The Director General

Maisons-Alfort, 24 January 2018

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

on the development of acute, subchronic and chronic TRVs by the respiratory route for ammonia (CAS No. 7664-41-7)

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

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

Its opinions are published on its website.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 24 January 2018 shall prevail.

On 18 March 2016, ANSES received a formal request from the Directorate General for Health (DGS) to undertake the following expert appraisal: Selection or establishment of toxicity reference values (TRVs) for trichloroethylene, perchloroethylene (tetrachloroethylene), ammonia and four chloroanilines.

1. BACKGROUND AND PURPOSE OF THE REQUEST

As part of the risk assessments carried out when examining reports relating to classified installations for environmental protection (ICPE) or to the management of polluted sites and soils, the Regional Health Agencies (ARSs) or consultancies send questions to the DGS about the choice of TRVs for certain chemicals. This choice is made with regard to information note No. DGS/EA1/DGPR/2014/307 of 31 October 2014 on the methods for selecting chemical substances and choosing TRVs in order to conduct health risk assessments in the framework of impact and management studies for polluted sites and soils.
In this note, ANSES is designated as the expert agency for selecting and establishing TRVs. In the management of certain dossiers, the choice of TRV may prove crucial for assessing the risks and shifting a risk from acceptable to unacceptable. This has already happened several times in the context of applications for authorisation to operate an ICPE releasing ammonia.

A toxicity reference value, or TRV, is a toxicological index for qualifying or quantifying a risk to human health. It establishes the link between exposure to a toxic chemical and the risk of occurrence of an adverse health effect. TRVs are specific to a duration (acute, subchronic or chronic) and route (oral or respiratory) of exposure. The way TRVs are established depends on the knowledge or assumptions made about the substances' mechanisms of action. Currently, the

default assumption is to consider that the relationship between exposure (dose) and effect (response) is monotonic. In the current state of knowledge and by default, it is generally considered that for non-carcinogenic effects, toxicity is only expressed above a threshold dose (ANSES, 2015a).

In practice, establishing a TRV involves the following five steps:

- choice of the critical effect;
- choice of a good quality scientific study generally enabling establishment of a dose-response relationship;
- choice or establishment of a critical dose from experimental doses and/or epidemiological data;
- application of uncertainty factors to the critical dose to take uncertainties into account for the threshold TRVs,
- conducting a linear extrapolation to the origin to determine an excess risk per unit for non-threshold TRVs.

TRVs are established according to a highly structured and rigorous approach involving collective assessments by groups of specialists.

2. ORGANISATION OF THE EXPERT APPRAISAL

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

The collective expert appraisal was undertaken by the Expert Committee (CES) on "Characterisation of substance hazards and toxicity reference values" until August 2017 and then by the CES on "Health reference values".

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.
The experts' declarations of interests are made public via the https://dpi.sante.gouv.fr website.

3. ANALYSIS AND CONCLUSIONS OF THE CES

■ Summary of toxicology data

Ammonia is a gas that causes severe irritation and even burns to the mucous membranes of the skin, eyes and respiratory tract due to its alkaline properties. Several short-term studies on volunteers are available but their quality is uneven and they present varying exposure patterns. Data from these studies in different categories of individuals (workers, healthy or asthmatic volunteers) confirm the irritant nature of ammonia.

Following chronic exposure, the respiratory tract is the main target of inhaled ammonia toxicity in humans, as well as in animals. The available studies in humans show respiratory symptoms (cough, rhinitis, etc.), irritative effects and effects on lung function. Animal studies have also identified immunological effects, histopathological changes in the liver, effects on the kidney and spleen, as well as the development of myocardial fibrosis.

Ammonia does not have any effect on reproduction or development.

Only one study has examined the genotoxic effect of ammonia in workers (22 exposed versus 42 non-exposed workers) (Yadav and Kaushik, 1997, cited in ATSDR, 2004). This showed an increase in the frequency of chromosomal aberrations and sister chromatid exchanges and in the mitotic index. However, given its limitations (small number of individuals, low levels of ammonia concentration in the ambient air, probable co-exposure, etc.), this study cannot be used to draw any conclusions about the mutagenicity of ammonia. Drawing on earlier data, the ATSDR[1] considers that ammonia and the ammonium ion may have clastogenic and mutagenic properties.

The carcinogenic potential of ammonia by inhalation has not been assessed in humans or animals.

- **Acute TRV**
  - Choice of the critical effect

The available data, both in humans and in animals, provide strong evidence that acute inhalation exposure to ammonia can cause lesions at the contact site, mainly the eyes and respiratory tract.

In humans, from a concentration in air of 5 ppm, a few subjective symptoms such as eye discomfort, headaches, dizziness and a feeling of intoxication were felt (Sundblad *et al*., 2004). From 25-50 ppm, a greater number of subjective symptoms were observed in volunteers exposed at rest or with alternating periods of rest and physical exercise: feelings of irritation in the eyes, nose, throat and chest, a need to cough, a penetrating odour, nasal dryness, difficulty breathing, headache, fatigue, nausea, dizziness and a feeling of intoxication (Silverman *et al*., 1949; Verberk, 1977; Wallace, 1978; Sundblad *et al*., 2004; Pacharra *et al*., 2016).

Objective respiratory symptoms have been identified at higher doses, such as an increase in respiratory rate, an increase in nasal airway resistance and changes in ventilation and spirometric parameters (change in minute volume, tidal volume) from 85 ppm (60.1 mg/m^3) (Silverman *et al*., 1949; Cole *et al*., 1977: MacLean *et al*., 1979; Douglas and Coe, 1987). Other studies concluded as to a lack of objective respiratory effect at concentrations ranging between 16-20 and 50 ppm (Verberk, 1977; MacEwen *et al*., 1970; Sigurdarson *et al.,* 2004; Sundblad *et al.,* 2004).
The CES therefore decided to select the objective respiratory effects as the critical effect.

  - Analysis of the existing TRVs

Three acute TRVs are available: one TRV developed by the OEHHA[2] in 1999, one by the ATSDR in 2004 and one by the TCEQ[3] in 2015. These were not selected by the CES for the following reasons:

- Choice of the critical effect: the existing TRVs were established on the basis of subjective symptoms, which were not selected as the critical effect by the CES.
- Choice of the key study: the TRVs from the OEHHA and the ATSDR were established from studies in volunteers. These studies have the following limitations: lack of a control group (Verberk, 1977; MacEwen *et al.*,1970), no statistical analysis of the results (Verberk, 1977), higher response rate in "naive" subjects compared to previously exposed subjects, which may indicate a bias related to the smell (Verberk, 1977), publication or studies not available (MacEwen *et al.* (1970); Industrial Bio-Test Laboratories, 1973).
- Choice of the critical dose: the OEHHA compiled the results of several different studies using modelling of the benchmark dose. This approach raises the question of the relevance of aggregating different experimental data (different experimental protocols, access to the individual data, *etc.*).

[1] Agency for Toxic Substances and Disease Registry
[2] Office of Environmental Health Hazard Assessment
[3] Texas Commission on Environmental Quality

- The choice of uncertainty factors selected by the TCEQ does not follow ANSES's method for establishing TRVs (ANSES, 2017).

As a consequence, given these limitations, the CES did not retain the existing values and proposes establishing an acute TRV by inhalation.

- Establishment
  - Choice of the key study and critical dose

Several controlled exposure studies in volunteers have shown subjective or objective respiratory effects. Two of them were considered to be of sufficient quality to establish a TRV: Cole *et al*. (1977) and Sundblad *et al.* (2004). The study by Cole *et al.* (1977) identified objective effects on respiratory function from 150 ppm for 18 volunteers during exercise (increased average respiration rate, decreased minute volume, increased tidal volume at 150 ppm only) (NOAEC[4] = 101 ppm). Sundblad *et al*. (2004) observed only a few transient subjective effects (eye discomfort, solvent odour, headache, dizziness and a feeling of intoxication) at the lowest dose (5 ppm) while all the subjective effects were noted at 25 ppm in volunteers exposed for 3 h with alternating rest and physical exercise increasing pulmonary ventilation. No objective effect (spirometry, bronchial hyperreactivity, concentration of IL-6 and 8 interleukins, change in cell composition in nasal lavage fluids, change in differential leukocyte count in peripheral blood, exhaled nitrogen oxide) was shown at the highest concentration of 25 ppm, which therefore represents the NOAEC concerning the respiratory effects of ammonia.
Other studies concluded as to a lack of objective respiratory effect at concentrations ranging between 16-20 and 50 ppm (Verberk, 1977; MacEwen *et al*., 1970; Sigurdarson *et al.,* 2004; Sundblad *et al.,* 2004). However, another study found objective effects at 85 ppm (increased airway resistance) (Douglas and Coe, 1987).

In view of this entire corpus of data, the CES decided to select as the key study the one by Sundblad *et al*. (2004) identifying an absence of objective effects on lung function (NOAEC = 25 ppm), supported by the study by Cole *et al.* (1977). The CES notes that the critical dose is located in the odour detection threshold range of 0.04 to 53 ppm.

  - Adjustments

No temporal adjustment was made because ammonia causes local irritant effects that seem to be dependent on the concentration rather than the total dose and/or duration of exposure.
No allometric adjustment is necessary because the key study was conducted in humans.

  - Choice of uncertainty factors

The TRVs were calculated from a $NOAEC_{ADJ}$ of 25 ppm using an inter-individual uncertainty factor (UF_H) of 3. The majority of studies on volunteers were carried out with small samples of healthy individuals. Several controlled human exposure studies found no difference in respiratory sensitivity to ammonia between healthy individuals and individuals with a respiratory condition (MacLean *et al*., 1979; Sigurdason *et al*., 2004; Petrova *et al*., 2008; Pachara *et al*., 2017). The NRC[5] considers that a different response in asthmatics compared to non-asthmatics is not expected (NRC, 2007).
Two studies found greater sensory irritation in naive volunteers (not familiar with the smell or effects of ammonia) than in non-naive volunteers (Verberk, 1977; Ihrig *et al*., 2006).

[4] No Observed Effect Concentration
[5] National Research Council

However, despite the absence of any study enabling the respiratory effects of ammonia to be compared between adults and children, it can be assumed that children may be more vulnerable to corrosive agents than adults because of the smaller diameter of their airways (ATSDR, 2004), which justifies the application of an uncertainty factor of 3.

- Proposed acute TRV by inhalation

$$TRV = 5.9\ mg.m^{-3}\ (8.3\ ppm)$$

In the framework of TRVs and in line with the scenarios generally taken into account when assessing health risks in humans, ANSES considers that the period of application of TRVs for acute exposure is one to 14 days. However, for irritating substances such as ammonia, the CES decided to select a period of application of 24 h.
Nevertheless, the CES points out that this TRV does not protect from effects associated with possible exposure peaks.

- Confidence level

The overall confidence level **moderate/high** was assigned to this acute TRV, based on the following four criteria: nature and quality of the data (high confidence level)**,** choice of the critical effect and the mode of action (high confidence level), choice of the key study (moderate confidence level) and choice of the critical dose (moderate confidence level).

- **Chronic TRV**
  - Choice of the effect

Following exposure by inhalation, the respiratory system is the target organ of ammonia, both in humans and in animals. Cross-sectional studies in the workplace have shown respiratory toxicity in humans, particularly changes in lung function and an increase in the prevalence of respiratory symptoms (cough, chest tightness, nasal discharge, sputum, dyspnea, wheezing, asthma, etc.). These studies are supported by studies in workers exposed to ammonia as a disinfectant or cleaning product, in the agricultural environment, controlled exposure studies and animal studies, which also show effects on the respiratory system.
The CES therefore decided to select the respiratory effects as the critical effect.

  - Analysis of the existing TRVs

The four chronic TRVs identified for ammonia are all based on respiratory effects and on the same good-quality key study by Holness *et al.* (1989) (an epidemiological study in the workplace), which showed no effect on lung function.

The differences between the proposed TRVs are based on:

- the studies selected as support studies: the OEHHA selected the study by Broderson *et al.* (1976) conducted in rats as the support study. The support studies selected by the US EPA and the TCEQ were studies of workers: Rahman *et al*. (2007), Ballal *et al*. (1998) and, only for the US EPA, Ali *et al.* (2001);
- the daily and hourly temporal adjustment by the ATSDR *versus* daily and respiratory adjustment (higher respiratory volume at the time of the occupational activity than at rest) by the US EPA, the TCEQ and the OEHHA. The daily temporal and respiratory adjustment was considered relevant by the CES when relating to a study in the workplace;

- the application of an uncertainty factor of 3 for the lack of data, by the ATSDR. The CES considers the available data on ammonia sufficient; it is therefore not necessary to add an additional uncertainty factor to take into account the lack of data;
- the determination of the critical concentration. The OEHHA and the ATSDR selected as the NOAEC the average concentration in exposed workers, while the TCEQ and the US EPA considered the concentration in the most highly exposed group. Lastly, the US EPA modelled the exposure of the most highly exposed group and selected as the critical dose the lower limit of the confidence interval of the mean concentration. The modelling performed by the US EPA was deemed to be of good quality and relevant.

The CES has therefore selected the TRV of the US EPA. An uncertainty factor UF_H of 10, which differs from the UF_H of 3 chosen by the CES for establishing the acute TRV, has been applied. However, the CES considers that inter-individual variability is higher for long-term than short-term exposure.

- Proposed chronic TRV by inhalation

TRV = 0.5 mg.m^{-3} (0.71 ppm)

- Confidence level

The overall confidence level **moderate/high** was assigned to this chronic TRV based on the following four criteria: nature and quality of the data (moderate confidence level)**,** choice of the critical effect and the mode of action (high confidence level), choice of the key study (moderate confidence level) and choice of the critical dose (moderate confidence level).

■ Subchronic TRV

There is no subchronic TRV available.
The only study subjecting healthy volunteers to subchronic exposure, by Ferguson *et al*. (1977), cannot be used because of the difficulty in determining a LOAEC/NOAEC due to different exposure durations and an inconsistency between the exposure durations described in the text and those described in a summary table of the publication. The animal studies available were carried out with higher concentrations than the LOAEC identified for acute exposure and are not of high enough quality to derive a subchronic TRV. **On the basis of the above justifications, the CES has not established a subchronic TRV for ammonia and proposes applying the chronic TRV in the event of subchronic exposure.**

- Confidence level

The overall confidence level **moderate** was assigned to this subchronic TRV based on the following four criteria: nature and quality of the data (low confidence level)**,** choice of the critical effect and the mode of action (high confidence level), choice of the key study (moderate confidence level) and choice of the critical dose (moderate confidence level).

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on "Health reference values" on the formulation of toxicity reference values by inhalation for ammonia.

The nature of the TRVs (acute, subchronic, chronic) is partly determined by the duration of exposure in the toxicological studies but also by the health risk assessment needs. The CES points out that the acute TRV does not protect from effects due to possible exposure peaks.

As a reminder, when dealing with TRVs and in line with the scenarios generally taken into account when assessing health risks in humans, ANSES distinguishes between three types of exposure duration:

- Acute exposure, from 1 to 14 days. For irritating substances such as ammonia, the CES has decided to select a period of application of 24 h;
- Subchronic exposure, from 15 to 364 days;
- Chronic exposure, for 365 or more days.

The carcinogenic potential of ammonia by inhalation has not been assessed in humans or animals. Therefore, only a chronic threshold TRV is proposed.

Type of TRV	Organisation	Critical effect (key study)	Critical concentration	UF	TRV
Acute TRV	ANSES	Respiratory irritation *Sundblad et al., 2004* supported by *Cole et al., 1977*	NOAEC = 25 ppm (17.7 mg/m^3) No temporal adjustment	3 UF_H = 3	5.9 mg/m^3 (8.3 ppm) Confidence level moderate/high
Subchronic TRV	US EPA (2016)	Decrease in lung function and increase in respiratory symptoms (cough, wheezing, other asthma-related symptoms) *Holness et al., 1989 supported by Rahman et al., 2007; Ballal et al., 1998 and Ali et al., 2001: studies in workers*	Modelling of exposure (log normal) → lower limit of the $CI_{95\%}$ of the most highly exposed exposure group → NOAEC = 13.6 mg/m^3 Temporal adjustment $NOAEC_{ADJ}$ = NOAEC x 5/7 x 10/20 = 4.9 mg/m^3	10 UF_H = 10	0.5 mg/m^3 (0.71 ppm) Confidence level Moderate
Chronic TRV	US EPA (2016)	Decrease in lung function and increase in respiratory symptoms (cough, wheezing, other asthma-related symptoms) *Holness et al., 1989 supported by Rahman et al., 2007; Ballal et al., 1998 and Ali et al., 2001: studies in workers*	Modelling of exposure (log normal) → lower limit of the $CI_{95\%}$ of the most highly exposed exposure group → NOAEC = 13.6 mg/m^3 Temporal adjustment $NOAEC_{ADJ}$ = NOAEC x 5/7 x 10/20 = 4.9 mg/m^3	10 UF_H = 10	0.5 mg/m^3 (0.71 ppm) Confidence level moderate/high

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KEY WORDS

Valeur toxicologique de référence, VTR, ammoniac, inhalation, respiratoire, aiguë, subchronique, chronique

Toxicity reference value, TRV, ammonia, inhalation route, respiratory effects, acute, subchronic, chronic