ANSES Opinion
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The Director General

Maisons-Alfort, 10 October 2024

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

on the establishment of long-term oral TRVs for 2,3-dinitrotoluene (CAS No 602-01-7), 2,4-dinitrotoluene (CAS No 121-14-2), 2,5-dinitrotoluene (CAS No 619-15-8), 2,6-dinitrotoluene (CAS No 606-20-2), 3,4-dinitrotoluene (CAS No 610-39-9) and 3,5-dinitrotoluene (CAS No 618-85-9)

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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 all necessary information concerning these risks as well as the requisite expertise and scientific 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 10 October 2024 shall prevail.

As part of the memorandum of understanding drawn up in December 2022 between ANSES, the Directorate General for Health (DGS) and the Directorate General for Risk Prevention (DGPR) for the implementation of the scientific expert appraisal work programme on toxicity reference values (TRVs), it was agreed to carry out the expert appraisal work necessary for the establishment of long-term oral TRVs for dinitrotoluene.

1. BACKGROUND AND PURPOSE OF THE REQUEST

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 chemical agent and the occurrence of an adverse health effect. By definition, TRVs are intended to protect the entire population, including susceptible population groups such as children, from the effects of a substance following short-, medium- or long-term exposure.

TRVs are specific to a chemical agent and to a route (oral, respiratory, dermal) and duration (short, medium or long term) of exposure. TRVs are therefore established for:

- short-term exposure, from one to 14 days;
- medium-term exposure, from 15 to 364 days;
- long-term exposure, for more than 365 days.

They can be used as part of quantitative health risk assessments (QHRAs) carried out at population level, exclusively in a given exposure context, and thus help in the choice of risk management measures. They can also be used to establish guidance values or maximum regulatory levels in food. Lastly, they can serve to prioritise chemical agents according to the hazard level they represent, in which case they often enable the toxicity of such agents to be assessed (ANSES, pending publication).

The way TRVs are established differs depending on the knowledge acquired 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. For carcinogenic effects, threshold or no-threshold TRVs can be established depending on the mode of action of the chemical agent studied (ANSES, pending publication).

In practice, establishing a TRV involves the following steps:

- identifying the target organ(s) and critical effect based on the toxicological profile;
- identifying the assumption on which it is based: with or without a threshold dose, depending on the substance's mode of action;
- undertaking a critical analysis of each of the TRVs identified to determine whether one of them can be used (choice). This analysis takes account of various analytical criteria described in ANSES's methodological guide (ANSES, pending publication), such as transparency and arguments, the various choices made when establishing the TRV (choice of critical effect, key study and point of departure (PoD), use of time and allometric adjustments, choice of uncertainty factors for threshold-dose effects or the low-dose extrapolation method for TRVs without a threshold dose), and the year in which the TRV was established or revised. When none of the available TRVs are deemed relevant, work is undertaken to establish a new TRV;
- selecting one or more key studies of good scientific quality and greatest relevance from among the epidemiological or toxicological studies available, in order to establish a dose-response relationship;
- defining a PoD for humans or animals based on this study/these studies;
- applying time and allometric adjustments if necessary;
- for a threshold TRV, applying uncertainty factors (UFs) to this PoD so as to derive a reference value (RV) that is applicable to the entire population;
- for a no-threshold TRV, determining the slope and/or concentrations/doses associated with several different risk levels;
- setting a confidence level.

The DGS entrusted ANSES's Nancy Laboratory for Hydrology (LHN) with the implementation of a national exploratory campaign in raw and treated water between 2020 and 2022 in order

to characterise the presence of pesticides, pesticide metabolites, explosives residues and 1,4-dioxane at national level. The initial results showed concentrations above the limits of quantification for several isomers of dinitrotoluene (DNT), in particular 2,3-, 2,6- and 3,4-DNT. Lower levels of 2,4-DNT were also found, albeit more frequently.

In this context, the DGS issued a formal request to ANSES on 17 November 2022, asking it to:

- assess the health risks associated with the presence of these DNT derivatives in drinking water (DW);
- determine a health-based guidance value for drinking water (DW HBGV).

Therefore, the aim of this expert appraisal was to propose long-term TRVs for the six isomers of DNT and for total DNT, so as to derive a DW HBGV.

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 on Health reference values (HRV Committee). ANSES entrusted the expert appraisal to several rapporteurs. The methodological and scientific aspects of the work were presented to the HRV Committee between 14 March and 27 June 2024. The work was adopted by the HRV Committee at its meeting on 27 June 2024.

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 following website: https://dpi.sante.gouv.fr/.

3. ANALYSIS AND CONCLUSIONS OF THE HRV COMMITTEE

To establish TRVs, ANSES refers to its guide for developing and choosing reference values (ANSES, pending publication). First, the data required to characterise these substances were collected (identification, physico-chemical properties, classifications), general information was gathered on uses, sources and exposure, and the experts identified the oral TRVs previously established by health & safety organisations recognised at supranational, European or national/regional level (see Chapter 2 of the report). A toxicological profile was drawn up to define the effects, observed in humans and animals, associated with various types of exposure to the different DNT isomers.

3.1. Summary of the toxicological data

The summary of oral toxicity data for DNT isomers was based on summary reports produced by internationally recognised organisations (ATSDR 2016; US EPA 2013; IARC 1996),

supplemented by a literature review performed by querying the PubMed and Scopus databases up to April 2024 (no starting date was defined).

3.1.1. Toxicokinetics

Digestive absorption of 2,4-DNT and 2,6-DNT is rapid and relatively complete in rats (Rickert *et al.* 1983). According to studies in rats, rabbits, dogs and monkeys, it is between 55% and 90% and occurs mainly during the first 24 hours after oral administration (Long and Rickert 1982; Rickert *et al.* 1981; Lee *et al.* 1978).

A study in rats showed that, after oral administration, peak plasma, erythrocyte, hepatic and renal concentrations of 2,4-DNT and its metabolites were proportional to the dose of 2,4-DNT administered orally (Rickert *et al.* 1980). Concentrations in the liver and kidneys were five to 10 times higher than those in plasma and erythrocytes. They were lower than plasma concentrations in other tissues.

DNT is primarily metabolised in the liver and intestines (via the microbial flora) (Long and Rickert 1982; Rickert *et al.* 1981).

Studies in rats have shown that 2,4-DNT and 2,6-DNT are excreted mainly in urine, with urinary excretion levels ranging from 55% to 90% (Long and Rickert 1982; Rickert *et al.* 1981; Medinsky and Dent 1983).

3.1.2. Acute toxicity

The experts did not identify any studies in humans assessing the acute effects of DNT isomers following short-term oral exposure.

The study by Lent *et al*. found that all DNT isomers were associated with haematotoxic effects in Sprague-Dawley rats, following 14 days of oral exposure. Decreased red blood cell counts were observed in animals exposed to 2,4-DNT (LOAEL[1]: 142 mg·kg bw^{-1}·day^{-1}), 2,5-DNT (LOAEL: 77 mg·kg bw^{-1}·day^{-1}), 2,6-DNT (LOAEL: 14 mg·kg bw^{-1}·day^{-1}) and 3,4-DNT (LOAEL: 14 mg·kg bw^{-1}·day^{-1}) (Lent *et al*. 2012). Extramedullary haematopoiesis was found following exposure to all the isomers, with LOAELs of 275 mg·kg bw^{-1}·day^{-1} for 2,3-DNT, 36 mg·kg bw^{-1}·day^{-1} for 2,4-DNT, 39 mg·kg bw^{-1}·day^{-1} for 2,5-DNT, 68 mg·kg bw^{-1}·day^{-1} for 2,6-DNT, 57 mg·kg bw^{-1}·day^{-1} for 3,4-DNT and 77 mg·kg bw^{-1}·day^{-1} for 3,5-DNT. At higher doses, the authors also observed hepatotoxic effects (relative increase in liver weight for 2,3- and 3,4-DNT and onset of neoplastic lesions for 2,4- and 2,6-DNT), reprotoxic effects (increase in relative testes weight and onset of non-neoplastic lesions for 2,3-, 3,4-, 2,4- and 2,6-DNT), muscular impairment (2,4-, 3,4- and 2,5-DNT), nephrotoxicity (increase in relative kidney weight, onset of non-neoplastic lesions for 2,3-, 2,4-, 2,6- and 3,4-DNT), and neurotoxicity and cardiac lesions (2,5- and 3,5-DNT).

3.1.3. Subchronic and chronic toxicity

In humans, only one early study reported various symptoms in workers exposed to 2,4-DNT for 12 months; these included fatigue, headaches, loss of appetite, dizziness, nausea, insomnia, paraesthesia, vomiting, cyanosis and anaemia. However, as concomitant exposure to other

[1] Lowest Observed Adverse Effect Level

compounds could not be ruled out, these symptoms could not be attributed to 2,4-DNT with certainty (McGee *et al*. 1942).

In animals, data concerning the subchronic and chronic toxicity of DNT were only identified for 2,4- and 2,6-DNT. Most of these data came from a series of studies in mice, rats and dogs conducted for the US Army (Lee *et al*. 1976; 1978; Ellis *et al*. 1979). They primarily involved haematological, hepatic and neurological effects.

- 2,4-DNT

Following medium-term exposure, haematological effects (anaemia, reticulocytosis, haemosiderosis, Heinz bodies) have been observed in mice (LOAEL: 468 mg·kg bw^{-1}·day^{-1}), rats (LOAEL: 93 mg·kg bw^{-1}·day^{-1}) and dogs (LOAEL: 25 mg·kg bw^{-1}·day^{-1}) (Ellis *et al*. 1979). Methaemoglobinaemia has also been observed in rats (LOAEL: 371 mg·kg bw^{-1}·day^{-1}) (Kozuka *et al*. 1979). Following long-term exposure, these effects, as well as a reduction in red blood cell counts, have also been observed in mice, rats and dogs, with LOAELs of 898, 3.9 and 0.2 mg.kg bw^{-1}.day^{-1} respectively.

An increase in various liver parameters (aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, triglycerides) was observed in rats exposed to 2,4-DNT for six months (LOAEL: 371 mg·kg bw^{-1}·day^{-1}) (Kozuka *et al*. 1979). Hepatocellular degeneration, vacuolisation and cellular alterations were associated with long-term exposure to this substance in rats (LOAEL: 27 mg·kg bw^{-1}·day^{-1}) (Leonard *et al*. 1987). Hepatocellular dysplasia was observed in mice exposed for 90 days (mild dysplasia) or two years (LOAEL: 14 mg·kg bw^{-1}·day^{-1}) (Hong *et al*. 1985), while biliary hyperplasia was reported in dogs exposed for two years (LOAEL: 10 mg·kg bw^{-1}·day^{-1}) (Ellis *et al*. 1985).

Several studies have highlighted neurotoxic effects on the control and coordination of the hind legs in rats exposed for 91 days (LOAEL: 93 mg·kg bw^{-1}·day^{-1}) or six months (Kozuka *et al*. 1979; Lee *et al*. 1985), mice exposed for two years (LOAEL: 898 mg·kg bw^{-1}·day^{-1}) (Hong *et al*. 1985) and dogs exposed for 91 days (LOAEL: 25 mg·kg bw^{-1}·day^{-1}) (Ellis *et al*. 1985). Convulsions have also been reported in rats (Kozuka *et al*. 1979; Ellis *et al*. 1985).

- 2,6-DNT

The US Army conducted tests with groups of eight Beagle dogs, which were given daily doses of 0, 4, 20 or 100 mg·kg bw^{-1}·day^{-1} of 2,6-DNT for 13 weeks (Lee *et al.* 1976). Six animals from the most exposed group died before the end of the eighth week, and treatment was discontinued for the other two groups after four weeks, following the onset of serious clinical signs. Extramedullary erythropoiesis was observed from the lowest dose. From 20 mg·kg bw^{-1}·day^{-1}, hepatic (biliary tract hyperplasia, degenerative and inflammatory changes), renal (dilated tubules, degenerative foci) and neurological (incoordination, loss of balance) effects were observed.

Splenic haemosiderosis and extramedullary haematopoiesis have been reported in rats. Extramedullary haematopoiesis has also been associated with 2,6-DNT in exposed mice (Lee *et al.* 1976).

Groups of male Sprague-Dawley rats were exposed to 2,6-DNT by gavage for 29 days (Rothfuss *et al.* 2010). The following hepatic effects were observed in the animals in the most exposed group (33 mg·kg bw^{-1}·day^{-1}): diffuse hepatocellular hypertrophy, hepatocellular vacuolisation, single-cell necrosis and bile duct hyperplasia.

3.1.4. Reprotoxicity and developmental toxicity

All six DNT isomers are classified as Category 2 reprotoxic substances (H361f: Suspected of damaging fertility) in Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (known as the CLP Regulation).

- Reprotoxicity

Exposure to 2,4-DNT has led to reduced fertility and damage to the seminiferous tubules of the testes in male rats (LOAEL: 34.5 mg·kg bw^{-1}·day^{-1}), mice (LOAEL: 14 mg·kg bw^{-1}·day^{-1}) and dogs (LOAEL: 25 mg·kg bw^{-1}·day^{-1}) (Lee *et al*. 1978; Ellis *et al*. 1979). In female rats, a decrease in fertility and difficulties with parturition have been observed (LOAEL: 45.3 mg·kg bw^{-1}·day^{-1}). Ovarian atrophy and follicular dysfunction have been highlighted in mice (LOAEL: 898 mg·kg bw^{-1}·day^{-1}).

Although fewer data are available for 2,6-DNT, those in the literature have shown testicular damage in rats (LOAEL: 35 mg·kg bw^{-1}·day^{-1}) and dogs (LOAEL: 20 mg·kg bw^{-1}·day^{-1}) and a decrease in spermatogenesis in mice (LOAEL: 51 mg·kg bw^{-1}·day^{-1}) and rats following short- and medium-term exposure (Lee *et al*. 1976).

In male rats exposed to 3,5-DNT for 14 days, a significant decrease in testes size and weight, degeneration of the seminiferous tubules and multinucleated giant cell formation in the testes were observed (LOAEL: 19 mg·kg bw^{-1}·day^{-1}) (Lent *et al*. 2012).

- Developmental toxicity

In a three-generation study in CD rats exposed to 2,4-DNT (Ellis *et al.* 1979), no anomalies were observed in the offspring, regardless of the generation. The decrease in viability observed in the offspring of the most exposed mothers (45.3 mg·kg bw^{-1}·day^{-1}) was the result of a high incidence of maternal death during birth and maternal neglect.

In a teratogenicity study in pregnant F344 rats exposed by gavage to technical-grade DNT (Tg-DNT) at doses ranging from 14 to 150 mg·kg bw^{-1}·day^{-1} (Price *et al.* 1985), no significant effects on foetal growth or development were observed. A slight change in haematological parameters as well as changes in the weight of certain organs, such as the spleen and liver, were observed in the foetuses of exposed mothers, although no dose-response relationship was found.

3.1.5. Genotoxicity

All six DNT isomers are classified as Category 2 mutagenic substances (H341: Suspected of causing genetic defects) in the CLP Regulation.

A study in workers, for whom inhalation was most likely not the primary route of exposure, showed a significant increase in chromosomal aberrations (deletions, translocations, breaks), suggesting a clastogenic effect in individuals exposed to DNT and TNT (2,4,6-trinitrotoluene) (Sabbioni *et al.* 2006).

Several *in vivo* genotoxicity studies have been carried out for the various isomers of DNT (comet assay, micronucleus test, UDS[2] assay, spot test). None of them showed any genotoxic

[2] Unscheduled DNA synthesis

effects for 2,3-, 2,5- or 3,5-DNT. Although not all the data on 2,4-DNT found it to be genotoxic, the majority of them did. The studies identified support the genotoxicity of 2,6-DNT.

In vitro, chromosomal aberrations have been observed in pulmonary fibroblasts of Chinese hamsters exposed to 2,6-DNT (Suzuki *et al.* 2011), as well as in human lymphocytes exposed to 2,4-DNT (Huang *et al.* 1996). A comet assay of rat Sertoli cells exposed to 2,4- or 2,6-DNT revealed DNA damage (Yang *et al.* 2005). Studies focusing on 2,3-, 2,5-, 3,5- and Tg-DNT did not show any genotoxicity (Abernethy and Couch 1982; Styles and Cross 1983).

Gene mutation tests have been undertaken for all six DNT isomers and for Tg-DNT. Their results were not all consistent and, from one study to the next, the variability of the results was probably due to differences in strain sensitivity and in the need for metabolic activation.

3.1.6. Carcinogenicity

All six DNT isomers are classified as Category 1B carcinogens (H350: May cause cancer) in the CLP Regulation.

A cohort study including 421 workers, whose exposure was not quantified, did not show any relationship between exposure to 2,4-DNT or Tg-DNT and the onset of cancer (reduced number of cancer cases) (Levine *et al.* 1986). In another retrospective cohort study with 4102 men who had worked at a munitions and rocket motor production facility, an association was observed between exposure to DNT and the risk of developing liver and biliary tract cancer (Stayner *et al.* 1993). A significant increase in mortality from hepatobiliary cancer was reported for exposed versus unexposed workers. The authors considered that exposure had mainly occurred through the skin and by inhalation. However, the interpretation of the results is limited by the small number of cancer cases noted (six), the relatively short period of exposure (less than four months for half of the cases), and likely exposure to other substances. In miners having handled explosive sticks containing Tg-DNT, most of whom were exposed for at least 20 years, six cases of urothelial cancer and 14 cases of kidney cancer were identified, corresponding to risks 4.5 and 14.3 times higher than those from cancer registries (Brüning *et al.* 1999; 2001; 2002). The exposure of the workers was assessed retrospectively by interviewing the individuals in question, who were then divided into four exposure categories (low, medium, high and very high). However, this did not demonstrate any dose-response relationship. All the individuals with urothelial cancer were slow acetylators. An increase in the risk of lung cancer was found in an epidemiological cohort study with 16,441 miners (Seidler *et al.* 2014). Although an increased risk of kidney and bladder cancer was observed in the individuals exposed over the longest period, this increase was not significant. A classification bias may have caused the actual risk to be underestimated.

Various studies in rats and mice have observed the development of kidney tumours, hepatocellular dysplasia, neoplastic liver nodules, hepatocellular carcinoma, skin tumours (males: subcutaneous mesenchymal and epithelial tumours, fibroma, lipoma, basal cell carcinoma, sarcoma, fibrosarcoma, carcinosarcoma and squamous cell carcinoma) and mammary gland tumours (females: adenoma, papilloma, fibroadenoma, fibroma, adenocarcinoma, carcinoma) in association with exposure to 2,4-DNT (Ellis *et al*. 1979; 1985; Lee *et al*. 1985).

One study highlighted an association between long-term exposure to 2,6-DNT and the development of neoplastic liver nodules and hepatocellular carcinoma (Leonard *et al*. 1987).

3.1.7. Susceptible population groups

People with an erythrocyte enzyme deficiency, in particular glucose-6-phosphate dehydrogenase deficiency, which is widespread in the Mediterranean basin, appear to be more susceptible to the haemolysing effects of DNT.

3.2. Proposed long-term threshold oral TRV

3.2.1. 2,4-DNT

- Choice of the critical effect

The analysis of the chronic studies investigating the toxicity of 2,4-DNT primarily identified haematotoxic and hepatotoxic effects. These effects occurred in various exposed species (rats, mice and dogs) and highlighted the haemolysing and methaemoglobinising nature of 2,4-DNT. A drop in red blood cell counts is one of the first signs of haemolysis. The observed effects on the liver, in particular biliary hyperplasia, may occur as a result of this (Ellis *et al.* 1979).

The HRV Committee therefore selected a decrease in red blood cell counts as the critical effect.

- Choice of assumption for establishment of the TRV

For most non-carcinogenic effects, it is considered, on the basis of current knowledge and by default, that toxicity is only expressed above a threshold dose. **The HRV Committee therefore considered that the critical effect resulted from a threshold dose mechanism.**

- Analysis of the existing TRVs

Two long-term oral TRVs are available: an RfD[3] of 0.002 mg·kg bw^{-1}·day^{-1} established by the United States Environmental Protection Agency (US EPA)[4] in 2008 and an MRL[5] of 0.001 mg·kg bw^{-1}·day^{-1} set by ATSDR[6] in 2016. The RfD proposed by the US EPA is based on various critical effects (neurotoxicity, presence of Heinz bodies and biliary tract hyperplasia) and was derived from a NOAEL[7], whereas it is preferable to refer to a benchmark dose (BMD) whenever possible (ANSES, pending publication). Although the ATSDR value is based on the critical effect selected by the HRV Committee and was derived from a BMDL (see below), no allometric adjustment had been performed and the BMD had not been modelled using model averaging, as currently recommended by ANSES.

As a consequence, given these limitations, the HRV Committee did not retain the existing TRVs for 2,4-DNT and proposes establishing a long-term oral TRV.

- Establishing the TRV
    - Choice of the key study

[3] Reference dose
[4] US Environmental Protection Agency
[5] Minimal Risk Level
[6] Agency for Toxic Substances and Disease Registry
[7] No Observed Adverse Effect Level

The study by Ellis *et al.* from 1979, deemed of good quality (Klimisch 1), tested relatively low doses in Beagle dogs compared with other studies undertaken in rats or mice (Hong *et al*. 1985; Lee *et al*. 1985). The study focusing on females exposed for one year was chosen with the aim of using data based on a constant number of subjects in the various groups (n = 6). Studies conducted in males or over a two-year period compared smaller numbers of subjects in the most exposed group, in particular due to the higher level of mortality within this group.

The HRV Committee chose the study by Ellis *et al.* (1979) as the key study.

- Choice of the point of departure

The data from the Ellis *et al.* study demonstrate a dose-response relationship between the drop in red blood cell counts and long-term exposure to 2,4-DNT. This was modelled using Bayesian BMD software (BBMD, version 0.0.0.9077) available on the EFSA[8] website for the establishment of a BMD using Bayesian model averaging to estimate the BMDL.

In the case of a continuous variable (measurement of a biological variable), the benchmark response[9] (BMR) is chosen based on a tiered approach recommended by EFSA (EFSA, 2022). In the absence of a pre-established BMR, the experts chose a protective default value of 5%, considering that, while this decrease can be considered small for an individual, it may be significant at population level.

Table 1 shows the BMD obtained for the critical effect and its 90% credible interval (BMDL-BMDU) using the model averaging method.

Table 1: Modelled BMD and its 90% credible interval based on the data of Ellis *et al.* (1979), indicating a decrease in red blood cell counts in female Beagle dogs exposed to 2,4-DNT for 12 months

BMDL	BMD	BMDU
0.129 mg·kg bw^{-1}·day^{-1}	0.243 mg·kg bw^{-1}·day^{-1}	0.926 mg·kg bw^{-1}·day^{-1}

BMDL: lower bound of the credible interval for the benchmark dose; BMDU: upper bound of the credible interval for the benchmark dose

The validation criteria (BMD > one-tenth of the lowest exposure dose; BMD/BMDL < 20; BMDU/BMDL < 50) are all met. **The HRV Committee therefore selected a $BMDL_5$ of 0.129 mg·kg bw^{-1}·day^{-1} as the point of departure.**

- Allometric adjustment

An allometric adjustment was performed to reduce the uncertainty regarding interspecies variability. A human equivalent dose (HED) was calculated, using the following equation[10]:

$$\text{Equivalent dose}_{\text{Humans}} = \text{Dose}_{\text{animals}} \times \left(\frac{\text{Weight}_{\text{animals}}}{\text{Weight}_{\text{Humans}}}\right)^{1/4}$$

The average weight of the female dogs was 8.99 kg at the end of the study (Ellis *et al.* 1979). The average human weight used for the calculation was 70 kg.

[8] European Food Safety Authority
[9] Maximum change in the response level considered as being physiological (or non-harmful) for the studied effect.
[10] This equation is taken from the recommendations of the US EPA (US EPA, 2006).

i.e. **$BMDL_{HED}$ = 0.077 mg·kg bw^{-1}·day^{-1}.**

- Choice of uncertainty factors

The TRV was calculated from the $BMDL_{HED}$ using the following uncertainty factors (UFs) (ANSES, pending publication):

- inter-species variability (UF_A): 2.5, to account for toxicodynamic variability and residual uncertainties;
- inter-individual variability (UF_H): 10, to take account of the fraction of the population that is highly susceptible to the effects associated with haemolysis (glucose-6-phosphate dehydrogenase deficiency, widespread in the Mediterranean basin);
- subchronic to chronic transposition (UF_S): 1, as the key study was a one-year chronic study[11];
- use of a point of departure (UF_L): 1, as the PoD is a BMDL;
- inadequacy of the data (UF_D): 1, as several chronic studies focusing on the effects of 2,4-DNT are available, as are reprotoxicity and developmental toxicity data.

An overall uncertainty factor of 25 was thus used to establish the TRV.

- Proposed long-term threshold oral TRV and confidence level

A long-term threshold TRV was calculated based on the ratio between the PoD and the overall UF.

TRV = 0.003 mg·kg bw^{-1}·day^{-1}

The overall confidence level for this TRV was estimated at 3.2/5; this is a **moderate confidence level**.

3.2.2. 2,6-DNT

- Choice of the critical effect

The analysis of studies investigating the toxicity of 2,6-DNT primarily highlighted its medium- and long-term haematotoxic and hepatotoxic effects in Beagle dogs, Swiss albino mice and CD rats (Lee *et al*. 1976).

The HRV Committee therefore selected extramedullary haematopoiesis[12] as the critical effect, as it occurs at the lowest doses.

[11] The Organisation for Economic Co-operation and Development (OECD) recommends considering studies carried out over at least one year as chronic studies (OECD 2014).
[12] Extramedullary haematopoiesis is the proliferation of haematopoietic tissue outside the bone marrow. This process is generally a response to various haematological disorders, in particular haemolytic anaemia. Extramedullary haematopoiesis mainly occurs in haematopoietic tissue such as the liver and spleen during embryonic development.

- Choice of assumption for establishment of the TRV

For most non-carcinogenic effects, it is considered, on the basis of current knowledge and by default, that toxicity is only expressed above a threshold dose. **The HRV Committee therefore considered that the critical effect resulted from a threshold dose mechanism.**

- Analysis of the existing TRVs

Two long-term oral TRVs are available: an RfD of 0.001 mg·kg bw^{-1}·day^{-1} established by the US EPA in 2008 and a provisional RfD of 0.0003 mg·kg bw^{-1}·day^{-1} set by the US EPA in 2013. The first RfD was based on various critical effects (neurotoxicity, presence of Heinz bodies, biliary tract hyperplasia, renal and hepatic lesions) and an overall uncertainty factor of 3000 was added to the point of departure, a NOAEL of 4 mg·kg bw^{-1}·day^{-1}. The value from 2013 was based on the chosen critical effect and was derived from a $NOAEL_{HED}$ of 3 mg·kg bw^{-1}·day^{-1}, to which an overall uncertainty factor of 10,000 was applied. Very high uncertainty factors were used to derive these two values. A UF_D of 10 was assigned, despite the fact that several studies have investigated and observed various toxicological effects. A UF_S of 10 was applied to account for subchronic exposure in the key study even though the duration of the study (13 weeks) was deemed satisfactory with regard to the chosen critical effect.

As a consequence, given these limitations, the HRV Committee did not retain the existing TRVs and proposes establishing a long-term oral TRV for 2,6-DNT.

- Establishing the TRV

  - Choice of the key study

The study by Lee *et al.*, deemed of good quality (Klimisch 1), is the only available repeated-dose toxicity study (13 weeks) that investigated the various toxic effects of 2,6-DNT (Lee *et al.* 1976). The study by Leonard *et al*. was undertaken in rats for one year, but only hepatic effects were investigated (Leonard *et al*. 1987). The study by Lee *et al*. found 2,6-DNT to have haematotoxic effects from the lowest tested dose of 4 mg·kg bw^{-1}·day^{-1} in Beagle dogs, whereas these occurred at higher doses in SD rats (37 mg·kg bw^{-1}·day^{-1}) and Swiss albino mice (51 mg·kg bw^{-1}·day^{-1}).

The HRV Committee chose the study by Lee *et al.* (1976) conducted in Beagle dogs as the key study. The choice of this species in the study is protective, as it is the species for which effects were observed at the lowest dose.

  - Choice of the point of departure

The data from the Lee *et al.* study show that mild extramedullary haematopoiesis was observed from the lowest tested dose, enabling a LOAEL of 4 mg·kg bw^{-1}·day^{-1} to be defined, given that a BMD could not be modelled based on the data.

The HRV Committee selected a LOAEL of 4 mg·kg bw^{-1}·day^{-1} as the point of departure.

  - Allometric adjustment

An allometric adjustment was performed to reduce the uncertainty regarding interspecies variability. An HED was calculated using the equation given in Section 3.2.1, considering an

average body weight of 10.85 kg for female dogs (Lee *et al*. 1976) and of 70 kg for humans, i.e. a **$LOAEL_{HED}$ = 2.5 mg·kg bw^{-1}·day^{-1}.**

- Choice of uncertainty factors

The TRV was calculated from the $LOAEL_{HED}$ using the following UFs (ANSES, pending publication):

- inter-species variability (UF_A): 2.5, to account for toxicodynamic variability and residual uncertainties;
- inter-individual variability (UF_H): 10 by default, as no scientific data justified reducing the default value;
- subchronic to chronic transposition (UF_S): √10, as the key study was a subchronic study (13 weeks);
- use of a point of departure (UF_L): √10, as the PoD was a LOAEL showing mild extramedullary haematopoiesis;
- inadequacy of the data (UF_D): √10, because although data are available concerning subchronic and chronic toxicity, genotoxicity, reprotoxicity and carcinogenicity, the studies undertaken are relatively small in number and were mainly carried out by the same team.

An overall uncertainty factor of 790 was thus used to establish the TRV.

- Proposed long-term threshold oral TRV and confidence level

A long-term threshold TRV was calculated based on the ratio between the PoD and the overall UF.

TRV = 0.003 mg·kg bw^{-1}·day^{-1}

The overall confidence level for this TRV was estimated at 2.6/5; this is a **moderate-low confidence level**.

3.2.3. Other isomers: 2,3-DNT, 2,5-DNT, 3,4-DNT and 3,5-DNT

No studies have investigated the effects of chronic or subchronic exposure to 2,3-DNT, 2,5-DNT, 3,4-DNT or 3,5-DNT. However, following short-term exposure, the study by Lent *et al.* showed haematotoxic effects for these four isomers (Lent *et al.* 2012).

In the absence of specific long-term data for these isomers, the HRV Committee chose the value of the TRVs established for 2,4-DNT and 2,6-DNT, i.e. 0.003 mg·kg bw^{-1}·day^{-1}, as the indicative toxicity value (iTV). 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 (ANSES, pending publication).

3.2.4. All dinitrotoluene isomers combined

In the absence of specific long-term data on the various possible mixtures of DNT isomers, the HRV Committee chose the TRV established for 2,4-DNT and 2,6-DNT and applied as the iTV

for the other isomers, i.e. **0.003 mg·kg bw^{-1}·day^{-1}, as the iTV for the sum of the different DNT isomers**.

3.3. Proposed carcinogenic oral TRV

3.3.1. 2,4-DNT

- Choice of the critical effect

The various epidemiological studies identified did not enable the exposure levels of workers or the isomers to which they were exposed to be characterised. They were therefore not chosen for the establishment of the TRV.

The studies identified in animals showed various carcinogenic effects associated with 2,4-DNT, primarily in the liver and mammary tissue but also in the skin (Lee *et al*. 1985). For mammary gland cancer and skin cancer, the association between exposure to 2,4-DNT and increased incidence disappeared when only malignant tumours were considered and mammary fibroadenoma was excluded. However, data in female rats showed a connection between the exposure level and the incidence of malignant liver tumours.

The HRV Committee therefore chose the increased incidence of neoplastic nodules and hepatocellular carcinoma as the critical effect.

- Choice of assumption for establishment of the TRV

The epidemiological study by Sabbioni *et al*. suggested that 2,4-DNT is genotoxic (Sabbioni *et al*. 2006). It was based on several tests in animals (UDS assay, micronucleus test) supporting the genotoxicity of the substance, especially in hepatocytes (Mirsalis *et al*. 1989; Suzuki *et al*. 2009; Takasawa *et al*. 2010).

The HRV Committee concludes that 2,4-DNT has genotoxic effects (gene mutations, deletions, etc.) and that these effects show a no-threshold dose-response relationship.

- Analysis of the existing TRVs

Two oral slope factors are available: 0.31 (mg·kg bw^{-1}·day^{-1})$^{-1}$ defined by OEHHA in 2005 and 0.667 (mg·kg bw^{-1}·day^{-1})$^{-1}$ established by the US EPA in 2008. An analysis of the first value, based on the cumulative incidence of liver and mammary gland tumours, identified inconsistencies in its establishment (confusion concerning the key study and the oral slope factor taken from the US EPA). The second value was derived from a BMDL modelled by US EPA software and was based on the increase in mammary gland tumours as the critical effect, without any distinction being made between malignant and benign tumours, even though the dose-response effect disappeared when only malignant tumours were considered.

As a consequence, given these limitations, the HRV Committee did not retain the existing TRVs and proposes establishing a long-term no-threshold oral TRV.

- Establishing the TRV

  - Choice of the key study

The study by Ellis *et al.* showed a significant increase in neoplastic liver lesions and hepatic carcinoma in female CD rats (Ellis *et al.* 1979). As intercurrent mortality was non-negligible, cumulative incidence levels were adjusted using the Poly-3 method described by Bailer and

Portier to account for the deaths of animals, free of site-specific lesions, occurring before the end of the trial (Bailer and Portier 1988). Although this study was deemed of good quality (Klimisch 1), it should be noted that there was a large difference between the two highest tested doses (5.1 and 45 mg·kg $bw^{-1}\cdot day^{-1}$).

The HRV Committee chose the study by Ellis *et al.* (1979) as the key study.

- Choice of the point of departure

The data from the Ellis *et al.* study demonstrate a dose-response relationship between the development of neoplastic nodules and hepatocellular carcinoma and long-term exposure to 2,4-DNT. This relationship was modelled using Bayesian BMD software (BBMD, version 0.0.0.9077) available on the EFSA website for the establishment of a BMD. For dichotomous data, a BMR of 10% extra risk is recommended by default.

Table 2 shows the BMD for the critical effect and its 90% credible interval (BMDL-BMDU), obtained using model averaging.

Table 2: Modelled BMD for 2,4-DNT and its 90% credible interval (BDML-BMDU) based on the study by Ellis *et al*. (1979), considering neoplastic lesions and hepatic carcinoma as the critical effect and a BMR of 10%.

BMDL	BMD	BMDU
2.908 mg·$kg^{-1}\cdot day^{-1}$	10.342 mg·$kg^{-1}\cdot day^{-1}$	31.66 mg·$kg^{-1}\cdot day^{-1}$

The validation criteria (BMD > one-tenth of the lowest exposure dose; BMD/BMDL < 20; BMDU/BMDL < 50) are all met. Nevertheless, the BDML is slightly below the dose of 5.1 mg·kg $bw^{-1}\cdot day^{-1}$ corresponding to the NOAEL. The HRV Committee points out the uncertainties associated with this BMDL, due in particular to the difference between the last two tested doses and the choice of the default BMR in relation to the response levels at the two lowest doses (11% for 0.7 mg·kg $bw^{-1}\cdot day^{-1}$ and 14% for 5.1 mg·kg $bw^{-1}\cdot day^{-1}$). **Despite these reservations and considering that Bayesian model averaging incorporates model uncertainty, the HRV Committee chose the BMDL of 2.908 mg·kg $bw^{-1}\cdot day^{-1}$ as the point of departure, as it is protective in terms of the severity of the effect.**

- Allometric adjustment

An allometric adjustment was performed to reduce the uncertainty regarding interspecies variability. An HED was calculated using the equation given in Section 3.2.1 and considering an average monthly body weight of 384 g for female rats (Ellis *et al*. 1979) and a body weight of 70 kg for humans, i.e. a **$BMDL_{HED}$ = 0.79 mg·kg $bw^{-1}\cdot day^{-1}$.**

- Proposed long-term no-threshold oral TRV and confidence level

An ERU was calculated based on the ratio between the BMR and the $BMDL_{HED}$.

$$\mathbf{ERU = 0.13\ (mg\cdot kg\ bw^{-1}\cdot day^{-1})^{-1}}$$

This ERU corresponds to doses of 770 ng·kg $bw^{-1}\cdot day^{-1}$ for a risk of 10^{-4}, 77 ng·kg $bw^{-1}\cdot day^{-1}$ for a risk of 10^{-5} and 7.7 ng·kg $bw^{-1}\cdot day^{-1}$ for a risk of 10^{-6}.

The overall confidence level for this ERU was estimated at 3.9/5; this is a **moderate-high confidence level**.

3.3.2. 2,6-DNT

- Choice of the critical effect

The various epidemiological studies identified did not enable the exposure levels of workers or the isomers to which they were exposed to be characterised. They were therefore not chosen for the establishment of the TRV.

Only one carcinogenesis study was identified, highlighting an increase in the incidence of cholangiocarcinoma and hepatocellular carcinoma in F344 rats in connection with exposure to 2,6-DNT.

The HRV Committee therefore chose the increased incidence of hepatocellular carcinoma as the critical effect.

- Choice of assumption for establishment of the TRV

Not all the data concerning the genotoxicity of 2,6-DNT are consistent. However, most of the tests carried out have found DNA damage, adduct formation and the development of chromosomal aberrations following exposure to this isomer, especially in hepatocytes (Lent *et al*. 2012; Rothfuss *et al*. 2010; Jones *et al*. 2005; Takasawa *et al*. 2010).

The HRV Committee concludes that 2,6-DNT has genotoxic effects and that these effects show a no-threshold dose-response relationship.

- Analysis of the existing TRVs

A provisional oral slope factor of 1.5 (mg·kg bw^{-1}·day^{-1})$^{-1}$ was derived by the US EPA in 2013. The point of departure was a BMDL modelled with US EPA software, using a multistage cancer model without model averaging.

As this value was provisional and the BMD used had not been modelled using model averaging, the HRV Committee did not retain the existing TRV and proposes establishing a long-term no-threshold oral TRV.

- Establishing the TRV

  - Choice of the key study

Only one carcinogenesis study was identified during the literature review. It was the study by Leonard *et al.*, conducted in F344 rats exposed for one year to a dose of 0, 7 or 14 mg·kg bw^{-1}·day^{-1} of 2,6-DNT (Leonard *et al.* 1987). The study had a Klimisch score of 2 and was therefore considered reliable with some restrictions.

Despite a fairly short exposure period, the HRV Committee chose the study by Leonard *et al.* (1987) as the key study.

  - Choice of the point of departure

The data from the Leonard *et al.* study demonstrate a dose-response relationship between the development of hepatocellular carcinoma and long-term exposure to 2,6-DNT. This relationship was modelled using Bayesian BMD software (BBMD, version 0.0.0.9077) available on the EFSA website for the establishment of a BMD. For dichotomous data, a BMR of 10% extra risk is recommended.

Table 3 shows the BMD and its 90% credible interval (BMDL-BMDU) obtained by model averaging for the critical effect, using a Markov chain Monte Carlo (MCMC) method with bridge sampling.

Table 3: Modelled BMD for 2,6-DNT and its 90% credible interval (BMDL-BMDU) based on the study by Leonard *et al*. (1987), considering the increased incidence of hepatocellular carcinoma as the critical effect and a BMR of 10%.

BMDL	BMD	BMDU
0.468 mg·kg bw^{-1}·day^{-1}	3.095 mg·kg bw^{-1}·day^{-1}	5.491 mg·kg bw^{-1}·day^{-1}

The validation criteria (BMD > one-tenth of the lowest exposure dose; BMD/BMDL < 20; BMDU/BMDL < 50) are all met. Nevertheless, the BMD is below the first tested dose due to the high incidence observed at the two tested doses, leading to some reservations with regard to this value. **Even so, and considering that model averaging incorporates the variability associated with the different models, the HRV Committee chose the $BMDL_{10}$ of 0.468 mg·kg bw^{-1}·day^{-1} as the point of departure.**

- Allometric adjustment

An allometric adjustment was performed to reduce the uncertainty regarding interspecies variability. An HED was calculated using the equation given in Section 3.2.1 and considering the average monthly body weight of male rats from the key study, i.e. 376 g, and a body weight of 70 kg for humans, i.e. a **$BMDL_{HED}$ = 0.13 mg·kg bw^{-1}·day^{-1}.**

- Proposed long-term no-threshold oral TRV and confidence level

An ERU was calculated based on the ratio between the BMR and the $BMDL_{HED}$.

$$\mathbf{ERU = 0.79\ (mg \cdot kg\ bw^{-1} \cdot day^{-1})^{-1}}$$

This ERU corresponds to doses of 130 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-4}, 13 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-5} and 1.3 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-6}.

The overall confidence level for this ERU was estimated at 3.3/5; this is a **moderate confidence level**.

3.3.3. Other isomers: 2,3-DNT, 2,5-DNT, 3,4-DNT and 3,5-DNT

In the absence of carcinogenesis studies for 2,3-DNT, 2,5-DNT, 3,4-DNT and 3,5-DNT, the HRV Committee chose the most protective value from the ERUs determined for 2,4-DNT and 2,6-DNT, **i.e. 0.79 (mg·kg bw^{-1}·day^{-1})$^{-1}$, as the iTV**. This corresponds to doses of 130 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-4}, 13 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-5} and 1.3 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-6}.

3.3.4. All dinitrotoluene isomers combined

For a mixture of DNT isomers whose proportions can vary, the ERUs defined above should be applied to each isomer (0.13 (mg·kg bw^{-1}·day^{-1})$^{-1}$ for 2,4-DNT and 0.79 (mg·kg bw^{-1}·day^{-1})$^{-1}$ for the other isomers). The overall risk can then be estimated by adding up the risk values calculated from the ERUs.

3.4. Conclusion of the HRV Committee

Several long-term threshold and no-threshold oral TRVs and iTVs have been proposed by the HRV Committee (Table 4 and Table 5).

The long-term threshold oral TRV for 2,4-DNT is based on the drop in red blood cell counts. A moderate confidence level was assigned to this TRV. The long-term threshold oral TRV for 2,6-DNT is based on extramedullary haematopoiesis. A moderate-low confidence level was assigned to this TRV. The long-term no-threshold oral TRV for 2,4-DNT is based on the increased incidence of hepatocellular carcinoma and neoplastic liver nodules. A moderate-high confidence level was assigned to this TRV. The long-term no-threshold oral TRV for 2,6-DNT is based on the increased incidence of hepatocellular carcinoma. A moderate confidence level was assigned to this TRV.

In the absence of data on chronic toxicity and carcinogenesis, threshold and no-threshold iTVs have been proposed for the other four isomers and for the sum of the isomers, considering the most protective TRV values established for 2,4- and 2,6-DNT. Their confidence levels are low.

As DNT isomers are not found isolated in the environment but are used in mixtures, an iTV for the sum of the DNT isomers is recommended for threshold-dose effects. It corresponds to the TRV determined individually for each of the different isomers, i.e. 0.003 mg·kg bw^{-1}·day^{-1}.

For carcinogenic effects without a threshold dose, in the case of a mixture of DNT isomers, the ERUs defined above should be applied to each isomer (0.13 (mg·kg bw^{-1}·day^{-1})$^{-1}$ for 2,4-DNT and 0.79 (mg·kg bw^{-1}·day^{-1})$^{-1}$ for the other isomers). The overall risk can then be estimated by adding up the risk values calculated from the ERUs.

Some epidemiological studies seem to suggest that the route of dermal absorption is not negligible and should also be taken into account in health risk assessments.

Table 4: Long-term threshold oral TRVs and iTVs for the six isomers of DNT and the mixture of isomers

		2,4-DNT	2,6-DNT	2,3-DNT	2,5-DNT	3,4-DNT	3,5-DNT	DNT
RV	**Organisation**	ANSES	ANSES	ANSES				
	Year	2024	2024	2024				
	Name	TRV	TRV	iTV				
	Value	**0.003 mg·kg bw^{-1}·day^{-1}**	**0.003 mg·kg bw^{-1}·day^{-1}**	**0.003 mg·kg bw^{-1}·day^{-1}**				
Target population		General population	General population	General population				
Critical effect		Decrease in blood cell counts	Extramedullary haematopoiesis	Value based on the TRVs established for 2,4-DNT and 2,6-DNT				
Key study	**Reference**	Ellis *et al.* 1979	Lee *et al.* 1976					
	Species	Female Beagle dogs	Beagle dogs					
	Exposure (duration, route)	Oral (capsules), 12 months	Oral (capsules), 13 weeks					
Point of departure (PoD)		$BMDL_5$ = 0.129 mg·kg bw^{-1}·day^{-1}	LOAEL = 4 mg·kg bw^{-1}·day^{-1}					
Time adjustment		/	/					
Allometric adjustment		$BMDL_{5\ HED}$ = 0.078 mg·kg bw^{-1}·day^{-1}	$LOAEL_{HED}$ = 2.51 mg·kg bw^{-1}·day^{-1}					
Uncertainty factors (UFs)		25 UF_A: 2.5, $UF_{H\text{-}TK}$: 10	790 UF_A: 2.5, $UF_{H\text{-}TK}$: 10, UF_L: √10, UF_S: √10, UF_D: √10					
Confidence level		Moderate	Moderate-low	Low				

Table 5: Long-term no-threshold oral TRVs and iTVs for the six isomers of DNT

		2,4-DNT	2,6-DNT	2,3-DNT	2,5-DNT	3,4-DNT	3,5-DNT
RV	**Organisation**	ANSES	ANSES	ANSES			
	Year	2024	2024	2024			
	Name	ERU	ERU	iTV			
	Value	**0.13 (mg·kg bw^{-1}·day^{-1})$^{-1}$** 770 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-4} 77 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-5} 7.7 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-6}	**0.79 (mg·kg bw^{-1}·day^{-1})$^{-1}$** 130 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-4} 13 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-5} 1.3 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-6}	**0.79 (mg·kg bw^{-1}·day^{-1})$^{-1}$** 130 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-4} 13 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-5} 1.3 ng·kg bw^{-1}·day^{-1} for a risk of 10^{-6}			
Target population		General population	General population	General population			
Critical effect		Increased incidence of hepatocellular carcinoma and neoplastic nodules	Increased incidence of hepatocellular carcinoma	Value based on the TRV established for 2,6-DNT			
Key study	**Reference**	Ellis *et al.* 1979	Leonard *et al.* 1987				
	Species	Female SD rats	Male F344 rats				
	Exposure (route, duration)	Oral, more than 1 year	Oral, 1 year				
Point of departure (PoD)		$BMDL_{10}$ = 2.91 mg·kg bw^{-1}·day^{-1}	$BMDL_{10}$ = 0.47 mg·kg bw^{-1}·day^{-1}				
Time adjustment		***/***	***/***				
Allometric adjustment		$BMDL_{10\ HED}$ = 0.79 mg·kg bw^{-1}·day^{-1}	$BMDL_{10\ HED}$ = 0.13 mg·kg bw^{-1}·day^{-1}				
Establishment		Linear extrapolation to low doses					
Confidence level		Moderate-high	Moderate	Low			

* For carcinogenic effects without a threshold dose, in the case of a mixture of DNT isomers, the defined ERU should be applied to each isomer. The overall risk can then be estimated by adding up the risk values calculated from the ERUs.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the proposed TRVs and iTVs established for dinitrotoluene (DNT) isomers and their sum, as well as the conclusions of the Expert Committee on Health reference values.

The Agency reiterates that a toxicity reference value (TRV) is a toxicological indicator for qualifying or quantifying a risk to human health. TRVs enable the potential health effects of exposure to substances to be assessed. They can be used as part of quantitative health risk assessments (QHRAs) carried out at population level, in a given exposure context, and thus help in the choice of risk management measures.

They can also be used to establish guidance values such as health-based guidance values for drinking water. An iTV is a toxicological benchmark that can also be used for assessing a risk. However, it is an indicative value that is less robust than the TRV and therefore has a low confidence level.

For the DNT isomers, the toxicological profiles of the substances led the experts to propose a threshold TRV and a no-threshold TRV for each isomer, to cover carcinogenic effects. Given that the isomers are seldom detected in isolation, the experts also proposed an approach for taking account of the presence of all the isomers. While this iTV approach is associated with a low confidence level, it is nonetheless based on two cautious choices: additivity of effects and the selection of the lowest determined TRV.

These TRVs were established in particular for the purpose of assisting in ANSES's determination of health-based guidance values for drinking water. The Agency also stresses the importance, in a quantitative assessment of the risks associated with this class of substances, of considering exposure levels that could result from other routes, in particular the dermal route, when establishing scenarios.

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Valeur toxicologique de référence, VTR, dinitrotoluène, voie orale, long terme, chronique, seuil, cancer, sans seuil, valeur sanitaire

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