COLLECTIVE EXPERT APPRAISAL: SUMMARY AND CONCLUSIONS

Regarding the "expert appraisal for recommending occupational exposure limits for chemical agents"

Assessment of health effects and methods for the measurement of exposure levels in workplace atmospheres for

Trimethylamine (CAS No. 75-50-3)

This document summarises the work of the Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee) and the Working groups on health effects and on metrology.

Presentation of the issue

On 12 June 2007, AFSSET, which became ANSES in July 2010, was requested by the Directorate General for Labour to conduct the expert appraisal work required for establishing recommendations on measures to be taken in the event of specific exposure profiles such as those with peaks.

A first report[1] published in June 2009 issued recommendations on measures to be taken in the event of an 8h-OELV with no short-term exposure limit (STELV).

In 2010, ANSES published a second report that recommended studying the 36 substances in France with a short-term exposure limit but no time-weighted average (TWA) to recommend health values taken from the most recent scientific literature (ANSES, 2010).

In this context, an assessment was undertaken for trimethylamine, which has a short-term exposure limit in France set at 25 $mg.m^{-3}$ in a Circular[2] of 1982 but no TWA.

Scientific background

The French system for establishing OELVs has three clearly distinct phases:

- Independent scientific expertise (the only phase entrusted to ANSES);
- Proposal by the Ministry of Labour of a draft regulation for the establishment of limit values, which may be binding or indicative;
- Stakeholder consultation during the presentation of the draft regulation to the French Steering Committee on Working Conditions (COCT). The aim of this phase is to discuss the effectiveness of the limit values and if necessary to determine a possible implementation timetable, depending on any technical and economic feasibility problems.

[1] http://www.anses.fr/ET/DocumentsET/VLEP_Picsdexpo_Avis_0906.pdf
[2] Circular of 19 July 1982 on the acceptable values for concentrations of certain hazardous substances in workplace atmospheres.

The organisation of the scientific expertise phase required for the establishment of Occupational Exposure Limits (OELVs) was entrusted to AFSSET in the framework of the 2005-2009 Occupational Health Plan (PST) and then to ANSES after AFSSET and AFSSA merged in 2010.

The OELs, as proposed by the Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee), are concentration levels of pollutants in workplace atmospheres that should not be exceeded over a determined reference period and below which the risk of impaired health is negligible. Although reversible physiological changes are sometimes tolerated, no organic or functional damage of an irreversible or prolonged nature is accepted at this level of exposure for the large majority of workers. These concentration levels are determined by considering that the exposed population (the workers) is one that excludes both children and the elderly.

These concentration levels are determined by the OEL Committee experts based on information available from epidemiological, clinical, animal toxicology studies, etc. Identifying concentrations that are safe for human health generally requires adjustment factors to be applied to the values identified directly by the studies. These factors take into account a number of uncertainties inherent to the extrapolation process conducted as part of an assessment of the health effects of chemicals on humans.

The Committee recommends the use of three types of values:

- 8-hour occupational exposure limit (8h-OEL): this corresponds to the limit of the time-weighted average (TWA) of the concentration of a chemical in the worker's breathing zone over the course of an 8-hour work shift. In the current state of scientific knowledge (toxicology, medicine, epidemiology, etc.), the 8h-OEL is designed to protect workers exposed regularly and for the duration of their working life from the medium- and long-term health effects of the chemical in question;
- Short-term exposure limit (STEL): this corresponds to the limit of the time-weighted average (TWA) of the concentration of a chemical in the worker's breathing zone over a 15-minute reference period during the peak of exposure, irrespective of its duration. It aims to protect workers from adverse health effects (immediate or short-term toxic effects such as irritation phenomena) due to peaks of exposure;
- Ceiling value: this is the limit of the concentration of a chemical in the worker's breathing zone that should not be exceeded at any time during the working period. This value is recommended for substances known to be highly irritating or corrosive or likely to cause serious potentially irreversible effects after a very short period of exposure.

These three types of values are expressed:

- either in $mg.m^{-3}$, i.e. in milligrams of chemical per cubic metre of air and in ppm (parts per million), i.e. in cubic centimetres of chemical per cubic metre of air, for gases and vapours;
- or in $mg.m^{-3}$, only for liquid and solid aerosols;
- or in $f.cm^{-3}$, i.e. in fibres per cubic centimetre for fibrous materials.

The 8h-OELV may be exceeded for short periods during the working day provided that:

- the weighted average of values over the entire working day is not exceeded;
- the value of the short term limit value (STEL), when it exists, is not exceeded.

In addition to the OELs, the OEL Committee assesses the need to assign a "skin" notation, when significant penetration through the skin is possible (ANSES, 2014a). This notation

indicates the need to consider the dermal route of exposure in the exposure assessment and, where necessary, to implement appropriate preventive measures (such as wearing protective gloves). Skin penetration of substances is not taken into account when determining the atmospheric limit levels, yet can potentially cause health effects even when the atmospheric levels are respected.

The OEL Committee assesses the need to assign an “ototoxic” notation indicating a risk of hearing impairment in the event of co-exposure to noise and the substance below the recommended OELs, to enable preventionists to implement appropriate measures (collective, individual and/or medical) (ANSES, 2014a).

The OEL Committee also assesses the applicable reference methods for the measurement of exposure levels in the workplace. The quality of these methods and their applicability to the measurement of exposure levels for comparison with an OEL are assessed, particularly with regards to their compliance with the performance requirements in the NF-EN 482 Standard and their level of validation.

Organisation of the expert appraisal

ANSES entrusted examination of this request to the Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee). The Agency also mandated:

- The working group on health effects to conduct the expert appraisal work on health effects;
- The working group on metrology to assess measurement methods in workplace atmospheres.

The methodological and scientific aspects of the work of this group were regularly submitted to the Expert Committee.

The report produced by the working group takes account of observations and additional information provided by the Committee members.

This expert appraisal was therefore conducted by a group of experts with complementary skills. It was carried out in accordance with the French Standard NF X 50-110 “Quality in Expertise Activities”.

Preventing risks of conflicts of interest

ANSES analyses interests declared by the experts before they are appointed and throughout their work in order to prevent potential conflicts of interest in relation to the points addressed in expert appraisals.

The experts’ declarations of interests are made public on ANSES's website (www.anses.fr).

Description of the method

For the assessment of health effects:

A summary report on the health effects of trimethylamine was prepared by the working group on health effects and submitted to the OEL Committee, which commented on it and added to it.

The summary report was based on bibliographic information taking into account the scientific literature that had been published on this substance up to 2013. The literature search was carried out using the summary report written by ACGIH[3] (2001), the interim document regarding AEGLs of the US-EPA (2008) and articles found in the Medline, Toxline and HSDB (ToxNet) databases.

For assessment of methods for measuring exposure levels in workplace:

A summary report was prepared by the working group on metrology and submitted to the OEL Committee, which added its own comments.

The summary report presents the various protocols for measuring trimethylamine in workplace atmospheres grouped together based on the methods they use. These methods were then assessed and classified based on the performance requirements set out particularly in the French Standard NF EN 482: "Workplace atmospheres - General requirements for the performance of procedures for the measurement of chemical agents" and the decision-making criteria listed in the methodology report (ANSES, 2014a).

A list of the main sources consulted is detailed in the methodology report (ANSES, 2014a).

These methods were classified as follows:

- Category 1A: the method has been recognized and validated (all of the performance criteria in the NF-EN 482 Standard are met);
- Category 1B: the method has been partially validated (the essential performance criteria in the NF-EN 482 Standard are met);
- Category 2: the method is indicative (essential criteria for validation are not clear enough);
- Category 3: the method is not recommended (essential criteria for validation are lacking or inappropriate).

A detailed comparative study of the methods in Categories 1A, 1B and 2 was conducted with respect to their various validation data and technical feasibility, in order to recommend the most suitable method(s) for measuring concentrations for comparison with OELs.

The collective expert appraisal work and its conclusions and recommendations were adopted on 17 May 2014 by the OEL Committee.

The collective expert appraisal work and the summary report were submitted to public consultation from 21/05/2015 to 21/07/2015. The people or organizations who contributed to the public consultation are listed in appendix of the report (only available in French). The comments received were reviewed by the OEL Committee (term of office 2014-2017) who adopted this version on 15 December 2015.

[3] American Conference of Governmental Industrial Hygienists

Results of the collective expert appraisal on the health effects

Kinetics

No data were found in the literature regarding the absorption, metabolisation or excretion of trimethylamine by inhalation in humans or animals. Few absorption data were found for humans (oral and dermal routes). Dermal flux was calculated at 3.40 (± 1.60), 58.3 (± 30.6) and 265.0 (± 155.0) $\mu g.cm^{-2}.h^{-1}$ in rats and at 0.98 (± 0.75), 9.21 (± 3.06) and 92.7 (± 31.9) $\mu g.cm^{-2}.h^{-1}$ in humans for contact with 0.1, 1.0 and 10 mg for 0.32 cm^2 of skin (Kenyon *et al.*, 2004).

In rats, trimethylamine is rapidly distributed in tissues, particularly the liver, after intravenous injection (Schweinsberg and Sander, 1972 cited in ACGIH, 2001).

The metabolisation of trimethylamine in humans was studied for oral exposure only.

In humans, the oxidation of trimethylamine to trimethylamine N-oxide is the main metabolic pathway (it is the only one when exposure is not very high, but no quantitative information was found) (US-EPA, 2008). This oxidation is the result of the activity of the flavin-containing monooxygenase 3 (FMO3) in the liver. N-demethylation is a negligible metabolic pathway and becomes significant only with high exposure (Lang *et al.*, 1998 cited in US-EPA, 2008).

In rats, after intra-gastric administration of radiolabelled trimethylamine, 75% of the radioactivity was measured in the urine, including 45% as trimethylamine N-oxide, 3% as dimethylamine (by demethylation of trimethylamine) and 52% in unchanged form (Al-Waiz *et al.*, 1991 cited in US-EPA, 2008). The rest of the radioactivity was measured in the faeces, including 90% as trimethylamine and 10% as trimethylamine N-oxide.

A study in volunteers exposed orally (single dose) showed that within 24 hours, 95% of the amount of ingested trimethylamine was excreted in the urine as trimethylamine N-oxide and 5% was excreted in unchanged form (Al-Waiz *et al.*, 1987 cited in US-EPA, 2008).

General toxicity

Toxicity in humans

Acute and subacute toxicity

In humans, there have been reports of exposure (intentional or accidental) to trimethylamine solutions of varying concentrations causing damage to the skin barrier, dermal burns, hyperaemia, petechiae and reversible damage to the corneal epithelium (Rotenberg and Mashbits, 1967 cited in ACGIH, 2001; Grant, 1986 cited in US-EPA, 2008).

Chronic toxicity

The US-EPA document reports cases of workers with moderate irritation of the upper respiratory tract for exposure greater than or equal to 20 ppm, with no information about exposure times (AIHA, 2005 cited in US-EPA, 2008). The report also specifies that workers exposed to concentrations of trimethylamine ranging from 0.1 to 8 ppm (peaks), without exceeding the 8h-OEL of 5 ppm, showed no adverse effects.

Toxicity in animals

As for studies in humans, the toxicity data have primarily been taken from secondary sources (the publications were not found in the scientific literature).

Acute and subacute toxicity

It has been reported that trimethylamine is a dermal-mucosal irritant in animals.

In rats, effects occur from 75 ppm (no NOAEL[4]). A RD_{50}[5] was calculated in mice (61 ppm).

Exposure by inhalation to trimethylamine has effects on the airways (nasal discharge, respiratory problems) in animals exposed to high concentrations as well as neurological effects (no reaction to noise) (Kinney *et al.*, 1990). This same study showed that half of the rats (three in six) had died after four hours of exposure to 3500 ppm.

In this same study, the authors exposed, by inhalation (nose only), groups of ten eight-week-old male CD rats to 75, 250 or 750 ppm of trimethylamine, six hours per day, five days per week for 14 days and compared the results with the results obtained for a group of ten rats exposed to air. After sacrifice (five animals after the 14 days and five animals 14 days after the end of exposure), various clinical, biological and anatomopathological examinations were performed.

From the 1st exposure dose of 75 ppm and at the following doses of 250 and 750 ppm, irritation of the nasal cavities was observed. This irritation was characterised by hyperaemia, congestion with oedema, necrosis, atrophy and metaplasia of the nasal mucosa. In the group of rats exposed to 75 ppm and sacrificed after a 14-day period without exposure, these effects were no longer observed. However, in the groups of rats exposed to 250 or 750 ppm, these effects were still reported 14 days after the end of exposure.

The lungs of the rats exposed to 750 ppm had alveolar enlargement and focal interstitial pneumonia. At this same concentration, tracheal inflammation and necrosis were also observed. These effects were not observed in the group of rats sacrificed 14 days after the end of the exposure (to 750 ppm).

These effects were also not observed in the groups of rats exposed to 75 or 250 ppm (during or 14 days after the end of exposure).

According to the authors, the rats exposed to 250 and 750 ppm showed an increase in the number of erythrocytes, correlated with the exposure levels. Furthermore, at 750 ppm, trimethylamine caused increases in haemoglobin concentrations, haematocrit levels and platelet counts in these rats. However, the changes in these parameters did not seem pathological. These changes were not observed after the 14-day recovery period, regardless of the dose group. After the recovery period, the rats exposed to 750 ppm showed a significant decrease in lymphocyte, white blood cell and platelet counts.

Rotenberg and Mashbits (1967) reported, like Kinney *et al.* (1990)[6], neurological effects (aggressiveness, hyperactivity) at 10 and 30 ppm (approximately) in the first month of exposure (five hours/day and seven days/week). From two months of exposure, the behaviour of the exposed animals was no longer different from that of the animals in the control group.

Chronic toxicity

[4] No Observed Adverse Effect Level
[5] Concentration that induces a 50% decrease in respiratory rate
[6] study conducted by industry

Rotenberg and Mashbits (1967) reported changes in blood counts that did not occur immediately after the start of the exposure period. A decrease in lymphocytes and increase in neutrophils were noted in rats exposed to 31 ppm from the fourth month of exposure and until the end of the study. The anatomopathological examination performed at the end of the study showed that, in both groups, the exposed animals had bronchopneumonia and haemorrhage in the lung, liver, kidney and spleen tissues.

Based on this study, it is difficult to draw any conclusions as to the medium- or long-term systemic effects of trimethylamine. The systemic lesions observed at the end of the study may have been caused from the start of exposure (intense irritation) or from co-exposure to ethyl alcohol[7] throughout the study.

No data showing carcinogenic potential were identified in animals, despite dimethylnitrosamine being obtained *in vitro* from trimethylamine.

No mutagenic effects of trimethylamine were found in *Salmonella* Typhimurium with or without metabolic activation (ACGIH, 2001).

According to the report of the US-EPA[8] (2008), studies in female rats exposed before and during gestation (8 or 40 $mg.kg^{-1}.day^{-1}$) do not report any reproductive effects[9] (Takashima *et al.*, 2003 cited in US-EPA, 2008). The last exposure dose (200 $mg.kg^{-1}.day^{-1}$) had lethal toxicity for gestating rats.

Establishment of OELs

Trimethylamine is irritating to the skin, eyes and respiratory system in humans and animals. It is also corrosive to skin.

No conclusions can be drawn from the few data available in the literature as to the medium- or long-term systemic toxicity of trimethylamine in humans.

Thus, according to the methodological document on the establishment of limit values for irritants and corrosives, a 15min-STEL can be recommended to protect workers from the irritating effects of trimethylamine but it does not appear relevant to recommend an 8h-OEL (ANSES, 2014b).

15min-STEL

The study by Kinney *et al.* (1990) described above has some methodological advantages (use of a control group, exposure by inhalation). Therefore, despite some limitations (small study population and wide range of concentrations measured in the inhalation chambers), it was used to establish the 15min-STEL.

Irritation of the nasal mucosa, observed from 75 ppm, was selected as the critical effect. This irritation was characterised by hyperaemia, congestion with oedema, necrosis, atrophy and metaplasia of the nasal mucosa. Even though these effects were not observed in rats sacrificed 14 days after the end of exposure, the OEL Committee's experts consider that this type of effect is sufficiently characterised to consider this exposure level a LOAEL[10]. The authors indicate that these effects were reversible at 75 ppm (they were no longer observed in animals sacrificed 14

[7] During the study, the authors administered 1 ml of 40% ethyl alcohol to the rats by the intra-gastric route to show a decrease in the threshold for excitability in the rats exposed to trimethylamine.
[8] United States Environmental Protection Agency
[9] Oral exposure of Wistar rats from two weeks before breeding through day four of lactation.
[10] lowest observed adverse effect level

days after the end of exposure) but were still found at 250 and 750 ppm even after the 14-day recovery period. However, the results were not reported in the publication.

Thus, based on the LOAEL of 75 ppm, the OEL Committee proposes applying the following adjustment factors:

- a LOAEL-to-NOAEL adjustment factor of 3;
- an adjustment factor of 3 to take into account the small database;
- an adjustment factor of 3 to take into account inter-individual variability.

No adjustment factors were introduced to take into account inter-species variability; according to the methodology of the OEL Committee, in the case of a non-systemic effect (irritation and corrosion), the mechanism of action of the chemical substance varies little, irrespective of the species in question (bioavailability, metabolism, excretion and detoxification mechanisms do not impact the occurrence of local effects).

Therefore: 75 ppm / 27 = 2.77 ppm or 6.83 $mg.m^{-3}$ (20°C conversion factor and 101 kPa).

This value of 6.83 $mg.m^{-3}$ is rounded to recommend a 15min-STEL of 7 $mg.m^{-3}$.

"Skin notation"

In the absence of a conclusion on systemic toxicity, the "skin" notation was not assigned for trimethylamine.

Results of the collective expert appraisal on measurement methods in workplace atmospheres

Three methods for measuring concentrations of trimethylamine in workplace atmospheres were identified and assessed. (see Table 1).

Table 1: Assessment of methods for measuring concentrations of trimethylamine in workplace atmospheres

No.	Principle	Protocols[11]	Category[12]	
			For regulatory technical control of the 15min-STEL	For monitoring short-term exposure

[11] BGIA: Berufsgenossenschaftliches Institut für Arbeitsschutz; INRS: National Research and Safety Institute; OSHA: Occupational Safety and Health Administration

[12] Validation and performance criteria for methods for monitoring STELs are defined in the NF EN 482 Standard from 0.5 to 2 times the STEL. Under the French regulations, for the technical control of the exposure limit, the measurement method must be able to measure one-tenth of the 15min-STEL (Ministerial Order of 15 December 2009 on technical controls of occupational exposure limits in workplace atmospheres and conditions for accrediting the organisations in charge of controls, published in the OJ of 17 December 2009). As such, when a method cannot measure one-tenth of the 15min-STEL, it cannot be classified in category 1A or 1B for regulatory control of the 15min-STEL. However, it may be classified in category 1A or 1B solely for assessing occupational exposure.

1	Sampling in a glass tube containing Chromosorb P, impregnated with sulphuric acid - Neutralisation of the acid with soda - Analysis by gas chromatography with thermoionic detection.	Amines by gas chromatography. INRS MétroPol Sheet 025 (2004)	**3**	**3**
2	Sampling in an XAD-7 tube impregnated with 10% phosphoric acid - Desorption with a methanol/water mixture. Addition of an NaOH/methanol mixture. Analysis by gas chromatography with flame ionisation detection.	Triethylamine/ trimethylamine OSHA PV2060 (1993)	**1 B**	
3	Sampling in an active charcoal tube impregnated with sulphuric acid - Water desorption - Analysis by ion chromatography	BGIA 7853: 2005	**2**	**1B**

The graph below presents the ranges for which the various methods were tested, as well as their limits of quantification in relation to the 15min-STEL recommended by the OEL Committee.

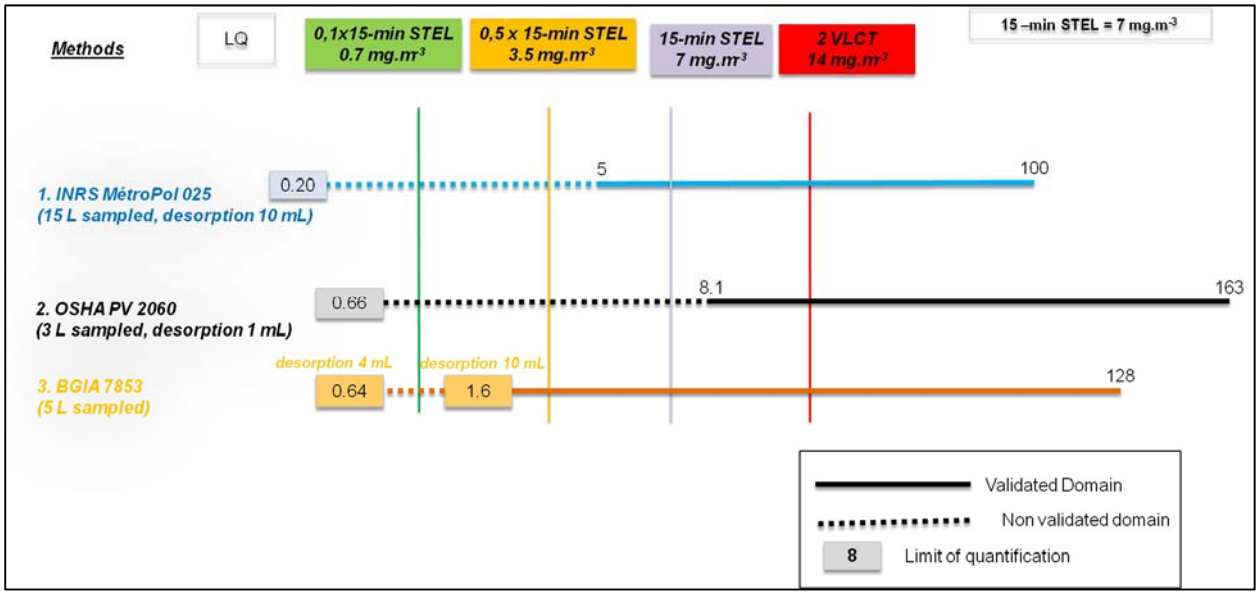

Figure 1: Ranges of validity and limits of quantification for the various compared methods from 0.1 to 2 times the 15min-STEL recommended by the OEL Committee for trimethylamine

Method 1 has some validation data covering the range of 0.1 to 2 times the 15min-STEL. However, environmental conditions and uncertainty data are not available. The method has therefore been classified in category 3 for regulatory technical control and the monitoring of short-term exposure.

Method 2 meets the main requirements of the NF EN 482 Standard and the limit of quantification can reach one-tenth of this 15min-STEL. The method has therefore been

classified in category 1B for regulatory technical control and the monitoring of short-term exposure.

Method 3 meets the main requirements of the NF EN 482 Standard. It has therefore been classified in category 1B for the monitoring of short-term exposure. This method cannot reach one-tenth of the 15min-STEL recommended by the OEL Committee. However, with a desorption volume of 4 mL instead of 10 mL, the limit of quantification can be adapted. It has therefore been classified in category 2 for technical control of the 15min-STEL.

Conclusions of the collective expert appraisal

Based on the data currently available, the OEL Committee recommends establishing a 15min-STEL of 7 $mg.m^{-3}$ and does not recommend an 8h-OEL considering the lack of expected medium- or long-term systemic effects.

The OEL Committee does not recommend a “skin” notation.

In light of the assessment of measurement methods, the OEL Committee recommends, for the regulatory technical control of the 15min-STEL and the monitoring of short-term exposure, method 2, described by the OSHA PV2060: 1993 protocol. This method classified in category 1B involves active sampling in an XAD-7 tube impregnated with 10% phosphoric acid, desorption with a methanol/water mixture followed by addition of an NaOH/methanol mixture, and then analysis by gas chromatography with flame ionisation detection.

For the monitoring of short-term exposure, the OEL Committee also recommends the method described by the BGIA 7853 protocol involving active sampling in an active charcoal tube treated with sulphuric acid, water desorption and then analysis by ion chromatography with conductivity detection. This method is partially validated (category 1B classification) for the monitoring of short-term exposure but indicative (category 2 classification) for the technical control of the 15min-STEL.

Further details: General information on the substance

1) Identification of the substance:

Name	**Trimethylamine**
CAS No	75-50-3
EINECS No	200-875-0
Molecular weight	59.1
Empirical formula	C_3H_9N
Physical form, appearance	Gas with a strong ammonia odour

2) Physico-chemical properties:

Flash point	- 6.5°C
Melting point	- 117°C
Conversion factor	1 $mg.m^{-3}$ = 0.406 ppm
Boiling point	3°C
Solubility in water (hydrolysis?)	complete
Relative density (air=1)	2
Relative density (water=1)	0.6
Explosive limits (in % vol air)	2 - 11.6

3) Professional uses:

Trimethylamine is used for the production of choline and quaternary ammonium salts, and as a catalyst in the petrochemical industry.

Trimethylamine is marketed in the form of compressed gas or as an aqueous solution.

Trimethylamine is a degradation product of plants and animals. It is the agent most responsible for the decomposition odour of marine organisms.

References

Anses. (2014a). Reference Document for the derivation and the measurement of exposure limit values for chemical agents in the workplace (OELs). (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, France). 115 p.

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Anses. (2010). Recommandation en vue de limiter l'importance et du nombre de pics d'exposition dans une journée (partie 2). (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, France), Fr. 36 p.

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US-EPA. (2008). Acute exposure guideline level (AEGLs) for trimethylamine - Interim. (United-State Environmental Protection Agency: Washington, USA). 45 p.

Metrology section

BGIA 7853 (2005) : Methylamine – IFA Arbeitsmappe Kennzahl 7853

Fiche MétroPol 025 INRS (2004) : Amines par chromatographie en phase gazeuse (http://www.inrs.fr//inrs-pub/inrs01.nsf/inrs01_metropol_view/C42DBF58BC58F795C1256D5C0041CEDD/$File/025.pdf , accédé le 17/05/2013)

NF EN 482 (2012) : Exposition sur les lieux de travail - Exigences générales concernant les performances des procédures de mesure des agents chimiques.

OSHA PV2060 (1993) : Triethylamine/trimethylamine(https://www.osha.gov/dts/sltc/methods/partial/pv2060/2060.html, accédé le 17/05/2013)