mg/kg bw for acute oral toxicity.

The available data do not warrant a change in the current classification for acute dermal toxicity (Acute Tox. 1, H310). It is proposed to assign an ATE of 50 mg/kg bw for acute dermal toxicity.

According to Regulation (EC) No. 1272/2008 on Classification, Labelling and Packaging, nicotine should be classified as Acute Tox. 2, H330. It is proposed to assign an ATE of 0.25 mg/L for acute inhalation toxicity.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not assessed in this dossier.

4.4 Irritation

Not assessed in this dossier.

4.5 Corrosivity

Not assessed in this dossier.

4.6 Sensitisation

Not assessed in this dossier.

4.7 Repeated dose toxicity

Not assessed in this dossier.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not assessed in this dossier.

4.9 Germ cell mutagenicity (Mutagenicity)

Not assessed in this dossier.

4.10 Carcinogenicity

Not assessed in this dossier.

4.11 Toxicity for reproduction

Not assessed in this dossier.

4.12 Other effects

Not assessed in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not assessed in this dossier.

6 OTHER INFORMATION

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