The Director General

Maisons-Alfort, 30 July 2018

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

on the development of chronic TRVs for the oral and respiratory routes for 4-chloroaniline (CAS No. 106-47-8), 3-chloroaniline (CAS No. 108-42-9), 2-chloroaniline (CAS No. 95-51-2) and 2,5-dichloroaniline (CAS No. 95-82-9)

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 30 July 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 development 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 dossiers concerning classified installations for environmental protection (ICPE) or 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 substances. 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 above-mentioned information note, ANSES is designated as the expert agency for selecting and establishing TRVs.

On 18 March 2016, ANSES received a formal request from the DGS to propose acute, subchronic and chronic TRVs by inhalation (with and without a threshold) for the following four chloroanilines: 4-chloroaniline (CAS No. 106-47-8), 3-chloroaniline (CAS No. 108-42-9), 2-chloroaniline (CAS No. 95-51-2), and 2,5-dichloroaniline (CAS No. 95-82-9). The request asked the Agency to analyse the relevance of selecting a tracer substance for the risk. In view of the data available on these

substances, the CES decided to propose TRVs not only for the respiratory route but also for the oral route.

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 threshold TRV involves the following steps:

- identifying and analysing the available toxicity data, based on epidemiological and/or experimental studies;
- identifying the target organ(s) and critical effect;
- identifying the assumption according to which it is established: with or without a threshold dose, depending on the substance's mode of action,
- choosing a good quality scientific study generally enabling establishment of a dose-response relationship;
- defining a critical dose for humans or animals from this study, and if required, in the case of a critical dose obtained in animals, adjusting this dose to humans;
- applying uncertainty factors to this critical dose so as to derive a TRV that is applicable to the entire population in question.

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

An indicative toxicity value (iTV) may be proposed when the necessary conditions for establishing a TRV are not met and a quantitative health risk assessment (QHRA) is required in a given exposure context:

1. if there are **insufficient data** available on the substance to characterise the hazard it presents or if there is **doubt as to the harmful nature of the effect**. In this case, ANSES conducts a literature review on these substances with a view to replacing the indicative toxicity values by TRVs if new data allow it;
2. in the event of **time and/or resource constraints**. In this case, the indicative toxicity value is developed as far as possible within the time available, with additional work being carried out subsequently, if appropriate, in order to propose a TRV.

An iTV is a toxicological benchmark that can be used for assessing a risk. It is an indicative value that is less robust than the TRV and therefore has a low confidence level.

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".

The work was adopted by the CES on "Health reference values" at its meeting on 23 and 24 November 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.

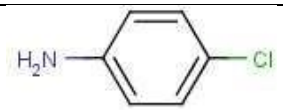
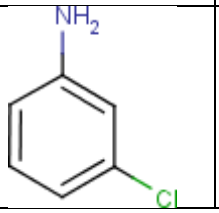
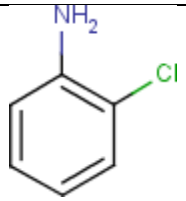
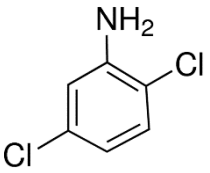
The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

■ Identification of the substances (isomers of chloroaniline and 2,5-dichloroaniline)

The identifying characteristics of 4-chloroaniline, 3-chloroaniline, 2-chloroaniline and 2,5-chloroaniline are shown in Table 1.

Table 1: Identification of chloroaniline isomers

Chemical substance	4-chloroaniline (para-chloroaniline)	3-chloroaniline (meta-chloroaniline)	2-chloroaniline (ortho-chloroaniline)	2,5-dichloroaniline
CAS number	106-47-8	108-42-9	95-51-2	95-82-9
Molecular formula	C_6H_6ClN	C_6H_6ClN	$ClC_6H_4NH_2$	$C_6H_5Cl_2N$
Molecular structure				

■ Summary of the toxicological data

- Acute toxicity: Several cases of acute human poisoning with 4-chloroaniline have been reported. They followed exposure by ingestion, inhalation and/or dermal contact (IPCS, 2003; Pizon *et al.*, 2009). All resulted in methaemoglobinaemia. One of these cases of acute poisoning was fatal.

- **Repeated toxicity**: Acute, subchronic and chronic studies show that the ortho, meta and para isomers (2-, 3- and 4-chloroaniline) are all haematotoxic and that their effects are identical in rats and mice, with 4-chloroaniline being the most documented isomer of the three.
No studies were identified for 2,5-dichloroaniline.

- Genotoxicity: According to the literature, neither 3-chloroaniline nor 2-chloroaniline have mutagenic activity. Studies with 4-chloroaniline conclude that there is little evidence of its genotoxicity.

- Carcinogenicity: The International Agency for Research on Cancer (IARC) has classified 4-chloroaniline in Group 2B (possibly carcinogenic to humans) (IARC, 1993) based on insufficient evidence in humans and sufficient evidence in animals.

- **Effects on reproduction and development:** According to the literature, 2-, 3- and 4-chloroaniline have no effect on reproduction and development.

■ Proposed TRVs

1. Choice of the critical effect for the oral route

The isomers of chloroaniline are haematotoxic. In particular, a dose-dependent increase in blood methaemoglobin levels and a haemolysing effect were shown in rats and mice, resulting in the formation of haemosiderin deposits in the liver, spleen and kidneys. The methaemoglobinising effect is the critical effect selected: the available experimental studies indicate that it appears at the lowest doses tested, regardless of the duration of acute, subchronic or chronic exposure.

2. Compilation and analysis of the existing TRVs

No TRV by inhalation is available in the databases of the recognised organisations listed in the methodological guide (ANSES, 2017).

The World Health Organisation (WHO) and the US EPA have proposed chronic oral TRVs for 4-chloroaniline. The WHO proposes a value of $2 \cdot 10^{-3}$ mg/kg/d based on haematotoxicity in rats (increased blood methaemoglobin levels) with a LOAEL[1] of 2 mg/kg/d. The US EPA proposes a value of $4 \cdot 10^{-3}$ mg/kg/d based on the occurrence of non-neoplastic spleen lesions in rats with a LOAEL of 12.5 mg/kg/d.

The CES decided not to retain the oral TRVs of the WHO and US EPA because although effects on the spleen and on haematotoxicity are regarded as relevant effects, the TRVs were not established according to the ANSES methodology (establishment of a benchmark dose, allometric adjustment).

The CES decided not to retain the non-threshold TRV of the Office of Environmental Health Hazard Assessment (OEHHA) because the experts had selected a threshold dose mechanism of action.

The CES therefore proposed establishing acute, subchronic and chronic TRVs (with and/or without a threshold) for the oral and respiratory routes.

3. Acute oral and respiratory TRVs

- **Acute TRVs for 2- and 3-chloroaniline**
  - **Choice of the key study and critical dose**

No experimental respiratory exposure studies are available in the literature.

The NTP[2] (1998) conducted comparative studies of 3, 23 and 93 days (up to 13 weeks) on the toxicity of 2-chloroaniline and 3-chloroaniline in Fisher rats (10 males and 10 females) and B6C3F1 mice (10 males and 10 females) at doses of 0, 10, 20, 40, 80 or 160 mg/kg bw/d administered by gavage. The NTP reported a significant increase in blood methaemoglobin levels after 3 days of exposure for 2-chloroaniline and 3-chloroaniline.

For 2-chloroaniline, the CES selected the lowest critical dose (determined in female rats) and the one established by the model that best fitted the experimental data (Hill model). Benchmark dose (BMD) and BMD lower confidence limit (BMDL) values were 11 and 8.97 mg/kg bw/d respectively, based on a 5% increase in blood methaemoglobin levels compared to the non-exposed group.

For 3-chloroaniline, the CES selected the lowest critical dose (determined in female rats) and the one established by the model that best fitted the experimental data (Hill model). BMD and BMDL

[1] Lowest Observed Adverse Effect Level

[2] National Toxicology Program

values were 1.6 and 1.37 mg/kg bw/d respectively, based on a 5% increase in blood methaemoglobin levels compared to the non-exposed group.

- **Allometric adjustment**

An allometric adjustment was performed to reduce the value of the uncertainty regarding interspecies variability, and enabled calculation of a Human Equivalent Dose (HED), using the following equation[3]:

$$\text{Human Equivalent Dose} = \text{Animal dose} \times \left(\frac{\text{Animal weight}}{\text{Human weight}}\right)^{1/4}$$

The corresponding average weight of the rats (female) in the NTP study (1998) on 2-chloroaniline and 3-chloroaniline was 124 g. The average human weight used for the calculation was 70 kg.

This corresponds to a critical dose in humans of:

- **for 2-chloroaniline: $BMD_{5\%}L_{90\%\ HED}$ = 1.83 mg/kg bw/d**
- **for 3-chloroaniline: $BMD_{5\%}L_{90\%\ HED}$ = 0.28 mg/kg bw/d**

- **For calculating the TRV for the respiratory route, a route-to-route extrapolation was performed.**

This calculation was based on an absorption assumption of 100% for the oral[4] and respiratory routes (for both humans and animals). The critical dose established in humans was converted to a critical concentration **($BMC_{5\%}L_{90\%\ HEC}$)** using a 24-hour respiratory volume of 20 m^3 of air for an average weight of 70 kg (ECHA, 2012), i.e. 6.4 $mg \cdot m^{-3}$ for 2-chloroaniline and 0.98 $mg \cdot m^{-3}$ for 3-chloroaniline.

- **Choice of uncertainty factors**

The TRVs for the 2 and 3 isomers of chloroaniline were calculated while applying 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. To account for toxicodynamic variability and residual uncertainties, the UF_A factor was set at 2.5 according to WHO/IPCS[5] recommendations (WHO/IPCS, 2005) and based on the ANSES methodological guide (ANSES, 2017).
- Inter-individual variability **(UF_H): 10**. Because there were no scientific data available to reduce the default value, the value of 10 was selected by the experts.

An overall uncertainty factor of 25 was thus used to determine the acute TRVs.

- **Confidence level for acute TRVs for 2- and 3-chloroaniline**
  - **for the oral route**

The overall confidence level **moderate/high** was assigned to the acute TRVs for 2- and 3-chloroaniline based on the following four criteria: nature and quality of the data (moderate), choice of the critical effect and the mode of action (high), choice of the key study (moderate) and choice of the critical dose (high).

[3] This equation is taken from the recommendations of the US EPA (US EPA, 2006)
[4] Toxicokinetic data on 4-chloroaniline showed that this compound was fully absorbed orally
[5] International Programme on Chemical Safety

- **for the respiratory route**

The overall confidence level **moderate** was assigned to the acute TRVs for 2- and 3-chloroaniline based on the following four criteria: nature and quality of the data (low[6]), choice of the critical effect and the mode of action (high), choice of the key study (high) and choice of the critical dose (high).

- **Acute Reference Values for 4-chloroaniline and 2,5-dichloroaniline**

For 4-chloroaniline, the data on the effects associated with acute exposure are not detailed in the NTP study (1989). The CES therefore selected the lowest critical dose from among the values determined for 2-chloroaniline and 3-chloroaniline.

For 2,5-chloroaniline, there are no acute exposure studies. The CES therefore selected the lowest critical dose from among the values determined for 2-chloroaniline and 3-chloroaniline.

In the absence of data on the effects of acute exposure to 4-chloroaniline and 2,5-dichloroaniline, the CES selected the lowest critical dose from among the values determined for 2-chloroaniline and 3-chloroaniline, i.e. the TRV for 3-chloroaniline, which is 11.2 µg/kg bw/d for the oral TRV and 39.2 µg/m^3 for the respiratory TRV. These values correspond to iTVs.

An indicative toxicity value (iTV) is a toxicological benchmark that can be used for assessing a risk. It is however an indicative value that is less robust than the TRV and therefore has a **low confidence level**.

The acute oral and respiratory TRVs and iTVs for the three isomers of chloroaniline and 2,5-dichloroaniline are summarised in Table 2.

[6] The literature review revealed that there were few or no studies on the subacute toxicity of 2-chloroaniline and 3-chloroaniline for the respiratory route. This led to a route-to route extrapolation being performed.

Table 2: Acute oral and respiratory TRVs for the three isomers of chloroaniline and 2,5-dichloroaniline

Substance	Critical effect (reference)	Critical dose	UF	TRV/iTV
ORAL ROUTE				
2-chloroaniline	5% increase in blood methaemoglobin levels in female F344 rats NTP (1998): 13-week study	$BMD_{5\%}L_{90\%}$ = 8.97 mg/kg/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED}$ = 1.83 mg/kg bw/d	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 73 µg/kg bw/d** **Confidence level: moderate**
3-chloroaniline	5% increase in blood methaemoglobin levels in female F344 rats NTP (1998): 13-week study	$BMD_{5\%}L_{90\%}$ = 1.37 mg/kg/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED}$ = 0.28 mg/kg bw/d	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 11.2 µg/kg bw/d** **Confidence level: moderate**
4-chloroaniline	TRV for 3-chloroaniline			**iTV = 11.2 µg/kg bw/d** **Confidence level: low**
2,5-dichloroaniline	TRV for 3-chloroaniline			**iTV = 11.2 µg/kg bw/d** **Confidence level: low**
RESPIRATORY ROUTE				
2-chloroaniline	5% increase in blood methaemoglobin levels in female F344 rats NTP (1998): 13-week study	$BMC_{5\%}L_{90\%}$ = 8.97 mg/kg/d Allometric adjustment $BMC_{5\%}L_{90\%\ HEC}$ = 1.83 mg/kg bw/d Route-to-route extrapolation $BMC_{5\%}L_{90\%\ HEC}$ = 6.4 mg·m^{-3}	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 256 µg·m^{-3}** **Confidence level: low**
3-chloroaniline	5% increase in blood methaemoglobin levels in female F344 rats NTP (1998): 13-week study	$BMC_{5\%}L_{90\%}$ = 1.37 mg/kg/d Allometric adjustment $BMC_{5\%}L_{90\%\ HEC}$ = 0.28 mg/kg bw/d Route-to-route extrapolation $BMC_{5\%}L_{90\%\ HEC}$ = 0.98 mg·m^{-3}	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 39.2 µg·m^{-3}** **Confidence level: low**
4-chloroaniline	TRV for 3-chloroaniline			**iTV = 39.2 µg·m^{-3}** **Confidence level: low**
2,5-dichloroaniline	TRV for 3-chloroaniline			**iTV = 39.2 µg·m^{-3}** **Confidence level: low**

4. Subchronic oral and respiratory TRVs

○ Choice of the key study and critical dose for the oral route

No respiratory studies are available. For the oral route, the NTP studies (1998 and 1989) on chloroaniline isomers are of sufficient quality to be regarded as key studies.

Regarding 2-chloroaniline and 3-chloroaniline, the NTP (1998) reported a significant increase in blood methaemoglobin levels after 13 weeks of exposure in Fisher rats (10 males and 10 females) and B6C3F1 mice (10 males and 10 females) at doses of 0, 10, 20, 40, 80 or 160 mg/kg bw/d administered by gavage.

The NTP (1989) study reported an increase in blood methaemoglobin levels in rats and/or mice receiving 4-chloroaniline for 3 months at doses of 0, 5, 10, 20, 40 and 80 mg/kg bw/d and 0, 7.5, 15, 30, 60 and 120 mg/kg bw/d, respectively.

For 2-chloroaniline, the CES selected the lowest critical dose (determined in male rats) and the one established by the model that best fitted the experimental data (Hill model). BMD and BMDL values were 1.9 and 1.5 mg/kg/d respectively, based on a 5% increase in blood methaemoglobin levels compared to the non-exposed group.

For 3-chloroaniline, the CES selected the lowest critical dose (determined in male rats) and the one established by the model that best fitted the experimental data (Hill model). BMD and BMDL values were 0.65 and 0.56 mg/kg/d respectively, based on a 5% increase in blood methaemoglobin levels compared to the non-exposed group.

For 4-chloroaniline, the CES was unable to establish a robust BMD. According to the NTP's results, there was a statically significant increase in blood methaemoglobin levels in male and female rats at the first dose tested, 5 mg/kg/d. The CES considered this value to be a LOAEL.

For 2,5-chloroaniline, there are no studies on effects after subchronic exposure. The CES selected the lowest critical dose from among the values determined for 2-chloroaniline, 3-chloroaniline and 4-chloroaniline.

○ Allometric adjustment

An allometric adjustment was performed to reduce the value of the uncertainty regarding interspecies variability, and enabled calculation of a Human Equivalent Dose (HED), using the following equation[7]:

$$\text{Human Equivalent Dose} = \text{Animal dose} \times \left(\frac{\text{Animal weight}}{\text{Human weight}}\right)^{1/4}$$

The average weight of the rats (females) corresponding to the weight of the females at 13 weeks was 326 g, 321 g and 334 g in the 2-chloroaniline, 3-chloroaniline and 4-chloroaniline studies, respectively. The average human weight used for the calculation was 70 kg.

This corresponds to a critical dose of:

- for 2-chloroaniline: $BMD_{5\%}L_{90\%\ HED}$ = 0.39 mg/kg bw/d
- for 3-chloroaniline: $BMD_{5\%}L_{90\%\ HED}$ = 0.15 mg/kg bw/d
- for 4-chloroaniline: $LOAEL_{\ HED}$ = 1.31 mg/kg bw/d

[7] This equation is taken from the recommendations of the US EPA (US EPA, 2006)

- **For calculating the TRV for the respiratory route, a route-to-route extrapolation was performed.**

This calculation was based on an absorption assumption of 100% for the oral and respiratory routes (for both humans and animals). The critical dose established in humans was converted to a critical concentration **($BMC_{5\%}L_{90\%\ HEC}$)** using a 24-hour respiratory volume of 20 m^3 of air for an average weight of 70 kg (ECHA, 2012), i.e. 1.365 $mg \cdot m^{-3}$ for 2-chloroaniline, 0.525 $mg \cdot m^{-3}$ for 3-chloroaniline and 4.585 $mg \cdot m^{-3}$ for 4-chloroaniline. The critical concentration in humans is therefore 1.365 $mg \cdot m^{-3}$ for 2-chloroaniline, 0.525 $mg \cdot m^{-3}$ for 3-chloroaniline and 4.585 $mg \cdot m^{-3}$ for 4-chloroaniline.

- **Choice of uncertainty factors**

The TRVs for the 2 and 3 isomers of chloroaniline were calculated while applying 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 account for toxicodynamic variability and residual uncertainties, the UF_A factor was set at 2.5 according to IPCS recommendations (IPCS, 2005) and based on the ANSES methodological guide (ANSES, 2017).
- Inter-individual variability **(UF_H): 10.** Because there were no scientific data available to reduce the default value, the value of 10 was selected by the experts.

For 4-chloroaniline, the CES chose to apply an uncertainty factor (10 by default) associated with the use of a LOAEL.

An overall uncertainty factor of 25 was therefore used to establish the subchronic TRVs for 2- and 3-chloroaniline, and an overall uncertainty factor of 250 was used to establish the subchronic TRV for 4-chloroaniline.

Regarding 2,5-dichloroaniline, in the absence of data on the effects of subchronic exposure, the CES selected the lowest of the values determined for 2-chloroaniline, 3-chloroaniline and 4-chloroaniline, i.e. the TRV for 4-chloroaniline, which is 5.2 µg/kg bw/d for the oral TRV and 18.3 $µg/m^3$ for the respiratory TRV. These values correspond to iTVs.

- **Confidence level for TRVs for 2-, 3- and 4-chloroaniline**
  - **for the oral route**

The overall confidence level **moderate/high** was assigned to the subchronic TRVs for 2-, 3- and 4-chloroaniline based on the following four criteria: nature and quality of the data (moderate), choice of the critical effect and the mode of action (high), choice of the key study (moderate) and choice of the critical dose (high for 2- and 3-chloroaniline, moderate for 4-chloroaniline).

  - **for the respiratory route**

The overall confidence level **moderate** was assigned to the subchronic TRVs for 2- and 3-chloroaniline and **low** for 4-chloroaniline based on the following four criteria: nature and quality of the data (low), choice of the critical effect and the mode of action (high), choice of the key study (high) and choice of the critical dose (high for 2- and 3-chloroaniline, low for 4-chloroaniline).

- **Confidence level for the iTV for 2,5-dichloroaniline**

An iTV is a toxicological benchmark that can be used for assessing a risk. It is however an indicative value that is less robust than the TRV and therefore has a **low confidence level**.

The subchronic oral and respiratory TRVs and iTVs for the three isomers of chloroaniline and 2,5-dichloroaniline are summarised in Table 3.

Table 3: Subchronic oral and respiratory TRVs for the three isomers of chloroaniline and 2,5-dichloroaniline

Substance	Critical effect	Critical dose	UF	TRV/iTV
ORAL ROUTE				
2-chloroaniline	5% increase in blood methaemoglobin levels in male F344 rats NTP (1998): 13-week study	$BMD_{5\%}L_{90\%}$ = 1.5 mg/kg/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED}$ = 0.39 mg/kg bw/d	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 15.6 µg/kg bw/d** **Confidence level: moderate**
3-chloroaniline	5% increase in blood methaemoglobin levels in male F344 rats NTP (1998): 13-week study	$BMD_{5\%}L_{90\%}$ = 0.56 mg/kg/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED}$ = 0.15 mg/kg bw/d	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 6 µg/kg bw/d** **Confidence level: moderate**
4-chloroaniline	Increase in blood methaemoglobin levels in male and female F344 rats NTP (1989): 13-week study	LOAEL = 5 mg/kg/d Allometric adjustment $LOAEL_{HED}$ = 1.31 mg/kg bw/d	250 UF_L = 10 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 5.2 µg/kg bw/d** **Confidence level: moderate**
2.5-chloroaniline	TRV for 4-chloroaniline			**iTV = 5.2 µg/kg bw/d** **Confidence level: low**
RESPIRATORY ROUTE				
2-chloroaniline	5% increase in blood methaemoglobin levels in male F344 rats NTP (1998): 13-week study	$BMC_{5\%}L_{90\%}$ = 1.5 mg/kg/d Allometric adjustment $BMC_{5\%}L_{90\%\ HEC}$ = 0.39 mg/kg bw/d Route-to-route extrapolation $BMC_{5\%}L_{90\%\ HEC}$ = 1.365 $mg{\cdot}m^{-3}$	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 54.6 µg·m^{-3}** **Confidence level: moderate**
3-chloroaniline	5% increase in blood methaemoglobin levels in male F344 rats NTP (1998): 13-week study	$BMC_{5\%}L_{90\%}$ = 0.56 mg/kg/d Allometric adjustment $BMC_{5\%}L_{90\%\ HEC}$ = 0.15 mg/kg bw/d Route-to-route extrapolation $BMC_{5\%}L_{90\%\ HEC}$ = 0.525 mg/m^3	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 21 µg·m^{-3}** **Confidence level: moderate**
4-chloroaniline	Increase in blood methaemoglobin levels in male and female F344 rats NTP (1989): 13-week study	LOAEL = 5 mg/kg/d Allometric adjustment $LOAEL_{HED}$ = 1.31 mg/kg bw/d Route-to-route extrapolation $LOAEC_{HEC}$ = 4.585 mg/m^3	250 UF_L = 10 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 18.3 µg·m^{-3}** **Confidence level: low**
2.5-chloroaniline	TRV for 4-chloroaniline			**iTV = 18.3 µg·m^{-3}** **Confidence level: low**

5. Chronic oral and respiratory TRVs

o Chronic TRVs for 4-chloroaniline

▪ Choice of the key study and critical dose for the oral route

In the carcinogenicity study of 4-chloroaniline hydrochloride (NTP, 1989), groups of 50 male and female rats and mice received 4-chloroaniline hydrochloride by gavage for five days per week for 103 weeks. The doses administered to rats were 0, 2, 6 or 18 mg/kg/d. The doses administered to mice were 0, 3, 10 or 30 mg/kg/d. The results in terms of increased blood methaemoglobin levels in mice were not examined beyond an exposure duration of 13 weeks.

The CES selected the lowest critical dose (determined in female rats) from the model that best fitted the experimental data (the exponential model). BMD and BMDL values were 0.16 and 0.11 mg/kg/d respectively, based on a 5% increase in blood methaemoglobin levels compared to the non-exposed group.

▪ Allometric adjustment

An allometric adjustment was performed to reduce the value of the uncertainty regarding interspecies variability, and enabled calculation of a Human Equivalent Dose (HED), using the following equation[8]:

$$\text{Human Equivalent Dose} = \text{Animal dose} \times \left(\frac{\text{Animal weight}}{\text{Human weight}}\right)^{1/4}$$

The average weight of the rats (female) corresponding to the weight of the females at 13 weeks was 292 g in the 4-chloroaniline study. The average human weight used for the calculation was 70 kg.

This corresponds to a critical dose for 4-chloroaniline of: $BMD_{5\%}L_{90\%\ HED}$ = 0.03 mg/kg bw/d

▪ For calculating the respiratory TRV, a route-to-route extrapolation was performed.

This calculation was based on an absorption assumption of 100% for the oral[4] and respiratory routes (for both humans and animals). The critical dose established in humans was converted to a critical concentration **($BMC_{5\%}L_{90\%\ HEC}$)** using a 24-hour respiratory volume of 20 m^3 of air for an average weight of 70 kg (ECHA, 2012). The critical concentration in humans is therefore 0.105 $mg \cdot m^{-3}$ for 4-chloroaniline, 1.365 $mg \cdot m^{-3}$ for 2-chloroaniline and 0.525 $mg \cdot m^{-3}$ for 3-chloroaniline.

▪ Choice of uncertainty factors

The TRVs were calculated while applying 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 account for toxicodynamic variability and residual uncertainties, the UF_A factor was set at 2.5 according to IPCS recommendations (IPCS, 2005) and based on the ANSES methodological guide (ANSES, 2017).
- Inter-individual variability **(UF_H): 10.** Because there were no scientific data available to reduce the default value, the value of 10 was selected by the experts.

An overall uncertainty factor of 25 was thus used to determine the chronic TRV for 4-chloroaniline.

[8] This equation is taken from the recommendations of the US EPA (US EPA, 2006).

In the absence of any subchronic or chronic toxicity study for **2,5-dichloroaniline, the CES decided to assign it the most protective TRV value calculated for the other three isomers**, i.e. the TRV for 4-chloroaniline, which is 1.2 µg/kg bw/d for the oral TRV and 4.2 µg/m3 for the respiratory TRV. These values correspond to iTVs.

- **Confidence level for the chronic TRV for 4-chloroaniline**
  - **for the oral route**

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

- **for the respiratory route**

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

- **Chronic TRVs for 2- and 3-chloroaniline**

There are no studies of chronic toxicity. The CES decided to establish chronic TRVs from the BMDLs modelled from subchronic studies and adjusted for humans, i.e. 0.39 mg/kg/d for 2-chloroaniline and 0.15 mg/kg/d for 3-chloroaniline. The TRV was calculated 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 account for toxicodynamic variability and residual uncertainties, the UF_A factor was set at 2.5 according to WHO/IPCS recommendations (WHO/IPCS, 2005) and based on ANSES practices (ANSES, 2017).
- Inter-individual variability **(UF_H): 10**. Since no scientific data are available to reduce the default value, the value of 10 was used.
- Transition from a subchronic to chronic exposure duration **(UF_S)**: **3**. The CES decided to establish chronic TRVs from subchronic studies for these two compounds by applying a UF_S of 3 for the transition from a subchronic to chronic exposure duration.

- **Confidence level for chronic TRVs for 2- and 3-chloroaniline**
  - **for the oral route**

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

- **for the respiratory route**

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

- **Chronic RVs for 2,5-dichloroaniline**

In the absence of any subchronic or chronic toxicity study for **2,5-dichloroaniline, the CES decided to assign it the most protective TRV value calculated for the other three isomers**, i.e. the TRV for 4-chloroaniline, which is 1.2 µg/kg bw/d for the oral TRV and 4.2 µg/m3 for the respiratory TRV. These values correspond to iTVs.

An iTV is a toxicological benchmark that can be used for assessing a risk. It is however an indicative value that is less robust than the TRV and therefore has a **low confidence level**.

The chronic oral and respiratory TRVs and iTVs for the three isomers of chloroaniline and 2,5-dichloroaniline are shown in Table 4.

Table 4: Chronic oral and respiratory TRVs for the three isomers of chloroaniline and 2,5-dichloroaniline

Substance	Critical effect	Critical dose	UF	TRV
ORAL ROUTE				
4-chloroaniline	5% increase in blood methaemoglobin levels in female F344 rats NTP (1998): 104-week study	$BMD_{5\%}L_{90\%}$ = 0.11 mg/kg/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED}$ = 0.03 mg/kg bw/d	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 1.2 µg/kg bw/d** **Confidence level: moderate/high**
2-chloroaniline	5% increase in blood methaemoglobin levels in male F344 rats NTP (1998): 13-week study	$BMD_{5\%}L_{90\%}$ = 1.5 mg/kg/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED}$ = 0.39 mg/kg bw/d	75 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10 UF_S = 3	**TRV = 5.2 µg/kg bw/d** **Confidence level: moderate/low**
3-chloroaniline	5% increase in blood methaemoglobin levels in male F344 rats NTP (1998): 13-week study	$BMD_{5\%}L_{90\%}$ = 0.56 mg/kg/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED}$ = 0.15 mg/kg bw/d	75 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10 UF_S = 3	**TRV = 2 µg/kg bw/d** **Confidence level: low**
2,5-dichloroaniline	TRV for 4-chloroaniline			**iTV = 1.2 µg/kg bw/d** **Confidence level: low**
RESPIRATORY ROUTE				
4-chloroaniline	5% increase in blood methaemoglobin levels in female F344 rats NTP (1998): 104-week study	$BMC_{5\%}L_{90\%}$ = 0.11 mg/kg/d Allometric adjustment $BMC_{5\%}L_{90\%\ HEC}$ = 0.03 mg/kg bw/d Route-to-route extrapolation $BMC_{5\%}L_{90\%\ HEC}$ = 0.105 mg/m³	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 4.2 µg·m^{-3}** **Confidence level: moderate/low**
2-chloroaniline	5% increase in blood methaemoglobin levels in male F344 rats NTP (1998): 13-week study	$BMC_{5\%}L_{90\%}$ = 1.5 mg/kg/d Allometric adjustment $BMC_{5\%}L_{90\%\ HEC}$ = 0.39 mg/kg bw/d Route-to-route extrapolation $BMC_{5\%}L_{90\%\ HEC}$ = 1.365 mg·m^{-3}	75 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10 UF_S = 3	**TRV = 18.2 µg·m³** **Confidence level: low**
3-chloroaniline	5% increase in blood methaemoglobin levels in male F344 rats NTP (1998): 13-week study	$BMC_{5\%}L_{90\%}$ = 0.56 mg/kg/d Allometric adjustment $BMC_{5\%}L_{90\%\ HEC}$ = 0.15 mg/kg bw/d Route-to-route extrapolation $BMC_{5\%}L_{90\%\ HEC}$ = 0.525 mg/m³	75 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10 UF_S = 3	**TRV = 7 µg·m^{-3}** **Confidence level: low**
2,5-dichloroaniline	TRV for 4-chloroaniline			**iTV = 4.2 µg·m^{-3}** **Confidence level: low**

To sum up, the **acute, subchronic and chronic oral and respiratory TRVs and iTVs were established for 2-chloroaniline, 3-chloroaniline**, **4-chloroaniline and 2,5-dichloroaniline** based on increased blood methaemoglobin levels (Tables 2 to 4). Chronic TRVs based on the tumour-inducing effects of 4-chloroaniline would have led to higher (and therefore less protective) values than those established from increased blood methaemoglobin levels.

In the absence of acute toxicity studies for **4-chloroaniline and 2,5-dichloroaniline, the CES decided to assign them the value of the most protective TRV, namely that for 3-chloroaniline (however, they are regarded as iTVs)**.

In the absence of any subchronic or chronic toxicity study for **2,5-dichloroaniline, the CES decided to assign it the value of the most protective TRV calculated for the other three isomers (regarded as iTVs)**.

Conclusion

The CES developed oral and respiratory TRVs for acute, subchronic and chronic application durations for the four isomers of chloroaniline, and did not consider it relevant to select a tracer substance for the risk from the four chloroaniline isomers.

The CES recommends applying the following values (Tables 5 and 6), i.e. using the TRV for 3-chloroaniline also for 4-chloroaniline (similar toxicity) and 2,5-dichloroaniline (no data available).

Table 5: Reference values for the oral route

		TRV for the oral route in µg/kg/d[(a)]		
Substance	**CAS number**	**acute**	**subchronic**	**chronic**
2-chloroaniline	95-51-2	73	16	5
3-chloroaniline	108-42-9	11	5	1
4-chloroaniline	106-47-8	11[(b)]	5	1
2.5-chloroaniline	95-82-9	11[(b)]	5[(b)]	1[(b)]

(a) These values are applicable to each of the substances taken individually
(b) This value corresponds to an iTV

Table 6: Reference values for the respiratory route

		TRV for the respiratory route in µg·m^{-3} [(a)]		
Substance	**CAS number**	**acute**	**subchronic**	**chronic**
2-chloroaniline	95-51-2	256	55	18
3-chloroaniline	108-42-9	39	18	4
4-chloroaniline	106-47-8	39[(b)]	18	4
2.5-chloroaniline	95-82-9	39[(b)]	18[(b)]	4[(b)]

(c) These values are applicable to each of the substances taken individually
(d) This value corresponds to an iTV

In the event of exposure to a mixture of chloroaniline isomers, the CES recommends that the principle of additivity linked to a mixture be applied (considering that chloroaniline isomers cause the same effects on the same tissues) (SCHER, 2012).

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 development of toxicity reference values for the oral and respiratory routes for isomers of chloroaniline.

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KEYWORDS

Valeur toxicologique de référence, VTR, chloroaniline, oral, inhalation, respiratoire, aiguë, subchronique, chronique

KEY WORDS

Toxicity reference value, TRV, chloroaniline, oral, inhalation, respiratory effects, acute, subchronic, chronic