ANSES Opinion
Request No 2014-SA--0110

The Director General

Maisons-Alfort, 7 October 2015

Opinion of 4 May 2015 revised in July 2015 of the French Agency for Food, Environmental and Occupational Health & Safety

on the validation of TRVs developed by the Berre petrochemical company as part of a quantitative assessment of the health risks associated with groundwater pollution in the commune of Berre l'Etang

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring 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 made public.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 7 October 2015 shall prevail.

On 11 April 2014, ANSES received a formal request from the French Directorate General of Health (DGS) to assess the establishment of toxicity reference values (TRVs) developed as part of a quantitative assessment of the health risks associated with groundwater pollution of the commune of Berre l'Etang (post code F-13130).

1. BACKGROUND AND PURPOSE OF THE REQUEST

In 2007, the residents of the district of La Molle in the commune of Berre l'Etang sent a complaint about unpleasant odours related to the water in their wells to the Provence-Alpes-Cote-d'Azur (PACA) Regional Directorate of the Environment, Land-Use Planning and Housing (DREAL). An investigation revealed widespread pollution of groundwater by pesticides. In addition, the groundwater abstracted by the residents was also polluted by hydrocarbon derivatives that could have been synthesised in the solvent manufacturing unit of the Berre petrochemical centre.

In the framework of the partial closure of this unit, the regional health agency (ARS) and the DREAL asked the operator to assess the health risks to residents arising from the presence of solvents in the groundwater. This assessment incorporated all the routes of exposure (inhalation of vapours indoors and outdoors, ingestion of garden produce, accidental ingestion of groundwater). For certain substances, however, no TRV was identified. To address this absence of values, a consultancy was commissioned to develop TRVs. The substances concerned are presented in the table below.

Cancels and replaces the Opinion of 4 May 2015; the changes made are summarised in the Annex.

Substance	CAS No.
Tert-butanol (TBA)	75-65-0
Diisobutylene (DIB)	25167-70-8
Diisopropyl ether (DIPE)	108-20-3
4-vinylcyclohexene (4-VCH)	100-40-3
Sec-butyl ether (s-BE)	6863-58-7
Ethyl acetate	141-78-9

The DGS called on ANSES to:

1. issue an opinion on the validity of the establishment of these TRVs and their conditions of use and, where applicable,
2. establish TRVs for the compounds for which the TRVs developed by the consultancy do not correspond to ANSES's methodology.

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 lies within the sphere of competence of the Expert Committee (CES) on Characterisation of substance hazards and toxicity reference values (hereafter the "CES on Substances").

In answer to the first question of this request, an analysis of the consistency between the method followed by the consultancy and that advocated by ANSES (AFSSET, 2010) was carried out by ANSES and the expert rapporteurs of the CES on Substances. The methodological and scientific aspects of the work were presented to the CES on 15 May, 12 June and 10 July 2014. The work was adopted by the CES on Substances at its meeting on 10 July 2014.

Regarding the second question of the request, the methodological and scientific aspects of the work were presented to the CES on 19 September 2014, 11 December 2014, 8 January 2015 and 12 February 2015. They were adopted by the CES on Substances at the meeting of 12 February 2015.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.
The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

- **Analysis of the consistency between the method followed by the consultancy and that advocated by ANSES**

TRVs are established according to a highly structured and rigorous approach involving collective assessments by groups of specialists (ANSES, 2010).

ANSES has published guides on the development of TRVs that detail the method used to develop TRVs. This method is recommended for establishing any new value:

- "Toxicity reference values for reprotoxic substances. Method for establishing TRVs based on toxic effects for reproduction and development" (AFSSET, 2007).

- "Toxicity reference values for carcinogenic substances. Method for establishing TRVs based on carcinogenic effects" (ANSES, 2010).

The CES thus compared the way in which the TRVs were established by the consultancy with the methods recommended by ANSES.

In response to the first question posed by the request, concerning the establishment of TRVs and their conditions of use, the CES believes that while the approach followed by the consultancy was consistent with the reference standard it chose (ECETOC, 2010), both in terms of the general approach and in the application of the method, it led to considerable methodological differences compared to ANSES's recommendations. Accordingly, not all of the TRVs proposed could be validated by ANSES in their current state.

Establishment of TRVs

As it was not possible to validate the TRVs proposed by the consultancy, ANSES commissioned the CES on Substances to propose new values for the substances of interest.

An analysis of the available data was carried out for each substance and route of exposure in order to identify the high-quality studies and assess which critical effects and critical doses could be used for formulating TRVs.
In general, the analysis of the literature data available for the substances concerned highlighted the small number of studies currently available for each of them.
For some substances, where data permitted, the decision was taken to establish several TRVs (literature data available for chronic, carcinogenic and/or reprotoxic effects).

The CES reaffirms that, concerning effects on development, it is generally accepted that a single exposure can be sufficient to induce the occurrence of the effect if exposure occurs during a critical phase of embryo-foetal development. This type of TRV is applicable for short exposure durations (from a few hours to a few days). Consequently, the exposure dose is to be used directly, without any adjustment for the duration of exposure.

The TRVs established by the CES on Substances have been summarised in the table below, incorporating:
- identification of the substance with its CAS number,
- type of TRV,
- route of exposure,
- choice of the critical effect and key study,
- details of how the TRV was calculated (critical dose),
- uncertainty factors applied,
- the final result, with an overall confidence level assigned to each TRV by taking the following criteria into account with due consideration for the strengths and weaknesses: nature and quality of the data, choice of the critical effect and mode of action, choice of the key study, choice of the critical dose.

Substance (CAS No)	Type of TRV	Route of exposure	Critical effect chosen	Critical dose	UF	TRV
Tert-butanol (TBA) (CAS 75-65-0)	Reprotoxic TRV	Oral route	Death of neonate rats shortly after birth Study Report by Lyondell Chemical Company (2004): Study of reproductive toxicity in Sprague Dawley rats exposed for 9 weeks	$BMD_{10\%}$ $L_{90\%}$ = 173 mg.kg $bw^{-1}.d^{-1}$ <u>Allometric adjustment</u> $BMD_{10\%}$ $L_{90\%\ HED}$ = 47.6 mg.kg $bw^{-1}.d^{-1}$	25 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10	**TRV = 1.9 mg.kg $bw^{-1}.d^{-1}$** **Confidence level: Moderate/High**
	Chronic TRVs	Oral route	Renal toxicity in female F344 rats Study Report of the NTP (1995): Carcinogenicity study	LOAEL = 10 mg.$kg^{-1}.d^{-1}$ <u>Allometric adjustment</u> $LOAEL_{HED}$ = 46 mg.kg $bw^{-1}.d^{-1}$	75 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10 UF_L = 3	**TRV = 0.6 mg.kg $bw^{-1}.d^{-1}$** **Confidence level: Moderate/High**
		Respiratory route		<u>Allometric adjustment</u> $LOAEL_{HED}$ = 46 mg.kg $bw^{-1}.d^{-1}$ <u>Route-to-route extrapolation</u> $LOAEC_{HED}$ = 161 $mg.m^{-3}$	75 $UF_{A\text{-}TD}$ = 2.5 UF_H = 10 UF_L = 3	**TRV = 2.1 $mg.m^{-3}$** **Confidence level: Low**
Diisobutylene (DIB) (CAS 25167-70-8)	Chronic TRVs	Oral route	Hepatic and renal toxicity (increase in the relative weight of the liver and kidneys) Huntingdon Life Science (1997a): repeated toxicity study in Sprague Dawley rats	LOAEL = 1000 mg.kg $bw^{-1}.d^{-1}$ NOAEL= 300 mg.kg $bw^{-1}.d^{-1}$ <u>Allometric adjustment</u> $NOAEL_{HED}$= 61 mg.kg $bw^{-1}.d^{-1}$	75 UF_A = 2.5 UF_H = 10 UF_S = 3	**TRV = 0.8 mg.kg $bw^{-1}.d^{-1}$** **Confidence level: Moderate/Low**
		Respiratory route		<u>Allometric adjustment</u> $NOAEL_{HED}$= 61 mg.kg $bw^{-1}.d^{-1}$ <u>Route-to-route extrapolation</u> $NOAEC_{HED}$ = 213.5 $mg.m^{-3}$	75 UF_A = 2.5 UF_H = 10 UF_S = 3	**TRV = 3 $mg.m^{-3}$** **Confidence level: Low**

Diisopropyl ether (DIPE) (CAS 108-20-3)	Chronic TRVs	Respiratory route	Hepatic toxicity Dalbey and Feuston 1996: 13-week study in Sprague Dawley rats	$BMC_{5\%}$ $L_{95\%}$ = 6052.7 mg.m^{-3} Allometric adjustment $BMC_{5\%}$ $L_{95\% HEC}$= 6052.7 mg.m^{-3} Time adjustment $BMC_{5\%}$ $L_{95\% HEC ADJ}$ = 1081.3 mg.m^{-3}	75 UF_A = 2.5 UF_H = 10 UF_S = 3	**TRV = 14.5 mg.m^{-3}** **Confidence level: Moderate**
		Oral route		Route-to-route extrapolation $BMD_{5\%}$ $L_{95\% HED ADJ}$ = 308.9 mg.m^{-3}	75 UF_A = 2.5 UF_H = 10 UF_S = 3	**TRV = 4 mg.kg bw^{-1}.d^{-1}** **Confidence level: Low**
	TRVs on development	Respiratory route	Increase in the number of rudimentary ribs Dalbey and Feuston 1996: 13-week study in Sprague Dawley rats	$BMC_{5\%}$ $L_{95\%}$ = 716.7 mg.m^{-3} Allometric adjustment $BMC_{5\%}$ $L_{95\% HEC}$= 716.7 mg.m^{-3} Time adjustment $BMC_{5\%}$ $L_{95\% HED ADJ}$ = 176 mg.m^{-3}	25 UF_A = 2.5 UF_H = 10	**TRV = 7.2 mg.m^{-3}** **Confidence level: Moderate**
		Oral route		Route-to-route extrapolation $BMD_{5\%}$ $L_{95\% HED ADJ}$ = 50.3 mg.kg bw^{-1}.d^{-1}	25 UF_A = 2.5 UF_H = 10	**TRV = 2 mg.kg bw^{-1}.d^{-1}** **Confidence level: Low**
	Carcinogenic TRVs	Oral route	Neoplasms of the lymphoreticular system (female) Belpoggi *et al.,* 2002: carcinogenicity study in Sprague Dawley rats	From data in animals $BMD_{10\%}$ $L_{95\%}$ = 114 mg.kg bw^{-1}.d^{-1} Time adjustment $BMD_{10\%}$ $L_{95\% ADJ}$ = 49 mg.kg.bw^{-1}.d^{-1} Allometric adjustment $BMD_{10\%}$ $L_{95\% ADJ HED}$ = 13 mg.kg bw^{-1}.d^{-1}		After linear extrapolation to the origin **ERU = 7.8.10^{-6} (µg.kg bw^{-1}.d^{-1})$^{-1}$** 13 µg.kg bw^{-1}.d^{-1} for a risk of 10^{-4} 1.3 µg.kg bw^{-1}.d^{-1} for a risk of 10^{-5} 0.13 µg.kg bw^{-1}.d^{-1} for a risk of 10^{-6}

						Confidence level: Low
		Respiratory route		From data in animals $BMD_{10\%}$ $L_{95\%}$ = 114 mg.kg $bw^{-1}.d^{-1}$ Time adjustment $BMD_{10\%}$ $L_{95\% ADJ}$ = 49 mg.kg.$bw^{-1}.d^{-1}$ Allometric adjustment $BMD_{10\%}$ $L_{95\% ADJ HED}$ = 13 mg.kg $bw^{-1}.d^{-1}$ Route-to-route extrapolation $BMC_{10\%}$ $L_{95\% ADJ HED}$ = 45 $mg.m^{-3}$		After linear extrapolation to the origin **ERU = $2.2.10^{-6}$ ($\mu g.m^{-3}$)$^{-1}$** 45 $\mu g.m^{-3}$ for a risk of 10^{-4} 4.5 $\mu g.m^{-3}$ for a risk of 10^{-5} 0.45 $\mu g.m^{-3}$ for a risk of 10^{-6} **Confidence level: Low**
4-vinylcyclohexene (4-VCH) (CAS 100-40-3)	Chronic TRVs	Respiratory route	Increased ovarian atrophy Bevan *et al*. (1996): 13-week study in mice	LOAEC = 4500 $mg.m^{-3}$ NOAEC = 1125 $mg.m^{-3}$ Allometric adjustment $NOAEC_{HED}$ = 1125 $mg.m^{-3}$ Time adjustment $NOAEC_{HED ADJ}$ = 202 $mg.m^{-3}$	75 UF_{A-TD} = 2.5 UF_H = 10 UF_S = 3	**TRV = 2.7 $mg.m^{-3}$** **Confidence level: Moderate/High**
		Oral route	Decrease in the number of ovarian follicles Grizzle *et al*. (1994): two-generation developmental study in CD-1 mice	LOAEL = 500 mg.kg $bw^{-1}.d^{-1}$ Allometric adjustment $LOAEL_{HED}$ = 72 mg.kg $bw^{-1}.d^{-1}$	225 UF_A = 2.5 UF_H = 10 UF_L = 3 UF_S = 3	**TRV = 0.32 mg.kg.$bw^{-1}.d^{-1}$** **Confidence level: Moderate/High**
	Carcinogenic TRVs	Oral route	Ovarian granulosa cell tumours and/or ovarian carcinomas NTP (1996) published by Collins *et al.*, 1988: Carcinogenicity study in B6C3F1 mice	From data in animals $BMD_{10\%}$ $L_{95\%}$ = 73 mg.kg $bw^{-1}.d^{-1}$ Time adjustment $BMD_{10\%}$ $L_{95\% ADJ}$ = 52 mg.kg.$bw^{-1}.d^{-1}$ Allometric adjustment $BMD_{10\%}$ $L_{95\% ADJ HED}$ = 7.5 mg.kg $bw^{-1}.d^{-1}$		After linear extrapolation to the origin **ERU = $1.3.10^{-5}$ (µg.kg $bw^{-1}.d^{-1}$)$^{-1}$** 7.5 µg.kg $bw^{-1}.d^{-1}$ for a risk of 10^{-4} 0.75 µg.kg $bw^{-1}.d^{-1}$ for a risk of 10^{-5} 0.075 µg.kg $bw^{-1}.d^{-1}$ for a risk of 10^{-6} **Confidence level:**

						Moderate/High
		Respiratory route		$BMD_{10\%}L_{95\%\ ADJ\ HED}$ = 7.5 mg.kg $bw^{-1}.d^{-1}$ Route-to-route extrapolation $BMC_{10\%}L_{95\%\ ADJ\ HED}$ = 26 $mg.m^{-3}$		After linear extrapolation to the origin **ERU = 3.8.10^{-6} (µg.m^{-3})$^{-1}$** 26 µg.m^{-3} for a risk of 10^{-4} 2.6 µg.m^{-3} for a risk of 10^{-5} 0.26 µg.m^{-3} for a risk of 10^{-6} **Confidence level: Low**
Sec butyl ether (CAS 6863-58-7)	No TRV due to lack of data					
Ethyl acetate (CAS 141-78-9)	Chronic TRV	Respiratory route	Effect on neurotoxicity (decrease in female motor activity) Christoph *et al*. (2003): 13-week study in Sprague Dawley rats	NOAEC = 750 ppm Allometric adjustment $NOAEC_{HED}$ = 750 ppm Time adjustment $NOAEC_{HED\ ADJ}$ = 134 ppm	75 UF_{A-TD} = 2.5 UF_H = 10 UF_S = 3	**TRV = 6.4 mg.m^{-3}** **Confidence level: Moderate/High**

Key:

BMD/C	Benchmark dose/concentration
BMDxLy	Lower limit of the confidence interval at y% of the benchmark dose associated with x%
ERU	Excess Risk per Unit
HED/C	Human Equivalent Dose/Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOAEC	Lowest Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOAEC	No Observed Adverse Effect Concentration
UF	Uncertainty factor
UF_A	Inter-species uncertainty factor
UF_{A-TD}	Toxicodynamic component of the inter-species uncertainty factor
UF_H	Inter-individual uncertainty factor
UF_L	Uncertainty factor related to the use of a LOAEL
UF_S	Uncertainty factor related to sub-chronic to chronic transposition
TRV	Toxicity Reference Value

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on Characterisation of substance hazards and toxicity reference values, relating to the examination of the establishment of TRVs and their conditions of use and on the establishment of the following TRVs:

Substance (CAS No)	Type of TRV	Value of the TRV
Tert-butanol (TBA) (CAS 75-65-0)	Reprotoxic TRV (acute) by the oral route	**1.9 mg.kg bw^{-1}.d^{-1}**
	Chronic TRV by the oral route	**0.6 mg.kg bw^{-1}.d^{-1}**
	Chronic TRV by the respiratory route	**2.1 mg.m^{-3}**
Diisobutylene (DIB) (CAS 25167-70-8)	Chronic TRV by the oral route	**0.8 mg.kg bw^{-1}.d^{-1}**
	Chronic TRV by the respiratory route	**3 mg.m^{-3}**
Diisopropyl ether (DIPE) (CAS 108-20-3)	Chronic TRV by the oral route	**4 mg.kg bw^{-1}.d^{-1}**
	Chronic TRV by the respiratory route	**14.5 mg.m^{-3}**
	TRV on development (acute) by the oral route	**2 mg.kg bw^{-1}.d^{-1}**
	TRV on development (acute) by the respiratory route	**7.2 mg.m^{-3}**
	Carcinogenic TRV by the oral route	**7.8.10^{-6} (µg.kg bw^{-1}.d^{-1})$^{-1}$**
	Carcinogenic TRV by the respiratory route	**2.2.10^{-6} (µg.m^{-3})$^{-1}$**
4-vinylcyclohexene (4-VCH) (CAS 100-40-3)	Chronic TRV by the oral route	**0.32 mg.kg bw^{-1}.d^{-1}**
	Chronic TRV by the respiratory route	**2.7 mg.m^{-3}**
	Carcinogenic TRV by the oral route	**1.3.10^{-5} (µg.kg bw^{-1}.d^{-1})$^{-1}$**
	Carcinogenic TRV by the respiratory route	**3.8.10^{-6} (µg.m^{-3})$^{-1}$**
Sec-butyl ether (s-BE) (CAS 6863-58-7)	**Absence of TRV** (due to lack of data)	
Ethyl acetate (CAS 141-78-9)	Carcinogenic TRV by the respiratory route	**6.4 mg.m^{-3}**

The French Agency for Food, Environmental and Occupational Health & Safety recommends that steps be taken to acquire more data in order to develop a TRV for sec-butyl ether.

Marc Mortureux

KEYWORDS

TRV, toxicity reference value, tert-butanol, diisobutylene, diisopropyl ether, 4-vinylcyclohexene, sec-butyl ether, ethyl acetate.

ANNEX

Changes made in the revised opinion:

1- **Diisopropyl ether (DIPE) (CAS 108-20-3)**

Carcinogen TRV by the oral route, ERU=7.8 10^{-6} (µg.kg $bw^{-1}.d^{-1})^{-1}$

Carcinogen TRV by the respiratory route, ERU=2.2 10^{-6} $(\mu g.m^{-3})^{-1}$

2- **4-vinylcyclohexene (4 VCH) (CAS 100-40-3)**

Carcinogen TRV by the oral route, ERU=1.3 10^{-5} (µg.kg $bw^{-1}.d^{-1})^{-1}$

Carcinogen TRV by the respiratory route, ERU=3.8 10^{-6} $(\mu g.m^{-3})^{-1}$