Opinion
Request No 2015-SA-0251
Related Request no. 2014-SA-0148

The Director General

Maisons-Alfort, 12 March 2018

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

on the development of chronic TRVs by the respiratory route for ethyl acetate (CAS No. 141-78-6), methyl methacrylate (CAS No. 80-62-6) and n-butyl acetate (CAS No. 123-86-4)

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 12 March 2018 shall prevail.

On 23 November 2015, ANSES received a joint formal request from the Directorate General for Risk Prevention (DGPR) and the Directorate General for Health (DGS) to identify or establish chronic toxicity reference values (TRVs) by inhalation for the following three substances: ethyl acetate, methyl methacrylate and n-butyl acetate.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Following several reports and complaints of unpleasant odours, the Central Laboratory of the Paris Police Prefecture carried out measurements of indoor air pollutants in homes near manicure and nail salons in Paris. The measurement results showed high concentrations of certain substances in the indoor air of these dwellings, mainly ethyl acetate, methyl methacrylate and n-butyl acetate. The report of the Central Laboratory of the Paris Police Prefecture raised the question of a possible health risk associated with the deterioration of the indoor air quality of these dwellings. Informed of this situation on 30 July 2015, the DGPR and the DGS made a formal request to ANSES on 23 November 2015 to propose TRVs by inhalation for these substances. These TRVs are needed for assessing the health risk to people living in these homes near manicure and nail salons and exposed to ethyl acetate, methyl methacrylate and n-butyl acetate.

As a reminder, a toxicity reference value, or TRV, is a toxicological indicator for qualifying or quantifying a risk to human health. It establishes the link between exposure to a toxic substance and 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 differs depending 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, 2017). 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 from among a series of studies of good quality;
- 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 developed according to a highly structured and rigorous approach based on ad-hoc methodological expertise developed by ANSES (ANSES, 2017) in accordance with international standards.

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 expert appraisal falls within the sphere of competence of the Expert Committee (CES) on "Characterisation of substance hazards and toxicity reference values" (hereinafter referred to as the CES "Substances"). The methodological and scientific aspects of the work were presented to the CES between May 2016 and January 2017. It was adopted by the CES "Substances" at its meeting on 12 January 2017.

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 ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

3.1. TRV for ethyl acetate

For ethyl acetate (CAS No. 141-78-6), a chronic TRV by inhalation of 6.4 mg/m^3 has already been established by the Agency (Table 1) (ANSES, 2015b).

Table 1: Chronic TRV by the respiratory route for ethyl acetate

Critical effect Key study	Critical concentration	UF	TRV
Decreased motor activity in female Sprague Dawley rats Christophe *et al.*, 2003	NOAEC = 2696 mg/m^3 (750 ppm) <u>Allometric adjustment</u> $NOAEC_{HEC}$ = 2696 mg/m^3 (750 ppm) <u>Temporal adjustment</u> $NOAEC_{HEC\ ADJ}$ = 481 mg/m^3 (134 ppm)	75 $UF_{A\text{-}TD}$: 2.5 UF_H: 10 UF_S: 3	**TRV = 6.4 mg/m^3 (1.78 ppm)** **Confidence level: moderate/high**

3.2. TRV for methyl methacrylate (MMA)

Most of the data used to draw up the toxicological profile for methyl methacrylate (or MMA, CAS No. 80-62-6) come from animal studies.

3.2.1. Toxicokinetics of MMA

Toxicokinetic data in rats and humans show that MMA is metabolised by the same metabolic pathway. It is rapidly transformed by carboxylesterases into methacrylic acid, which is eliminated in the urine. Carboxylesterases are not specific and are distributed throughout all the organs and tissues of the body. The final product of metabolism is CO_2, which is eliminated in exhaled air.

However, experimental data show that carboxylesterase distribution in tissues is much more dispersed in the nasal epithelium of humans than of rats, and that human carboxylesterases are about 13 times less active *in vitro* than those in rats. If they could be confirmed by *in vivo* data, they would support a lower sensitivity of humans to MMA than rats.

3.2.2. Toxicity of MMA

<u>Data in humans</u>

MMA is a skin, eye and respiratory irritant, as well as a skin sensitiser. According to the harmonised European classification, MMA is classified as a Category 2 skin irritant and Category 3 respiratory tract irritant after single exposure (STOT SE[1]). It is also classified as a Category 1 skin sensitiser, but not as a respiratory sensitiser.
However, a number of asthma cases associated with occupational exposure to MMA have been reported in the literature. MMA can therefore be regarded as a respiratory sensitiser. Based on these data in particular, France placed MMA on ECHA's Registry of Intentions in 2016 with the aim of submitting a proposal for classification as a respiratory sensitiser in 2018.

[1] Specific target organ toxicity - Single exposure

Short- and long-term occupational exposure induces different symptoms depending on exposure levels and times, and include headaches, dizziness, fatigue, rhinitis and an impaired sense of smell. The threshold that trigger these effects have not been established. Similarly, the data available have not enabled a dose-response relationship to be established.

Epidemiological studies show that there is no excess mortality in workers exposed by inhalation to MMA.

Data in animals

In animal experiments, the LD_{50}[2] (oral, dermal, intraperitoneal routes) and LC_{50}[3] (inhalation) values of MMA are high in rodents, rabbits, guinea pigs and dogs, which supports low acute toxicity. Available values range from 5000 to 8000 mg/kg bw for the LD_{50}, and from 15,000 to 60,000 mg/m^3 for the LC_{50}, with exposure ranging from 2 to 5 hours. The most common effects observed are hypoactivity, dyspnea, respiratory depression and low blood pressure, which ends in respiratory and cardiac arrest.

MMA is a skin and eye irritant, and a skin sensitiser.

Via the oral route (administered in drinking water to rats, and in food capsules to dogs), a chronic study showed that MMA produced no effects up to doses of 2000 ppm for rats and 1000 ppm for dogs (Borzelleca *et al.,* 1964).

Experimental inhalation studies in rats show that the first effects appeared locally at high doses with subchronic exposure (up to 6 months of exposure to 1640 mg/m^3), and at lower doses with chronic exposure (18 or 24 months of exposure to 416 mg/m^3). The results of these studies converge on the finding that systemic effects (decreased body weight gain, increased weight of certain organs, and decreased intestinal activity) occur less frequently and at higher doses.

The earliest effect with chronic inhalation exposure occurred in the olfactory epithelium in rats exposed to 1640 mg/m^3 (400 ppm) from the 13th week of exposure (Lomax *et al.*, 1997). This effect was manifested by necrosis of the neuroepithelial olfactory cells in the nasal cavity. At a lower dose (416 mg/m^3 or 100 ppm), this effect appeared only in animals exposed for 2 years, and with lower severity. This effect was not seen in animals exposed to 104 mg/m^3 (25 ppm) for 2 years. This value of 104 mg/m^3 can be regarded as the NOAEC for chronic inhalation exposure. Moreover, the results of the study by Lomax *et al.* (1997) showed the onset of cases of chronic inflammation of the respiratory epithelium and goblet cell hyperplasia at the highest dose (1640 mg/m^3 or 400 ppm). This incidence was far higher in males (26 cases out of 42) than in females (9 cases out of 42).

With regard to other potential effects of MMA, the available data show that MMA:

- does not appear to have reprotoxic and developmental effects according to the available studies, which are relatively old and mainly focus on embryonic development,
- responds negatively in mutagenicity tests on bacterial cells with or without metabolic activation. *In vivo* tests on mammalian bone marrow cells are either negative up to 4520 mg/kg bw (in mice) or inconclusive (in rats),
- is not carcinogenic after chronic inhalation or oral exposure.

3.2.3. Choice of a chronic TRV by inhalation for MMA

Compilation and analysis of the existing TRVs

The literature review identified three TRVs published by Health Canada (1993), the WHO (1998) and the US EPA (1998, revised in 2006). These three TRVs are based on data from the same source study (Röhm and Haas, 1979), which was subsequently adopted by Lomax *et al*. (1997). This last

[2] Lethal dose for 50% of exposed animals
[3] Lethal concentration for 50% of exposed animals

study was then used by the US EPA to establish its TRV. The list and details of these three TRVs are summarised in Table 2.

Table 2: Chronic TRVs by inhalation for MMA

Organisation, year	Health Canada, 1993	WHO, 1998	US EPA, 1998 revised in June 2006
Value of the TRV	0.073 mg/m^3	0.2 mg/m^3	0.7 mg/m^3
Critical effect	Decreased body weight and mild rhinitis	Degeneration and atrophy of the olfactory epithelium	Degeneration and atrophy of the olfactory epithelium
Species	1. F344 rats 2. Hamsters	F344 rats	F344 rats
Route of exposure	Inhalation	Inhalation	Inhalation
Durations of exposure	1. 2 years 2. 18 months	2 years	2 years
Source critical dose	NOEC = 410 mg/m^3 (100 ppm)	NOEC = 102.5 mg/m^3 (25 ppm)	BMC_{10} = 143 mg/m^3 (35 ppm)
Parameters	Temporal adjustment $NOEC_{ADJ}$ = NOEC x (6/24) x (5/7) Allometric adjustment $NOEC_{ADJ\ HEC}$ = $NOEC_{ADJ}$ x 0.11 m^3/d x 0.35 kg where 0.11 m^3/d is the volume of air inhaled per day by an adult rat, and 0.35 kg is the weight of an adult rat	Temporal adjustment $NOEC_{ADJ}$ = 102.5 mg/m^3 x (6/24) x (5/7) = 18.3 mg/m^3	Temporal adjustment $BMC_{10}L_{95\ ADJ}$ = 143 x (6/24) x (5/7) = 25.6 mg/m^3 Allometric adjustment $BMC_{10}L_{95\ ADJ\ HEC}$ = $BMC_{10}L_{95\ ADJ}$ x RGDR* = 25.6 mg/m^3 x 0.28 = 7.2 mg/m^3
UF	1000 UF_H = 10 UF_A = 10 UF_D = 10	100 UF_H = 10 UF_A = 10	10 UF_A = √10 UF_H = √10
Confidence level	/	/	Source study: high Data: moderate to high TRV: moderate to high
Source study	Röhm and Haas, 1979	Röhm and Haas, 1979	Röhm and Haas, 1979; Lomax, 1992; Lomax *et al.*, 1997

* RGDR (regional gas deposition ratio) between animals and humans

Choice of the TRV

The literature review showed that there were no new studies that could be used to establish a new TRV. Of the three TRVs published, and summarised in Table 2, the US EPA's TRV (2006) appears to be the most relevant and was therefore selected by ANSES as the chronic TRV by inhalation. It was established using data published by Lomax *et al.* (1997). In this study, the authors re-examined the histological sections of the olfactory epithelium of rats that had been exposed to MMA for 2 years. Fisher 344 rats (70 per sex and exposure level) were exposed to concentrations of 0, 102.4, 408.6 or 1621.7 mg/m^3, for 6 hours/day, 5 days/week for 2 years.

The critical effect selected by the US EPA was the degeneration and atrophy of the neuroepithelium of the mucosa and submucosa that line the olfactory dorsal meatus. This effect is probably due to methacrylic acid produced by hydrolysis of MMA by non-specific carboxylesterases.

The data published by Lomax *et al.* (1997) enabled the US EPA to use a BMD[4] approach and determine a $BMC_{10}L_{95}$[5] of 35 ppm (143 mg/m^3).

The animals were exposed for 6 hours per day, 5 days per week. The value was therefore adjusted for continuous exposure: 143 x (6/24) x (5/7) = 25.6 mg/m^3.

Extrapolation from animals to humans was performed using a regional gas deposition ratio (RGDR), which is equal to 0.28.

The $BMC_{10}L_{95\ ADJ\ HEC}$ obtained for humans is thus equal to 25.6 x 0.28 = 7.2 mg/m^3. The authors of the TRV then applied an overall uncertainty factor of 10 to account for inter-species variability between rats and humans and intra-species sensitivity. The TRV value is therefore 0.7 mg/m^3.

Confidence level

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

The CES adopted the chronic TRV by inhalation for MMA developed by the US EPA as the chronic TRV.

Case reports have established a link between inhalation exposure to MMA and the occurrence of asthma. In France, the National Network for Monitoring and Prevention of Occupational Diseases (RNV3P), created in 2001 and managed by ANSES, collects more than 8,000 cases of occupational diseases each year. From this database, 37 cases of occupational asthma, specifically related to exposure to MMA, were identified. Based in particular on these data, France placed MMA on ECHA's Registry of Intentions in 2016 with the aim of submitting a proposal for classification as a respiratory sensitiser in 2018.

MMA is liable to generate respiratory sensitising effects. In the current state of knowledge, it is not possible to determine a TRV that guarantees an absence of respiratory sensitising effects. Thus, the proposed TRV does not protect against respiratory sensitisation effects.

[4] BMD: Benchmark dose, a dose that causes the critical effect to appear in a certain percentage of exposed animals.

[5] BMC_{10}: Benchmark concentration that causes the critical effect to appear in 10% of exposed animals. $BMC_{10}L_{95}$: Lower limit of the 95% confidence interval of the BMC_{10}.

3.3. TRV for n-butyl acetate (nBA)

Toxicity data for n-butyl acetate (nBA; CAS No. 123-86-4) are limited to a few experimental studies, several of which are unpublished industrial studies. Human toxicity data are rare and mainly related to irritation of the nasal and ocular mucosa.

3.3.1. Toxicokinetics of nBA

Exposure to nBA most often occurs via the inhalation and dermal routes. Its presence in certain fruits and other foods results in non-negligible oral exposure. The available data suggest that the substance is readily absorbed by the inhalation, oral and dermal routes. It is then rapidly hydrolysed to n-butanol and acetic acid and exhaled as CO_2.

3.3.2. Toxicity of nBA

Data in humans

There are very few data on the toxicity of nBA in humans. Some data are unpublished, but they were summarised in the WHO's 2005 evaluation report (WHO, 2005). They mainly concern clinical observations made in the workplace. These data show that nBA is an irritant to the throat, eyes, nose and respiratory tract. The threshold levels that trigger these effects have not been sufficiently established.

Data in animals

Most of the available data come from unpublished studies, which were summarised in the WHO evaluation report (2005) or on the ECHA[6] website (Table 3).

Table 3: Main effects observed after repeated exposure to n-butyl acetate

Type of study	Main effects	Reference
Subchronic toxicity study SD rats exposed to 2400, 7200 or 14,400 mg/m^3, 6 hours/day and 5 days/week for 13 weeks.	**7200 mg/m^3**: Decreased motor activity, body weight, food consumption and weight of certain organs; increased weight of adrenal glands, testicles and brain, and moderate olfactory epithelial necrosis. **14,400 mg/m^3**: In addition: cases of irritation of the glandular stomach and necrosis of the non-glandular stomach + cases of degeneration of the olfactory epithelium. No effect on sperm parameters. **NOAEC: 2400 mg/m^3**	David *et al.*, 2001
Neurotoxicity study SD rats exposed to 2400, 7200 or 14,400 mg/m^3, 6 hours/day and 5 days/week for 14 weeks.	**7200 and 14,400 mg/m^3**: Transient signs of sedation. **14,400 mg/m^3**: Significant increase in motor activity in males during week 4 only. No other treatment-related effects reported.	David *et al.*, 1998
Reprotoxicity study Female rats exposed to 7260 mg/m^3, 7 hours/day and 5 days/week for 3 weeks before mating.	The mating rate and the reproductive parameters studied (fertilisation rate, number of corpora lutea, implantations, resorptions of live foetuses) were not affected.	Hackett *et al.*, 1983

[6] https://echa.europa.eu/registration-dossier/-/registered-dossier/15948

Development study Female rats and rabbits exposed to 7260 mg/m^3, 7 hours/day from GD1 to GD6 or from GD1 to GD16 or 5 days/week for 3 weeks before mating with non-exposed males.	**In female rats:** - Maternal toxicity: decreased weight gain and food intake, increased kidney and lung weight, and decreased placental weight. - Foetal toxicity: rib dysmorphology, delayed pelvic ossification and dilated ureters. **In rabbits**: decreased food intake without any change in body weight, and signs of foetal toxicity (retinal folds and delayed pelvic ossification).	Hackett *et al.*, 1983
Development study Comparison of effects with nBA alone or in co-exposure with ethylbenzene or toluene.	Simultaneous exposure to nBA and the other two substances did not alter the maternal or foetal toxicity of nBA. The effects investigated were mainly clinical signs of maternal and infant toxicity, weight gain, food consumption, reproductive parameters, and foetal malformations.	Saillenfait *et al.*, 2007
Two-generation study Crl:CD rats exposed for 6 hours/day and 7 days/week, for 70 days before mating. 0, 750, 1500 and 2000 ppm (0, 3600, 7200 and 9600 mg/m^3) for the F0, F1 and F2 generations	- **For F0 and F1**: no effects on reproductive parameters at any of the doses (oestrous cycle, mating and fertility index, number of days between pairing and coitus, sperm parameters and gestation time). - **For F1 and F2**: decrease in average body weight and in body weight gain at 1500 and 2000 ppm (7200 and 9600 mg/m^3): degeneration of the olfactory epithelium of F0 and F1, and delay in preputial separation and vaginal opening. **NOAEC** = 2000 ppm (9600 mg/m^3) for fertility **NOAEC** = 750 ppm (3600 mg/m^3) for systemic and developmental toxicity.	Study summarised on the ECHA website[7]

*GD: gestation day

3.3.3. Establishment of a chronic TRV by inhalation

Compilation and analysis of the existing TRVs

Only one TRV by inhalation (WHO, 2005) is available (Table 4).

Table 4: TRV by inhalation for n-butyl acetate (WHO, 2005)

Organisation	Critical effect Key study	Critical concentration	UF	TRV
WHO (2005)	Systemic effects in Sprague Dawley rats exposed by inhalation for 13 weeks David *et al.*, 2001	LOAEC = 7200 mg/m^3 NOAEC = 2400 mg/m^3 Temporal adjustment $NOAEC_{ADJ}$ = 2400 x (6/24) x (5/7) = 420 mg/m^3	1000 UF_H = 10 UF_A = 10 UF_S = 10	**0.42 mg/m^3**

The critical effect selected by the authors of the TRV was formed by all the systemic effects observed at 7200 mg/m^3 (decreased activity, decreased body weight, change in the weight of certain organs, and cases of degeneration of the olfactory epithelium). None of these effects and no other treatment-related effects were observed at 2400 mg/m^3. This value was therefore used as the NOAEC. A time adjustment was made to take account of the discontinuity of exposure. An overall uncertainty factor of 1000 was applied (UF_H = 10, UF_A = 10 and UF_S = 10).

The critical effect selected does not seem appropriate since it combines different effects with no possibility of establishing a dose-response relationship. In addition, the application of three

[7] https://echa.europa.eu/en/brief-profile/-/briefprofile/100.001.180

uncertainty factors to the NOAEC is not sufficiently justified. For these reasons, this TRV was not used.

Establishing the TRV

- Choice of critical effect and key study

Data on the toxicity of nBA in humans are limited to a few on eye, throat and nose irritation. However, the threshold values causing this effect are not precisely known. There are no data on chronic or subchronic exposure to nBA in humans.

The most relevant study that can be used to derive a TRV for chronic inhalation exposure is that of David *et al.* (2001).
Sprague Dawley rats (15 animals per dose and per sex) were exposed to target concentrations of nBA vapours of 0, 2400, 7200 or 14,400 mg/m^3, 6 hours/day, 5 days/week for 13 consecutive weeks. No treatment-related mortality was observed in any of the exposure groups.
In addition to several systemic effects (decreased body weight gain and changes in the weight of certain organs, including liver, spleen, kidneys, adrenal glands, lungs and testes), this study showed an effect on the olfactory epithelium in animals of both sexes (degeneration of the olfactory epithelium along the dorsal meatus). This effect was observed in all animals (males and females) exposed to the 14,400 mg/m^3 dose, with mild to moderate severity, while it occurred in only 4 out of 10 males and 6 out of 10 females in the 7200 mg/m^3 group, with minimal to mild severity (Table 5). The value of 2400 mg/m^3 is therefore the NOAEC. This lesion was characterised by karyorrhexis, pyknosis and depletion of olfactory epithelial cells. This effect was selected as the critical effect for establishing the TRV.

Table 5: Proportion of animals with necrosis of the nasal olfactory epithelial (David *et al.*, 2001)

Concentration (mg/m^3)	0	2400	7200	14,400
Proportion of male rats with the critical effect	0	0	0.4	1
Proportion of female rats with the critical effect	0	0	0.6	1

- Choice of the critical concentration

A BMC_{10}, defined as the dose that produces a 10% increase in the incidence of the response (in this case, nasal olfactory epithelial necrosis) observed in the exposed group compared to the control group, was modelled. The lower limit of the 95% confidence interval of the BMC_{10} ($BMC_{10}L_{95}$) was determined by the model. This value was used in order to better protect against the critical effect.

Applying the Log-Logistic model used[8] to the data in Table 5 yielded the following values for the BMC_{10} and $BMC_{10}L_{95}$ (Table 6):

Table 6: Values for the BMC_{10} and $BMC_{10}L_{95}$

	Values for the BMC_{10}	**Values for the $BMC_{10}L_{95}$**
Male rats	1358 ppm or 6517 mg/m^3	767 ppm or 3681 mg/m^3
Female rats	1298 ppm or 6230 mg/m^3	579 ppm or 2778 mg/m^3

[8] US EPA (Environmental Protection Agency), 2015. Benchmark Dose Software (BMDS) Version 2.6.0.1 (Build 88, 6/25/2015). National Center for Environmental Assessment.

The value of 2778 mg/m^3 is the most protective and was therefore used to establish the TRV.

- Temporal adjustment

The animals were exposed for 6 hours per day, 5 days per week. A time adjustment was made to take account of the discontinuity of exposure:

$$BMC_{10}L_{95\ ADJ} = 2778 \times (6/24) \times (5/7) = 496\ mg/m^3$$

- Dose adjustment

An allometric adjustment was performed to reduce the value of the uncertainty regarding interspecies variability. nBA is a Category 1 gas because it is highly water soluble (solubility greater than 1g/L) and the toxic effect taken into account (necrosis of the nasal olfactory epithelium) is a local effect. The dose adjustment applied by default for a gas in this category is as follows:

$$BMD_{10}L_{95\ ADJ\ HEC} = BMD_{10}L_{95\ ADJ} \times (Ve/Set)_{Animal}/(Ve/Set)_{Human} = BMD_{10}L_{95\ ADJ} \times 0.28 = 138.9\ mg/m^3$$

Where: Ve: inhalation volume in cm^3/minute, equal to 0.25 L/min for rats, and 13.8 L/min for humans.

Set: surface area of the extra-thoracic region, equal to 11.6 cm^2 for rats and 177 cm^2 for humans.

- Choice of uncertainty factors

The TRV was calculated from the $BMC_{10}L_{95\ ADJ\ HEC}$ using the following uncertainty factors (ANSES, 2017):

- Inter-species variability (UF_A = 2.5). The dose adjustment performed enabled a human equivalent concentration to be calculated, using the previous equation. To take toxicodynamic variability and residual uncertainties into account, an additional uncertainty factor of 2.5 was applied.
- Inter-individual variability (UF_H): 10
- Subchronic to chronic transposition (UF_S): 3
- Use of a BMD ($UF_{B/L}$): 1

In total, an overall uncertainty factor of **75** was used to determine the TRV.

- Proposed chronic TRV by inhalation

TRV = 138.9/75 = 1.85 (rounded to 2 mg/m^3)

- Confidence level

The overall confidence level **moderate to high** was assigned to this 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 (high confidence level).

The report was validated unanimously by the experts present (17 out of the 17 experts present).

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES "Substances" on the proposed chronic toxicity reference values (TRVs) by inhalation for ethyl acetate, methyl methacrylate (MMA) and n-butyl acetate (nBA).

The analysis of the toxicological profiles of the three substances studied showed that their inhalation toxicity following acute exposure is relatively low. It was therefore not considered appropriate to establish acute respiratory TRVs for these substances.

MMA is liable to generate respiratory sensitising effects. In the current state of knowledge, it is not possible to determine a TRV that guarantees an absence of respiratory sensitising effects. **Thus, the proposed TRV does not protect against respiratory sensitisation effects.**

Table 7: Chronic respiratory TRVs for ethyl acetate, methyl methacrylate and n-butyl acetate.

Substance	Organi-sation	Critical effect Key study	Critical concentration	UF	TRV
Ethyl acetate	ANSES (2015)	Decreased motor activity in female Sprague-Dawley rats Christophe *et al.*, 2003	NOAEC = 2696 mg/m^3 (750 ppm) Allometric adjustment $NOAEC_{HEC}$ = 750 ppm Temporal adjustment $NOAEC_{HEC\ ADJ}$ = 134 ppm	75 $UF_{A\text{-}TD}$: 2.5 UF_H: 10 UF_S: 3	**TRV = 6.4 mg/m^3 (1.78 ppm)** **Confidence level: moderate/ high**
Methyl meth-acrylate	US EPA (2006)	Degeneration and atrophy of the olfactory epithelium in Fisher 344 rats Lomax *et al.* (1997)	$BMC_{10}L_{95}$ = 143 mg/m^3 (34.72 ppm) Temporal adjustment $BMC_{10}L_{95\ ADJ}$ =143 x (6/24) x (5/7) = 25.6 mg/m^3 Allometric adjustment $BMC_{10}L_{95\ ADJ\ HEC}$ = $BMC_{10}L_{95\ ADJ}$ x RGDR* = 25.6 mg/m^3 x 0.28 = 7.2 mg/m^3	10 UF_A = √10 UF_H = √10	**TRV = 0.7 mg/m^3 (0.17 ppm)** **Confidence level: high**
N-butyl acetate	ANSES (2017)	Degeneration of the olfactory epithelium in Sprague Dawley rats. (David *et al.*, 2001)	$BMC_{10}L_{95}$ = 2778 mg/m^3 (556 ppm) Temporal adjustment $BMC_{10}L_{95\ ADJ}$ = 496 mg/m^3 Allometric adjustment $BMC_{10}L_{95\ ADJ\ HEC}$ = $BMC_{10}L_{95\ ADJ}$ x RGDR* = 496 mg/m^3 x 0.28 = 138.9 mg/m^3	75 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10 UF_S = 3	**TRV = 1.85 (rounded to 2 mg/m^3)** **Confidence level: moderate/ high**

***** RGDR (regional gas deposition ratio) between animals and humans

Dr Roger Genet

KEYWORDS

Ethyl acetate, Methyl methacrylate, N-butyl acetate, toxicity reference value, inhalation.