Approved: 29 June 2026

DOI: 10.2903/j.efsa.2026.10227

SCIENTIFIC REPORT

efsa JOURNAL

Scientific Report on consumer health-based guidance values for trifluoroacetic acid

EFSA (European Food Safety Authority) | Adeline Cavelier | Tamara Coja | Andrea Gall | Antonio Hernandez-Jerez | Christiane Vleminckx | Elodie Bergsma | Marco Binaglia | Federica Crivellente | Mathilde Colas | Anna Lanzoni | Jochem Louisse | Rafaela Rodrigues De Jesus | Annetta Grillo | Iris Mangas | Juan Manuel Parra Morte

Correspondence: Ask a Question

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

The European Commission asked EFSA to provide a scientific report according to Article 31 of Regulation (EC) No 178/2002, in conjunction with Regulation (EC) No 1107/2009, to revise consumer health-based guidance values, i.e. acceptable daily intake (ADI) and acute reference dose (ARfD), for trifluoroacetic acid (TFA). EFSA launched a targeted call for data from 6 August to 7 October 2024 and established an EFSA Working Group. Based on the weight of evidence, the WG concluded that TFA and its salt, TFA Na, are unlikely to be genotoxic. The WG established an ADI of 0.014 mg/kg body weight (bw) per day (expressed as trifluoroacetic acid) derived from the parental benchmark dose lower confidence limit ($BMDL_{20}$) of 8.6 mg/kg bw per day. This value is based on changes in thyroid hormones, specifically decreased thyroxine (T4) levels, observed in the extended one-generation reproductive toxicity study in rats. A standard uncertainty factor of 100 was applied to account for inter- and intra-species differences. An additional factor of 5 was applied to address remaining uncertainties, namely the absence of a long-term toxicity/carcinogenicity study and the potential for developmental immunotoxicity, given the lack of functional immunotoxicity testing forTFA during the developmental phase. In addition, an ARfD of 0.07 mg/kg bw (expressed as trifluoroacetic acid) was derived based on the same $BMDL_{20}$ of 8.6 mg/kg bw per day, by applying a standard uncertainty factor of 100.

KEYWORDS

ADI, ARfD, common metabolite, health-based guidance values, mammalian toxicology, PFAS, trifluoroacetic acid

CONTENTS

1 INTRODUCTION

Trifluoroacetic acid (TFA) is formed as a degradation product of various chemicals such as hydrochlorofluorocarbons (HCFCs) and hydrofluorocarbons (HFCs), as well as pharmaceuticals. Additionally, TFA can result from the metabolism and breakdown of certain per- and polyfluoroalkyl substances (PFAS)[1] and some active substances used in plant protection products (Solomon et al., 2016).

The existing consumer health-based guidance values (HBGVs) for TFA were set during the peer review of the pesticide risk assessment of the active substance flurtamone (EFSA, 2017): an acceptable daily intake (ADI) of 0.05 mg/kg body weight (bw) per day (expressed as sodium trifluoroacetate (TFA Na)) was established based on a 90-day rat study. This value incorporates an uncertainty factor (UF) of 200, which includes a twofold factor to account for the extrapolation from subchronic to chronic exposure. No acute reference dose (ARfD) was considered necessary based on the available toxicological studies.

On 7 January 2021, a notification according to Article 56 of Regulation (EC) No 1107/2009[2] was submitted to EFSA, the European Commission and Member States Competent Authorities by the TFA task force, which consists of companies which market chemicals forming TFA in the European Union. The notification raised concerns regarding potentially harmful or unacceptable effects of TFA, as adverse developmental effects were observed in rabbits after exposure to TFA in a newly conducted prenatal developmental toxicity study (OECD Test Guideline (TG) 414). In 2023, this study was submitted in the context of the REACH registration procedure for TFA.[3]

Furthermore, in the recent assessment of TFA as part of the evaluation of tritosulfuron (EFSA, 2023a) and flufenacet (EFSA, 2024), a data gap was identified by EFSA for aneugenicity potential. The same HBGVs for consumer risk assessment as previously peer reviewed in the context of the EFSA conclusion offlurtamone (EFSA, 2017) were used. According to the latest scientific state of the art (EFSA Scientific Committee, 2021), the derivation of HBGVs could be affected by the conclusion on aneugenicity. However, this was not discussed during the peer review since the EFSA Scientific Opinion on the guidance on aneugenicity assessment was not available at the time of the dossier submission.

On 11 June 2024, in accordance with Regulation (EC) No 1272/2008,[4] Germany submitted a harmonised classification and labelling (CLH) dossier on TFA and its salt, TFA Na,[5] to the European Chemicals Agency (ECHA), proposing new classifications as a reproductive toxicant (Category 1B, H360fD[6]); persistent, mobile and toxic (PMT); and very persistent and very mobile (vPvM). The CLH proposal for reproductive toxicity was based on the prenatal developmental toxicity study in rabbits[3] and fertility effects in an extended one-generation reproductive toxicity study (EOGRTS) in rats, having an impact on the relevance assessment as a groundwater metabolite. In addition to the ADI, the effects observed in the developmental toxicity may trigger the need to set an ARfD for TFA. From 26 May 2025 to 25 July 2025, ECHA launched a public consultation on the CLH dossier[7] submitted by Germany, inviting stakeholders to provide comments on the new CLH proposals. The CLH proposals were discussed at the ECHA Risk Assessment Committee (RAC) 77 WG meeting on 27 April 2026 and at the RAC-77 Plenary meeting on 2 June 2026. The ECHA RAC recommended a classification for TFA as Acute Tax. 4 (oral, H302[8]) with an acute toxicity estimate (ATE) of 500 mg/kg bw; for TFA and TFA Na as Acute Tax. 3 (inhalation, H331[9]) with an ATE of 3 mg/L (vapour), as Reproductive toxicity category 1B for effects on development (H360Df[10]) and as PMT and vPvM.[11]

1.1 Background and Terms of Reference as provided by the requestor

In accordance with Article 31 of Regulation (EC) No 178/2002,[12] in conjunction with Regulation (EC) No 1107/2009, the European Commission requested EFSA to issue an EFSA output and to review the recommended consumer HBGVs, i.e. ADI and ARfD, for TFA.[13] The review should take into account the available studies: those previously submitted and evaluated as part of active substance assessments, new studies submitted by the TFA task force related to notification under Article

[1]According to OECD guideline (OECD, 2021) and ECHA's definition from restriction intention (ECHA, 2023), PFAS are identified based on their perfluorinated chemical structure.

[2]Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L309, 24.11.2009, p. 1-50.

[3]REACH registration dossier on TFA including the prenatal developmental toxicity study in rabbits (OECD TG 414).

[4]Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 76 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, pp. 1-1355.

[5]ECHA Registry of CLH intentions until outcome - Trifluoroacetic acid: https://echa.europa.eu/it/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236el 88 e6e587.

[6]May damage fertility. Suspected of damaging the unborn child.

[7]ECHA Harmonised classification and labelling consultations: https://echa.europa.eu/fr/harmonised-classification-and-labelling-consultation/-/substance-rev/80001/ term.

[8]Harmful if swallowed.

[9]Toxic if inhaled.

[10]May damage fertility. Suspected of damaging the unborn child.

[11]https://echa.europa.eu/documents/d/guest/rac77_fi nal_min utes_ en.

[12]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

[13]Refer to the EC Mandate and follow-up communications at: https://open.efsa.europa.eu/questions/EFSA-Q-2024-00502.

56 of Regulation (EC) No 1107/2009[14] received on 7 January 2021, data used by Germany for the CLH dossier on TFA and TFA Na, and, where available, studies submitted to Member States for the assessment of plant protection products. More specifically, EFSA was requested to conduct the following tasks:

1. **Term of reference (ToR) 1:** to collect evidence in addition to data on TFA already available from EFSA peer review processes, including: (i) additional data on TFA and TFA Na generated by the TFA task force, (ii) data used by Germany for the CLH dossier on TFA and TFA Na, and (iii) data submitted to Member States as part of the process for authorisation of plant protection products.
2. **Term of Reference (ToR) 2:** to assess the available evidence in relation to the toxicological properties of the metabolite TFA and TFA Na and, if possible, to derive HBGVs (ADI and ARfD) for TFA to be used in risk assessments.

As part of this work, EFSA was invited to consult Member States and, where relevant, experts from EFSA's Panels and their working groups, as well as ECHA's Committee on Risk Assessment (RAC).

1.2 Interpretation of the Terms of Reference

1.2.1 Term of Reference (ToR) 1

To address ToR 1, EFSA launched a targeted call for data, inviting Member States, the TFA task force and the Competent Authority Germany,[15] which prepared the CLH dossier on TFA and TFA Na, to submit toxicological and metabolism studies on TFA and TFA Na. Specifically, the following data was requested:

- For Member States, data from the PPP authorisation dossiers for pesticide active substances identified as PFAS[16] that either form or potentially form TFA, according to the outcome of EFSA peer review procedures. In this context, Member States were invited to submit any available data related to the toxicological hazard properties ofTFA or TFA Na covering the endpoints of Section 5 - Subsections 5.1. to 5.9.7 in Commission Regulation (EU) No 283/2013.[17]
- For the Competent Authority Germany, studies used in the context of the CLH report and not available at EFSA level and the confidential version of the CLH report, for the sake of ECHA/EFSA alignment on data package and assessment, and in line with the one substance one assessment concept (BAuA, 2025). As part of this procedure, the list of references used in the context of the CLH dossier was beforehand compared with the data available at EFSA level.
- For the TFA task force, the list of studies generated by the TFA task force and related original study reports or position papers.

In parallel to the targeted call for data, EFSA gathered all studies relevant for this Mandate from the dossiers submitted within the EFSA peer review processes.

The targeted call was launched from 6 August to 7 October 2024. A total of 170 studies and position papers were collected. The list of studies can be found in Annex 2 and at: https://open.efsa.europa.eu/questions/EFSA-Q-2024-00502.

1.2.2 Term of Reference (ToR) 2

An EFSA Working Group (WG) was established in order to address ToR 2.

The WG interpreted the ToR 2 by considering the following tasks in the first tier:

- To conduct the assessment, including relevance and reliability, of studies available for genotoxicity hazard identification;
- To conduct the assessment, including relevance and reliability of studies available for assessment of toxicokinetic and toxicodynamic properties ofTFA;
- To identify the most suitable basis for setting the ADI and ARfD, if applicable;
- To derive HBGVs (ADI and ARfD, if applicable) for TFA to be used in consumer risk assessments.

The WG defined the assessment questions as follows:

- **Assessment question 01:** Based on the results of the targeted call for data, do the available data raise a concern for genotoxicity?

[14]On 7 January 2021, a notification according to Article 56 ofRegulation 1107/2009 was submitted from the TFA task force to EFSA, the European Commission and Member States concerning potentially harmful or unacceptable effects ofTFA.

[15]The CLH dossier was drafted by the German federal agency, BAuA - Federal Institute for Occupational Safety and Health.

[16]According to their chemical structures (https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas).

[17]Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, pp. 1-84.

- **Assessment question 02:** Based on the results of the targeted call for data, what is the most suitable basis for setting the ADI?
- **Assessment question 03:** Based on the results of the targeted call for data, is there a need for setting an ARfD? If so, what is the most suitable basis?

1.3 Evaluations carried out under other regulatory contexts

TFA and TFA Na have been evaluated by several organisations including UBA (2020), DTU (2021), RIVM (2023), Department Zorg (2024) and JMPR (2024). TFA is registered under REACH Regulation and is manufactured in and/or imported to the European Economic Area. At the time EFSA was drafting this report, ECHA CLH procedure for TFA and TFA Na was underway for proposed classification as a reproductive toxicant (Category 1B, H360fD), PMT and vPvM.

There are ongoing activities to assess several PFAS including TFA under different regulatory frameworks. On 13 January 2023, five national authorities (Denmark, Germany, the Netherlands, Norway and Sweden) submitted a joint proposal to ECHA to introduce a restriction under the REACH Regulation covering approximately 10,000 PFAS (ECHA, 2023). The proposal sought to establish an EU-wide framework to address risks associated with the manufacture, placing on the market and use of this broad group of substances.

From 22 March 2023 to 22 September 2023, ECHA conducted a 6-month public consultation on the proposed restriction, enabling stakeholders to provide scientific, technical and socio-economic information. ECHA aims to complete its scientific evaluation of the proposed restriction by the end of 2026.

In the area of drinking water regulation, TFA has been proposed for inclusion in the most recent revision of the Drinking Water Directive,[18] with the objective of ensuring that TFA is considered among the PFAS substances subject to regular monitoring in drinking water. As of 12 January 2026,[19] PFAS analysis in drinking water (including TFA) has become mandatory across Member States.

Member States are required to monitor compliance using one of the following parametric values:

- Total PFAS (including TFA), with a limit value of 0.5 µg/L; or
- The sum of 20 specified PFAS, with a limit value of 0.1 µg/L.

Prior to this date, PFAS monitoring in drinking water was conducted on a voluntary basis, except where more stringent national or regional requirements applied. The transitional period has now ended, and mandatory monitoring requirements are in force.

As regards the assessment of TFA within the PFAS group, the WG did not assess substances other than TFA and TFA Na since this is considered out of the scope of the current mandate. In 2020, the EFSA Panel on Contaminants in the Food Chain (CONTAM) assessed the risks related to the presence of PFAS in food, focusing mainly on four PFAS: perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS), which were the most frequent PFAS detected in human blood and for which sufficient occurrence and toxicological data were available. Based on toxicological evidence, particularly effects on the immune system and measured human exposure, a tolerable weekly intake of 4.4 ng/kg bw per week was established for the sum of these four PFAS (EFSA, 2020). In that EFSA opinion, no data on TFA or its sodium salt were included, and no explicit reference was made to TFA, TFA Na or related synonyms (e.g. trifluoroacetate) in the main text, tables or annexes. This absence reflects that TFA was outside the defined scope of the EFSA's assessment from 2020, which focused on selected perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs) and related substances, mainly those with carbon chain ranging from C4 to C18, and for which adequate occurrence and toxicological data were available at the time.

2 DATA AND METHODOLOGIES

2.1 Data collection

Collection of evidence from targeted call for data

To address ToR 1, EFSA collected evidence through a targeted call for data launched from 6 August 2024 to 7 October 2024. This includes data on TFA and TFA Na already available from EFSA peer review processes, additional data on TFA and TFA Na generated by the TFA task force, data used by the dossier submitter (Germany) for the drafting of the CLH dossier on TFA and data submitted to Member States as part of the process for authorisation of plant protection products. EFSA noted that a literature search[20] had been conducted by the dossier submitter for the drafting of the CLH dossier and additional litera-

[18] Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption. http://data.europa.eu/eli/dir/2020/2184/oj.

[19] https://environment.ec.europa.eu/news/new-eu-rules-limit-pfas-drinking-water-2026-01-12_en.

[20] Please refer to CLH report, Section 6. Data Sources (electronic page 9) https://echa.europa.eu/documents/10162/0dae2d2a-2b4c-b63e-c669-bd3a76197aal.

ture studies were part of the data package submitted to EFSA by different stakeholders. Data generated by the TFA task force notably included REACH registration data. These data have also been considered by the WG.

The complete list of 170 studies and position papers can be found in Annex 2. In the interests of transparency, data owners were invited to submit redacted versions of original study reports or position papers that were not previously made publicly available. The studies are available on Open EFSA.[21]

The studies are identified and referenced throughout the present scientific report using the numbering #1 to #170, as provided in Column A of Annex 2.

Consolidated list of studies outside the window of call for data

During the development of this report, the WG was also made aware of new studies out of time frame of targeted call of data (6 August 2024 to 7 October 2024).

Formally, there is no legal obligation to consider newly available data submitted outside of the dedicated targeted call for data, unless such information qualifies as adverse data. A list of the collected newly available public literature or studies is reported in the format of an Excel spreadsheet and made publicly available as part of the background documentation to the EFSA Scientific report (Annex 9).

EFSA screened the newly submitted public literature or studies to determine their potential impact on the risk assessment and whether they would have altered the weight of evidence (WoE) in the current risk assessment. Overall, this exercise aimed to ensure that information from published literature or new studies, even if they became available at a later stage and outside the legislative frame, was screened to ensure that available evidence that could have led to a different outcome was considered.

2.2 Methodologies

Based on the collected data **(ToR 1),** a structured, systematic approach was used to assess all submitted data and to set consumer HGBVs, if appropriate **(ToR 2).** To address ToR 2, a weight of evidence (WoE) was applied, following EFSA's established principles for data handling and uncertainty analysis, with methods planned through a detailed protocol (see Annex 1).

All the evidence retrieved followed the same process, i.e. extraction of the data, appraisal of the evidence and uncertainty analysis. Furthermore, specific eligibility criteria for relevance of studies were defined by the WG and applied to all the submitted studies. For instance, only studies on TFA or TFA Na were included, whereas studies on mixtures containing TFA or TFA salts (e.g. TFSK: KTMS (potassium trifluoromethanesulfinate) TFAK (potassium trifluoroacetate) reaction mass), were excluded as it was not possible to distinguish toxic effects of TFAK from those of KTMS. In addition, for the in vivo studies, only the oral route was considered, since it is the relevant route of exposure for setting the consumer reference values. The detailed protocol is available in Annex 1.

The reasons for excluding 84 studies and position papers from the body of evidence according to the eligibility criteria can be found in Appendix H.

The WG divided studies providing relevant information for genotoxicity hazard identification, for assessment of toxicokinetic and toxicodynamic properties ofTFA, and studies providing mechanistic information on mode of action (MoA). All the studies were classified as either OECD TG (regulatory) or non-OECD TG studies and by streams of evidence (i.e. in vitro and in vivo animal, human observational studies).

The WG extracted the data from selected studies and appraised the evidence. The methodology for appraising evidence on the relationship between TFA exposure and:

1. Genotoxicity endpoints were based on the approach described by the EFSA cross-cutting Working Group on Genotoxicity (EFSA, 2023b).
2. Non-genotoxicity endpoints, coming from OECD TG studies, were based on the Klimisch et al. (1997) scoring system.
3. Non-genotoxicity endpoints, coming from non-OECD TG studies, were based on the risk of bias (RoB). The RoB methodology was tailored to three streams of evidence: in vitro, in vivo and human studies. The internal validity, or RoB, of each study was appraised using a customised version of the OHAT/NTP RoB assessment tool (NTP, 2015 ,2016). Each study was appraised by two independent reviewers from the WG and EFSA. Possible discrepancies not resolved by discussion were taken to the attention of the whole WG.

All studies, including already peer-reviewed studies, were subject to evaluation by the WG.

For further details on the methodology, please refer to the protocol in Annex 1.

The WG also considered the evaluations carried out under other regulatory contexts described under Section 1.3.

The draft output and annexes of the current Scientific Report on TFA were open for public consultation before its finalisation from 22 July 2025 to 22 September 2025. The outcome of the public consultation is available in Annex 8

[21] https://open.efsa.europa.eu/questions/EFSA-Q-2024-00502.

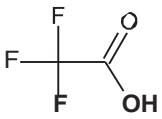
3 ASSESSMENT

3.1 Identity and method of chemical analysis

Most toxicological studies have been conducted using the salt of TFA, e.g. TFA Na. The purity of the test material in the toxicological studies ranged from 98% to 99.1%.

Trifluoroacetic acid and sodium trifluoroacetate are, respectively, the IUPAC chemical names for compound codified or named in this Scientific Report as TFA and TFA Na (see Table 1).

TABLE 1 Used compound codes.

Code/trivial name [a]	IUPAC name/SMILES notation/InChiKey [b]	Structural formula [c]
Trifluoroacetic acid, **TFA**	Trifluoroacetic acid FC(F)(F)C(=O)O DTQVDTLACAAQTR-UHFFFAOYSA-N	
Sodium trifluoroacetate, **TFA Na,** perfluoroacetate	Sodium trifluoroacetate [Na+].FC(F)(F)C([0-])=0 UYCAUPASBSROMS-UHFFFAOYSA-M	

Abbreviations: InChiKey, International Chemical Identifier; IUPAC, International Union of Pure and Applied Chemistry; SMILES, Simplified Molecular Input Line Entry System.

"The name in bold is the name used in this scientific report.

bACD/Name 2021.1.3 ACD/Labs 2021.1.3 (File Version N15E41, Build 723232, 7 July 2021).

'ACD/ChemSketch 2021.1.3 ACD/Labs 2021.1.3 (File Version C25H41, Build 123835, 28 August 2021).

The method of analysis ofTFA in the diet (#150) used in the key study to set HBGVs was considered reliable (i.e. fit for purpose).[22]

3.2 Hazard identification and characterisation

3.2.1 Toxicokinetics

No test guideline-compliant absorption, distribution, metabolism and excretion (ADME) studies were submitted to EFSA. It should be noted that under physiological pH, TFA and TFA salts dissociate, resulting in systemic exposure to the TFA anion. Toxicokinetic data are available from plasma measurements in the newly submitted rat studies (#99, #109) and rabbit study (#102). The rabbit developmental toxicity study #103 lacked the kinetic analyses needed to assess linearity but provided information on tissue distribution. In addition, a publicly available in vivo study (#97) was reviewed but excluded due to a non-oral route of administration, although it provided contextual information regarding TFA kinetics. Collectively, the toxicokinetic data derived from these oral toxicity and developmental studies (#99, #102, #103 and #109) were considered sufficient to characterise systemic exposure, distribution and general kinetic behaviour under the tested conditions.

In a 52-week rat drinking water study (#109), apparent steady-state plasma concentrations were achieved by week 52. Systemic exposure (Concentration maximum (Cmax), Area under the Curve (AUC)) increased approximately proportionally over the concentration range of 30-600 ppm, with no relevant sex-related differences. In contrast, a preliminary dietary reproductive study in rats (#99) showed non-linear, sub-proportional kinetics in dams, suggesting saturation of absorption and/or elimination processes at higher doses. FI offspring exhibited age-dependent toxicokinetic differences, with low and dose-independent plasma concentrations shortly after birth (postnatal day (PND) 4) and markedly higher systemic exposure by weaning (PND 21), consistent with increasing internal exposure as pups matured and because FI offspring generally consumed diet from mid-lactation.

In rabbits administered TFA by oral gavage (#102), absorption was evident, with median time to maximum concentration (Tmax) values of approximately 3-8 h on gestation day (GD) 6, decreasing to about 2-3 h by GD 28, indicating faster absorption later in gestation. Systemic exposure increased in a sub-proportional manner with dose, consistent with non-linear kinetics, likely reflecting saturation of absorption processes and/or a relative increase in elimination efficiency at higher doses. The rabbit developmental toxicity study (#103) confirmed systemic exposure and demonstrated efficient placental transfer, with fetal plasma levels comparable to maternal levels, as well as distribution to the ocular aqueous and vitreous humour, but did not allow assessment of kinetic linearity.

"Methods of analysis reports supporting other toxicological studies were also submitted (#149, #152, #155, #156, #160, #161); only methods of analysis supporting key studies were assessed in the current output.

Overall, TFA is absorbed after oral exposure and distributes systemically, including to the fetus and the eye. Its toxicokinetic behaviour is context-dependent, with approximately linear kinetics under long-term low-dose exposure in rats and non-linear kinetics observed at higher doses in developmental studies in both species. A more detailed assessment is provided in Appendix B. Integrating these toxicokinetic data is critical for interpreting both hazard and risk, particularly in contextualising systemic exposures in animal studies relative to human biomonitoring data. Serum concentrations at the No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) in the animal studies are several orders of magnitude higher than TFA levels reported in human populations (typically in the µM range, see Table 17). This indicates a substantial margin of exposure (MOE) between experimental exposures and environmental or background human exposure levels. Based on its physicochemical properties (low log Kaw, low bioconcentration factor and high polarity) and the absence of progressive systemic accumulation upon repeated dosing, TFA is expected to have a low potential for bioaccumulation or undergo significant metabolism. Although specific data on absorption, mass balance and elimination are limited, qualitative evidence supports high oral absorption, systemic distribution and rapid excretion, aligning with expectations for small, hydrophilic acids.

3.2.2 Genotoxicity

The genotoxic potential of TFA and TFA Na was investigated in vitro in several tests covering different endpoints (see Appendix A).

Induction of reverse mutations in bacteria was investigated in a total of five studies. Several strains of Salmonella typhimurium were tested in the presence or absence of metabolic activation. Three tests were performed in accordance with OECD TG 471, and the study results were considered highly relevant. One additional test was not fully in accordance with OECD TG 471, and the study results were considered of low relevance. A spot test was also reported in the literature, with a reliability score of 4 ('not assignable') and the results were considered of low relevance. All tests gave negative results with or without S9 (metabolic activation).

Induction of gene mutations in mammalian cells was investigated in five studies. All tests were performed in mouse lymphoma cells (L5178Y) in the presence or absence of metabolic activation. Four tests investigating mutations at the thymidine kinase locus and one at the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus were conducted in accordance with OECD TG 490 and OECD TG 476, respectively. All the study results with or without S9 were negative and considered of high relevance.

During the public consultation on this Scientific Report, comments were provided on the interpretation of the results from an in vitro clastogenicity assay (#141). The EFSA cross-cutting WG on genotoxicity was consulted on the clastogenic and aneugenic potential of TFA and TFA Na (see Appendix J). The TFA WG concurred with the advice of the EFSA Genotoxicity WG on chromosomal damage.

The potential ofTFA and TFA Na to induce chromosomal damage was evaluated with the chromosome aberration test performed in human peripheral blood lymphocytes and CHL cell line in the presence and absence of metabolic activation. Negative results were obtained in three studies of limited relevance carried out using TFA Na, while a highly relevant study conducted with TFA was positive.

The potential of TFA to induce chromosomal damage was also investigated in two in vitro micronucleus tests, conducted in the presence and absence of metabolic activation using the human lymphoblastoid TK6 and CHO-KI cell lines. Negative results were obtained in both studies, considered of high relevance. The WG noted that the former data gap on aneugenicity (EFSA, 2023a) is now fulfilled by applying the in vitro micronucleus test.

Overall, the studies carried out with TFA Na report no induction of chromosomal damage, while inconsistencies were observed among the studies performed with TFA showing a positive response in one chromosome aberration test and negative results in two in vitro micronucleus tests. In addition, the WG noted that studies with TFA showed considerable variability in cytotoxicity levels, both within individual studies (e.g. between range-finding and main experiments) and across different studies. The reasons for these inconsistencies remain unclear but could be related to the physico-chemical characteristics of TFA (a strong acid) (Morita et al., 1989) and technical factors (e.g. pH changes) modulating the effects of TFA. In contrast, no such variability was observed with TFA Na. Considering: (i) the substantial uncertainty surrounding the mechanisms underlying these findings with TFA compared to TFA Na and (ii) that exposure to TFA in its acid form is unlikely under physiological conditions, the WG assigned greater weight to the consistently negative results of chromosomal aberration studies conducted with the TFA salt than to those observed with the acid.

Overall, the weight of evidence does not raise a concern for chromosomal damage ofTFA and its Na salt.

Based on the in vitro studies, the weight of evidence suggests that genotoxicity is unlikely. There is no need to perform in vivo tests, in line with EFSA Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011).

In conclusion, based on available data, TFA or TFA Na are unlikely to be genotoxic.

3.2.3 Toxicity in experimental animals

3.2.3.1 *Single oral toxicity*

Based on the available information, TFA Na is of low acute oral toxicity to rats (#1, #122) with the lethal dose (LD) 50 value of TFA Na above 2000 mg/kg bw in female rats (#1). The WG noted that, at the ECHA RAC 77 Plenary meeting (2 June 2026), the ECHA RAC recommended a classification for TFA as Acute Tox. 4 (oral, H302) with an acute toxicity estimate (ATE) of 500 mg/kg bw and for TFA and TFA Na as Acute Tox. 3 (inhalation, H331 with an ATE of 3 mg/L (vapour)).[23]

3.2.3.2 *Repeated oral toxicity*

Repeated oral toxicity after sub-chronic(# 8, #15, #16, #124) and chronic (52-week, #109) exposure to TFA Na indicated the liver as the main target of toxicity in rats, as evidenced by increased organ weight and hepatocellular hypertrophy. Rats treated with TFA Na showed changes in some clinical chemistry parameters across all repeated dose studies (e.g. decrease in cholesterol, decrease in glucose and bilirubin levels in both sexes) (see Appendix C and **Section 3.2.7).** Nevertheless, in the absence of associated histopathological findings or significant increase in liver weight (higher than 15%), the WG did not consider the effects on clinical chemistry parameters relevant for setting the NOAEL. Changes in haematological parameters observed across studies were mild and not considered to reach the relevant level of adversity. There were no TFA Na treatment-related effects noted by the end of the 52-week rat study (#109) or following the 6-week recovery phase (i.e. the maximum tolerable dose (MTD) was not reached, as the highest dose tested (37.8 mg/kg bw per day) was not properly selected). Ophthalmological investigation in rats in 52-week study (#109) revealed no treatment-related effects, as most of the effects were observed in animals also during pretreatment. The ophthalmological observations were macroscopic and not related to folded retina observed in rabbit developmental studies.

Across all repeated-dose oral toxicity studies, the lowest NOAEL of 9.9 mg/kg bw per day was identified in the 90-day study in Wistar rats (#8) with the corresponding LOAEL of 98 mg/kg bw per day. At the LOAEL, effects included increased relative liver weight in both sexes and hepatocellular hypertrophy in males and a decrease in total bilirubin and glucose in both sexes (see Table 2).In Sprague Dawley rats, the NOAEL in the 90-day study (#124) was 89.2 mg/kg bw per day, whereas in the 52-week study (#109), the NOAEL was 37.8 mg/kg bw per day (highest dose level tested).

[23] https://echa.europa.eu/documents/d/guest/rac77_final_minutes en.

TABLE 2 A 90-day rat study (#8).

Parameter	Sex	N	Vehicle 0 ppm Mean/ Incidence	Vehicle 0 ppm SD±	Low dose - 160 ppm (9.9 mg/kg bw per day in males/12.2 mg/kg bw per day in females) Mean/ Incidence	Low dose - 160 ppm (9.9 mg/kg bw per day in males/12.2 mg/kg bw per day in females) SD±	Low dose - 160 ppm (9.9 mg/kg bw per day in males/12.2 mg/kg bw per day in females) %	Mid dose - 1600 ppm (98 mg/kg bw per day in males/123 mg/kg bw per day in females) Mean/ Incidence	Mid dose - 1600 ppm (98 mg/kg bw per day in males/123 mg/kg bw per day in females) SD±	Mid dose - 1600 ppm (98 mg/kg bw per day in males/123 mg/kg bw per day in females) %	High dose - 16,000 ppm (1043 mg/kg bw per day in males/1216 mg/kg bw per day in females) Mean/ Incidence	High dose - 16,000 ppm (1043 mg/kg bw per day in males/1216 mg/kg bw per day in females) SD±	High dose - 16,000 ppm (1043 mg/kg bw per day in males/1216 mg/kg bw per day in females) %
Absolute liver weight (g)	M	10	12.15	0.83	11.61	0.92	-4	13.25*	1.01	9	14.48**	0.95	19
	F	10	5.96	0.48	6.25	0.67	5	6.71*	0.46	13	7.36**	0.68	23
Relative liver weight %	M	10	2.327	0.119	2.258	0.083	-3	2.657**	0.148	14	3.102**	0.157	33
	F	10	2.243	0.089	2.343	0.165	4	2.520**	0.221	12	2.880**	0.149	28
Centrilobular to panlobular Hepatocellular hypertrophy, diffuse -minimal	M		1/10		0/10			3/9			1/10		
	F		0/10		0/10			0/10			5/10		
Centrilobular to panlobular Hepatocellular hypertrophy, diffuse - slight	M		0/10		0/10			2/9			6/10		
	F		0/10		0/10			0/10			4/10		
Centrilobular to panlobular Hepatocellular hypertrophy, diffuse - moderate	M		0/10		0/10			0/9			3/10		
	F		0/10		0/10			0/10			0/10		
Total bilirubin µmol/L	M	10[a]	1.6	0.4	1.1	0.2	-31	0.5**	0.1	−69	0.3**	0.2	−81
	F	10	2.1	0.5	1.8	0.4	-14	1**	0.6	−52	0.5**	0.3	−76
Glucose, mmol/L	M	10[a]	5.87	0.53	5.4	0.64	-8	4.21**	0.44	-28	4.14**	0.84	-29
	F	10	5.57	0.86	5.13	0.56	-8	4.19**	0.45	-25	4.62*	1.11	-17
Alanine AminoTransferase (ALT), IU/L	M	10[a]	47	25	47	20	0	87	84	85	65**	19	38
	F	10	38	9	40	10	5	48	17	26	45	5	18

Abbreviations:% Effect size/N, Number of animals examined; SD, standard deviation.

'Nine instead of 10 males was included in group 3 (1600 ppm).

**The group mean is significantly different from the control at $p < 0.01$. *The group mean is significantly different from the control at $p < 0.05$.

- If the Bartlett test was not significant ($p > 0.05$), means were compared using the analysis of variance (ANOVA). If the ANOVA was not significant ($p > 0.05$), the group means are considered to be homogeneous, and no further analysis were performed. If the ANOVA was significant ($p \leq 0.05$), the group means are considered to be homogeneous, and no further analysis were performed. If the ANOVA was significant ($p < 0.05$), means of the exposed groups were compared to the mean of the control group using the Dunnetttest (2-sided).

- If the Bartlett test was significant ($p < 0.05$), group means were compared using the non-parametric Kruskal-Wallis test. If the Kruskal-Wallis test was not significant ($p > 0.05$), the group means are considered to be homogeneous, and no further analysis were performed. If the Kruskal-Wallis test was significant ($p < 0.05$), means of the exposed groups were compared to the mean of the control group using the Dunn test (2-sided).

3.2.3.3 *Reproductive toxicity after oral exposure*

3.2.3.3.1 *Multigeneration reproductive toxicity*

A preliminary reproductive toxicity study in rats (#99) and an extended one-generation reproductive toxicity study (EOGRTS) in rats (#100) were conducted on TFA Na.

In the EOGRTS in rats (#100) conducted on TFA Na (see Appendix D), thyroid hormone (thyroxine, T4) concentrations were determined in F0 and FI adults at termination, as well as in FI offspring on PND22 (see Table 3).The **offspring NOAEL** was set at 8.65 mg/kg bw per day based on decreased T4 levels at 44.3 mg/kg bw per day and higher. Dose-response relationship and biological significance (benchmark of 20%, see Appendix G) were considered the main criteria for determining the NOAEL for T4 decrease. It is noted that, although an increased relative thyroid weight was observed in some groups, no dose relationship was observed; the absolute thyroid weight was not changed, and no treatment-related histopathological findings were reported in adults of both generations (see Table 4).

TABLE 3 Thyroid hormone (T4) levels, EOGRTS (#100).

Thyroxine (T4) concentrations (pg/ml)	ppm mg/kgbw perday *N*	0 0 10	120/60 8.65 10	600/300 44.3 10	3000/1500 223 10
F0 male adult terminal (Week 18)	Mean±SD CV% %	41,700± 7690 18.4	43,400 ± 9630 22.2 +4%	37,100± 7710 20.8 -11%	24,700± 7610** 30.8 -41%
F0 female adult terminal (Day 28 of lactation)	Mean±SD CV% % difference	32,200 ± 7390 23.0	31,400 ± 7970 25.4 -3%	31,800 ± 3630 11.4 -1%	22,000 ± 5090** 23.1 -32%
FI male offspring (Day 22 of age)	Mean±SD CV% %	56,900 ± 7370 13.0	60,200 ± 6510 10.8 +6%	47,300±6590* 13.9 -17%	40,700 (36600)[a] ±9680 (15800)[a]** 23.8 (43.2)[a] -36%
FI female offspring (Day 22 of age)	Mean±SD CV% % difference	53,800± 11,000 20.4	50,800 ± 8340 16.4 -6%	50,800 ± 8020 15.8 -6%	34,100±8340** 24.5 -37%
FI male adult terminal (Week 13)	Mean±SD CV% %	50,400± 7130 14.1	42,700±8980* 21.0 -15%	34,400 ± 10100** 29.4 -32%	24,400 ± 3690** 15.1 -52%
FI female adult terminal (Week 13)	Mean±SD CV% %	33,800 ± 13,700 40.5	34,000 ± 8480 24.9 +1%	35,900 ± 10,900 30.4 +6%	26,100±5750 22.0 -23%

Abbreviations:% Effect size/N, Number of animals examined; CV, coefficient of variation; SD, standard deviation.

*p <0.05 (Williams test).

**p<0.01 (Williams test).

'Data for animal 289-1 (below the limit of quantification) excluded from statistical calculations. Data in parenthesis include all data in statistical calculations.

TABLE 4 Thyroid weights, EOGRTS (#100).

Thyroid weights	ppm mg/kg bwper day	0 0	120/60 8.65	600/300 44.3	3000/1500 223
	F0 generation males (week 17)				
	N	25	25	25	25
Thyroids and parathyroids weight[a] - absolute (g)	Mean±SD	0.019±0.003	0.020 ± 0.003	0.107±0.437[a]	0.019±0.003
Thyroids and parathyroids weight - relative to bw (%)	Mean±SD	0.0042 ± 0.0008	0.0044 ± 0.0006	0.0215 ± 0.0859	0.0044 ± 0.0007
	F0 generation females (Day 28 post-partum)				
	N	23	23	24	23
Thyroids and parathyroids weight - absolute (g)	Mean±SD	0.016 ± 0.003	0.017±0.003	0.017±0.004	0.019±0.005*
Thyroids and parathyroids weight - relative to bw (%)	Mean±SD	0.0068 ± 0.0012	0.0071 ±0.0011	0.0070 ± 0.0013	0.0082 ± 0.0026**

TABLE 4 (Continued)

Thyroid weights	ppm mg/kgbwper day	0 0	120/60 8.65	600/300 44.3	3000/1500 223
	Fl generation males- cohort 1A {Week 13)				
	N	20	20	20	20
Thyroids and parathyroids weight - absolute (g)	Mean±SD	0.017 ± 0.002	0.019 ± 0.003	0.020 ± 0.004	0.018±0.003
Thyroids and parathyroids weight - relative to bw (%)	Mean±SD	0.0045 ± 0.0007	0.0053 ± 0.0008 *	0.0052±0.0011 *	0.0052 ± 0.0009 *
	Fl generation females - cohort 1A {Week 13)				
	N	20	20	20	20
Thyroids and parathyroids weight - absolute (g)	Mean±SD	0.015 ± 0.002	0.015 ± 0.001	0.015 ± 0.002	0.015±0.003
Thyroids and parathyroids weight - relative to bw (%)	Mean±SD	0.0068 ± 0.0012	0.0068 ± 0.0009	0.0068 ± 0.0008	0.0076 ± 0.0011*

Abbreviations: *N*, Number of animals examined; SD, standard deviation.

*p <0.05 (Williams' test).

**p <0.01 (Williams' test).

'One single animal showed a very high increased thyroid and parathyroids' weight (2.206 g).

The **parental NOAEL** was determined to be 8.65 mg/kg bw per day based on decreased T4 levels at 44.3 mg/kg bw per day and higher. This NOAEL is also supported by decreased body weight gain and minor histopathological findings in the stomach (mainly glandular dilatation). Similar to the findings in repeated dose toxicity studies following sub-chronic and chronic exposure, parental generations treated with TFA Na showed changes in clinical chemistry parameters (i.e. decreased levels of glucose, non-esterified fatty acids, triglycerides and cholesterol). Nevertheless, in the absence of associated histopathological findings or significant increase liver weight (higher than 15%), the WG did not consider the effects on clinical chemistry parameters at the dose level of 8.65 mg/kg bw per day as relevant for setting the NOAEL.

The WG considered whether the decrease in thyroid hormone levels observed in the EOGRTS (#100) lead to a concern for developmental neurotoxicity (DNT). In this study, no effect on brain weight was observed in pups on PND22. In young adults (13 weeks old, exposed in utero and from PND22), a decrease in body weight and a slight decrease in the absolute brain weight were reported in both sexes in the high dose group only; but no histopathological findings were noted in the brain. This finding on brain weight was considered of low biological relevance due to the slight magnitude of the finding. The sensitive populations, i.e. dams, fetuses and newborns, were not investigated for thyroid toxicity (effects on T4, TSH and thyroid histopathology) as would be the case in a specific study (i.e. Comparative Thyroid Assay). Nevertheless, the WG considered that the NOAEL (or $BMDL_{20}$) based on decreased T4 level in Fl offspring on PND22 is considered sufficiently conservative and protective of potential DNT effects, and that remaining uncertainties (lack of data in the target population) would be covered by the application of the standard UF of 100 (see Appendix G).

Reduced sperm/spermatid counts, as well as increased abnormal sperm, were reported at the highest dose of 223 mg/kg bw per day in both generations (see Table 5). Decreased absolute testes and epididymis weights were also noted in the Fl generation (see Table 6). It is noted that the effects observed in this study were not associated with marked systemic toxicity and may be consistent with the decreased testosterone production by human chorionic gonadotropin (hCG)-stimulated Leydig cells reported in the mechanistic in vitro rat study (#169, see **Section 3.2.5).** The changes in sperm motion parameters (average path velocity (VAP), curvilinear velocity (VCL), beat cross frequency (BCF)) observed in Fl generation only were of small magnitude, showed weak dose-response consistency and had no impact on overall sperm motility (see Table 5). However, when considered together with reduced sperm/spermatid counts, increased abnormalities and decreased testes and epididymis weights, these findings indicate a mild but coherent deterioration in male reproductive parameters at the highest dose (223 mg/kg bw per day). The WG noted that the assessment of sperm parameters (except sperm motility in both generations and sperm morphology in Fl generation) was conducted only at the highest dose. However, the WG considered the lack of data on sperm morphology at the low- and mid-dose levels in F0 generation as a low uncertainty since this parameter was examined at the low- and mid-dose levels in Fl generation and no treatment-related effects were reported. Regarding the lack of data on sperm/spermatid counts at the low- and mid-dose levels in both generations, the WG considered that, since treatment-related decreased sperm (F0)lspermatid (Fl) counts were observed at the high dose, there are uncertainties in setting a reproductive NOAEL for this study. The WG concluded that the reproductive LOAEL of this study is the highest dose level of 223 mg/kg bw per day. Usually, when a LOAEL is used for setting a health-based guidance value, an additional uncertainty factor of 3 is applied. This would give a value of 74 mg/kg bw per day, i.e. higher than the parental/offspring NOAEL identified for this study (8.65 mg/kg bw per day). Therefore, the WG considered that the parental/offspring NOAEL would be sufficiently protective of reproductive effects.

TABLE 5 Sperm parameters, EOGRTS (#100).

	ppm	0	120/60	600/300	3000/1500
Sperm parameters	**mg/kg bw per day**	**0**	**8.65**	**44.3**	**223**
	F0 generation males				
	N	**24**	**25**	**24**	**25**
Motile sperm (%)	Mean±SD	94±4	93±5	91±5	92±6
Progressively motile sperm(%)	Mean±SD	42±9	40±10	38±6	37±10
Cauda epididymis (left) - Weight (g)	Mean±SD	0.253± 0.035	-	-	0.231 ±0.027*
Cauda epididymis (left) - Sperm count (millions/g)	Mean±SD	484±138			437±116
Cauda epididymis (left) - Total sperm count (million)	Mean±SD	123±41			101 ±29 *
Testis (left) - Weight (g)	Mean±SD	1.98±0.15	-	-	1.95±0.18
Testis (left) - Spermatid count (millions/g)	Mean±SD	91±19			90±15
Testis (left) - Total spermatid count (million)	Mean±SD	180±36			175±33
Normal sperm (%)	Mean±SD	97.1 ± 1.5	-	-	95.8±2.3*
Total abnormal sperm(%)	Mean±SD	2.9±1.5	-	-	4.2±2.3*
Head abnormal (%)	Mean±SD	0.8±0.8			1.0±0.8
Head flat(%)	Mean±SD	0.7 ±0.7	-	-	1.0±0.8
	FI generation males				
	N	**20**	**20**	**20**	**20**
Motile sperm(%)	Mean±SD	93±5	92±5	94±3	94±4
Progressively motile sperm(%)	Mean±SD	44±9	41 ±10	43±12	43±12
Cauda epididymis (left) - Weight (g)	Mean±SD	0.204±0.019			0.191 ±0.025
Cauda epididymis (left) - Sperm count (millions/g)	Mean±SD	453±93			478±71
Cauda epididymis (left) - Total sperm count (million)	Mean±SD	92±18	-	-	92±21
Testis (left) - Weight (g)	Mean±SD	1.83±0.14			1.70±0.16**
Testis (left) - Spermatid count (millions/g)	Mean±SD	97±19			81 ±18**
Testis (left) - Total spermatid count (million)	Mean±SD	178±39	-	-	136±30**
Normal sperm(%)	Mean±SD	96.4±2.2	96.6±2.4	96.3±2.6	94.3±5.4
Total abnormal sperm(%)	Mean±SD	3.6±2.2	3.4±2.4	3.7±2.6	5.7± 5.4
Head abnormal(%)	Mean±SD	1.1 ± 1.0	1.0±0.7	1.0± 1.0	2.3±2.1*
Head flat(%)	Mean±SD	1.0±0.9	0.7±0.6	0.9±1.0	2.1 ± 1.9*
Average path velocity (VAP) (μm/s)	Mean±SD	146±12	137± 12*	138±14*	140±12*
Curvilinear velocity (VCL) (μm/s)	Mean±SD	357±28	337±37	328±7*	331 ±36 *
Beat cross frequency (BCF) (Hz)	Mean±SD	36±2	35±2	35±3	35±3*

Abbreviations: *N*, Number of animals examined;-, not measured; SD, standard deviation.

*$p<0.05$ (t-test).

**p<0.01 (t-test).

TABLE 6 Testes and epididymis weights, EOGRTS (#100).

	ppm	0	120/60	600/300	3000/1500
Testes and epididymis weights #	**mg/kg bw per day**	**0**	**8.65**	**44.3**	**223**
	F0 generation males				
	N	**25**	**25**	**25**	**25**
Testes weight - absolute (g)	Mean±SD	3.810±0.573	4.105 ± 0.295 *	3.751 ±0.731	3.864± 0.368
Testes weight - relative to body weight(%)	Mean±SD	0.842±0.145	0.894±0.076	0.845 ± 0.178	0.903 ± 0.105
Epididymis weight - absolute (g)	Mean±SD	1.459±0.172	1.522±0.115	1.389 ± 0.234	1.425±0.117
Epididymis weight - relative to body weight (%)	Mean±SD	0.322 ± 0.045	0.331±0.031	0.314 ± 0.055	0.333± 0.032
	FI generation males- cohort 1A				
	N	**20**	**20**	**20**	**20**
Testes weight - absolute (g)	Mean±SD	3.635 ± 0.263	3.572 ± 0.257	3.732 ± 0.245	3.352 ± 0.343**
Testes weight - relative to body weight(%)	Mean±SD	0.967 ± 0.086	0.968 ± 0.076	1.000 ± 0.100	0.986±0.091
Epididymis weight - absolute (g)	Mean±SD	1.252±0.101	1.234 ± 0.108	1.246 ± 0.119	1.156±0.136*
Epididymis weight - relative to body weight(%)	Mean±SD	0.333 ± 0.030	0.335 ± 0.030	0.333 ± 0.036	0.340±0.Q35
	FI generation males - cohort 1B				
	N	**20**	**20**	**20**	**20**
Testes weight - absolute (g)	Mean±SD	3.724 ± 0.423	3.740 ± 0.287	3.828±0.299	3.473 ± 0.276*
Testes weight - relative to body weight(%)	Mean±SD	0.956±0.126	0.963 ± 0.108	0.979±0.100	0.934±0.069
Epididymis weight - absolute (g)	Mean±SD	1.299±0.147	1.330±0.145	1.316±0.121	1.217±0.105
Epididymis weight - relative to body weight(%)	Mean±SD	0.334±0.048	0.342 ± 0.041	0.336 ± 0.035	0.328±0.031

Abbreviations: *N*, Number of animals examined; SD, standard deviation.

'Left and right.

*$p < 0.05$ (Williams' test).

**p<0.01 (Williams' test).

FI cohort IA showed changes in immunophenotyping. Results are discussed under immunotoxicity (see **Section 3.2.3.4.1**).

3.2.3.3.2 *Developmental toxicity*

Developmental toxicity was investigated in rats (#6, #7, #50) and rabbits (#87, #88, #101, #102, #103, #130, #162) (see Appendix E).

The most sensitive species to the developmental toxicity ofTFA Na is the rabbit as evidenced by different eye malformations, e.g. multiple folded retina, absent aqueous/vitreous humour in several studies. The relevant developmental toxicity NOAEL is 60 mg/kg bw per day in an OECD TG 414 (#103) based on different eye malformations at 250 mg/kg bw per day (see Table 7). Some skeletal findings (i.e. fused and partially fused ribs and sternebrae), reduced fetal weight and increased post-implantation loss were observed at the dose level of 750 mg/kg bw per day (Tables 8-10, respectively).

The maternal NOAEL is 30 mg/kg bw per day based on increased absolute liver and kidney weight with histopathological correlation in the liver at 60 mg/kg bw per day. In addition, satellite phase dams were investigated for toxicokinetics, blood chemistry, haematology and both maternal and fetal aqueous/vitreous humour. An additional developmental toxicity study in rabbits was submitted to ECHA RAC to characterise the changes of the visual pathway of rabbits.[24] In this study, two abortions were found at the dose level of 375 mg/kg bw per day (only one dose level was tested). Study results are not impacting the lowest NOAEL/LOAEL setting for developmental toxicity.

TABLE 7 Fetal examinations - major abnormality findings - group incidences/group mean percentage of litters (developmental toxicity study in rabbits, #103).

Dose (mg/kg bw per day)	**0**	**30**	**60**	**250**	**750**
Number examined (Litters)	**28**	**29**	**28**	**28**	**28**
	NI%	***NI%***	***NI%***	***NI%***	***NI%***
Multiple folded retina	0/0	0/0	0/0	2/7.1	9/32.1**
Absent aqueous/vitreous	0/0	0/0	0/0	1/3.6	7/25.0**
Misshapen lens(es)	0/0	0/0	0/0	0/0	2/7.1
Haemorrhage aq./vit.	0/0	0/0	0/0	0/0	5/17.9*

Abbreviations: *N*, number of affected animals.

*p <0.05. **p <0.01.

- The proportion of litters with at least one fetus affected were analysed using Fisher's (Thakur, 1985) exacttests (uppertail). The percentage of affected fetuses for each animal/litter were analysed using a Kruskal-Wallis nonparametric ANOVA (Lehmann, 1975).
- If the Kruskal-Wallis test was significant *(p,;0.05)*, pairwise comparisons of each treated group with the control group were made using the Wilcoxon rank sum test (Lehmann, 1975).
- If the Kruskal-Wallis test was not significant *(p>0.05)*, no further analyses were conducted. Where only two groups were available for analysis a Wilcoxon rank sum test was performed.

TABLE 8 Fetal examinations - skeletal abnormality findings (prenatal developmental toxicity study in rabbits, #103).

	Fetuses					**Litters**				
Dose (mg/kg bw per day)	**0**	**30**	**60**	**250**	**750**	**0**	**30**	**60**	**250**	**750**
	Group 1	**Group2**	**Group3**	**Group4**	**Group 5**	**Group 1**	**Group2**	**Group 3**	**Group4**	**Group 5**
Number examined	**209**	**244**	**219**	**193**	**178**	**28**	**29**	**28**	**28**	**28**
	NI%	*NI%*	*NI%*	*NI%*	*NI%*	*NI%*	*NI%*	*NI%*	*NI%*	*NI%*
Ribs Fused/partially fused	0/0	1/0.4	0/0	1/0.4	6/2.8	0/0	1/3.4	0/0	1/3.6	3/10.7
Sternebrae Fused/ partially fused	1/0.5	0/0	3/2.0	3/1.5	3/1.6	1/3.6	0/0	2/7.1	3/10.7	3/10.7

Abbreviations: *N*, number of affected animals.

*p <0.05. **p <0.01.

- The proportion of litters with at least one fetus affected were analysed using Fisher's (Thakur, 1985) exact tests (upper tail). The percentage of affected fetuses for each animal/litter was analysed using a Kruskal-Wallis non-parametric ANOVA (Lehmann, 1975).
- If the Kruskal-Wallis test was significant *(p,;0.05)*, pairwise comparisons of each treated group with the control group were made using the Wilcoxon rank sum test (Lehmann, 1975).
- If the Kruskal-Wallis test was not significant *(p>0.05)*, no further analyses were conducted. Where only two groups were available for analysis a Wilcoxon rank sum test was performed.

[24]Study submitted under Comment 47 of the ECHA public consultation: https://echa.europa.eu/documents/10162/e7cc66e0-aa3f-fd05-b35d-fe2647db3fbc: Anonymous, 2025. SODIUM TRIFLUOROACETATE: A PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS WITH 14-DAY RECOVERY.

TABLE 9 Overall fetal and litter weight in rabbits on Day 29 of gestation (prenatal developmental toxicity study in rabbits, #103).

	Litters				
Dose (mg/kg bw per day)	**0**	**30**	**60**	**250**	**750**
Mean/SD	**Group 1**	**Group 2**	**Group 3**	**Group4**	**Groups**
N	**28**	**29**	**28**	**28**	**28**
Total litter weight (g)	299.9/87.6	335.8/71.22	309.8/94.26	265.0/89.59	232.7**/84.36
Overall fetal weight (g)	41.1/5.39	40.0/3.59	40.4/4.97	40.3/5.76	37.4**/4.81

Abbreviations: *N*, Number of animals examined; SD, standard deviation.

***p* < 0.01 - **p* <0.05: treated groups compared with control using Williams' test.

TABLE 10 Post-implantation loss(%) in rabbits (prenatal developmental toxicity study in rabbits, #103).

	Litters				
Dose (mg/kg bw per day)	**0**	**30**	**60**	**250**	**750**
Mean/SD	**Group 1**	**Group2**	**Group 3**	**Group4**	**Groups**
N	**28**	**29**	**28**	**28**	**28**
Post-implantation loss(%)	8.1/13.13	7.7/9.41	4.6/7.74	16.4/21.89	19.4*/22.16

Abbreviations: N: Number of animals examined; SD, standard deviation.

Note: Data were square root transformed for the statistical analysis. ***p*< 0.01 - **p*< 0.05-treated groups compared with control using Williams' test.

In a supplementary study (#88), eye malformations were observed in rabbit fetuses at the lowest dose level of 180 mg/kg bw per day, whereas in dams, only minimal liver effects were noted.

Regarding the moderate severity of maternal toxicity in different studies, the findings in rabbit fetuses were not considered to be secondary to toxic effects observed in dams.

TFA did not induce maternal and developmental toxicity in rats up to 150 mg/kg bw per day (highest dose level tested, #7). The WG noted that the multiple anomalies observed were found in six individual fetuses from one single litter (1 out of 21) at 150 mg/kg bw per day (see Table 11).In the rat reproductive toxicity study (EOGRTS, #100), rosettes/folds in the retina were observed in three females of the FI generation at histopathological examination; however, it is noted that rosettes were already observed in the male control group of the F0 generation (see Table 12).

TABLE 11 Abnormalities observed in fetal and litters examinations - (developmental toxicity study in rats, #7).

Dose level (mg/kg per day)	Dam No.-fetus No.	External	Visceral	Skeletal
0				
37.5	2504-3		5ubclavian artery: retroesophageal	
75				
150	4501-1	Umbilicus: omphalocele Hindlimbs: flexure, malrotated		Ribs: partially fused
	4501-2	Umbilicus: omphalocele	Abdomen: omphalocele □: retina, bilateral, folded	
	4501-3	Umbilicus: omphalocele		Ribs: partially fused Sternum: partially cleft/split Thoracic vertebral arches: partially fused
	4501-4	Abdomen: gastroschisis Hindlimbs: flexure, malrotated		Ribs: partially fused Thoracic vertebral arches: partially fused
	4501-6	Umbilicus: omphalocele	Abdomen: omphalocele	
	4501-9	Umbilicus: omphalocele		Ribs: partially fused

TABLE 12 Histopathology of the eyes, EOGRTS in the rat (#100).

	Males				Females			
Dose(ppm)	**0**	**120/60**	**600/300**	**3000/1500**	**0**	**120/60**	**600/300**	**3000/1500**
Dose (mg/kg bw per day)	**0**	**8.65**	**44.3**	**223**	**0**	**8.65**	**44.3**	**223**
	F0 generation							
N	**25**	**0**	**0**	**25**	**23**	**0**	**0**	**23**
Eyes - Rosettes, Retina (minimal)	3			0	0			0
	FI generation - Cohort 1A							
N	**20**	**0**	**0**	**20**	**20**	**0**	**0**	**20**
Eyes - Rosettes/Folds, Retina (minimal)	0			0	0			3

Abbreviation: *N,* Number of animalsexamined.

3.2.3.4 *Other toxicological effects*

3.2.3.4.1 Immunotoxicity

The WG assessed whetherTFA affected immunological endpoints across available repeated-dose toxicity and reproductive toxicity studies. The assessment was based on relevant immunological endpoints as described by Chemicals Regulation Directorate (CRD, 2015). Within the available toxicity data package on TFA no consistent or dose-dependent effects on immunological endpoints were observed across studies (Annex 10). For example, a decrease in lymphocyte count was observed at the top dose in male rats in F0 and FI (#100) and top dose in female rats (#15). However, these findings were not observed in other studies up to the high dose level tested (more than 1000 mg/kg bw per day, #8) (see Tables 13-15).

Evidence from retrospective analysis on pesticide active substances (CRD, 2015; Gehen et al., 2014; US EPA, 2013) indicates that immunotoxicity, as assessed by the T-cell-dependent antibody response (TDAR) assays in adult animals, or by analysis of standard immunotoxicity endpoints, is rarely a major driver for deriving the reference point for risk assessment (see Appendix I).

TABLE 13 Female rat lymphocytes (14-day toxicity study in rat; #15).

Group	**Control**	**2**	**3**	**4**
Dose in mg/kg bw per day	**0**	**45.41**	**91.08**	**189.59**
N	**5**	**5**	**5**	**5**
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**
Female lymphocyte count	9.3 ± 1.3	8.3±1.7	7.1 ± 1.3	5.8± 1.5*

Abbreviations: *N,* Number of animalsexamined; SD, standard deviation.
**p,;* 0.05 (Dunnett).

TABLE 14 Male and female rat lymphocytes (90-day toxicity study in rat; #8).

	Male				**Female**			
Group		**2**	**3**	**4**		**2**	**3**	**4**
Dose in mg/kg bw perday	**0**	**9.9**	**98**	**1043**	**0**	**12.2**	**123**	**1216**
N	**10**	**10**	**9**	**10**	**10**	**10**	**10**	**10**
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
Lymphocyte count (10E9/L)	12.0±3.2	10.0±2.2	11.3±2.1	10.6±3.5	7.5±2.2	7.1±1.6	6.4± 1.5	6.1± 1.4
Lymphocyte count (%)	81±3	78±4	80±6	78±4	81±5	80±4	76±6	79±5

Note: Bart; NSg-05/Anova: NSg-05/No unplanned test performed.
Abbreviations: *N,* Number of animalsexamined; SD, standard deviation.

TABLE 15 Haematology- monocytes and lymphocytes changes in male rats (EORGTS, #100).

Dose(ppm)	0 Group 1M	120 Group2M	600 Group 3M	3000 Group4M
F0 generation				
N	**10**	**10**	**10**	**10**
Dose (mg/kg bw per day)	**0**	**9,71**	**49.1**	**248**
Lymphocytes (x 10^9/L) (mean/SD)	2.74/0.409	3.62/2.731	3.48/0.936	2.80/0.557
Monocytes (x 10^9/L) (mean/SD)	0.12/0.031	0.14/0.091	0.10/0.034	0.08*/0.031
FI generation				
N	**10**	**10**	**10**	**10**
Dose (mg/kg bw per day)	**0**	**9.37**	**47.3**	**242**
Lymphocytes (x 10^9/L) (mean/SD)	4.08/3.116	3.27/0.860	2.97/0.553	2.59*/0.558
Monocytes (x 10^9/L) (mean/SD)	0.12/0.094	0.12/0.033	0.11/0.057	0.07*/0.026

Abbreviations: *N*, Number of animalsexamined; SD, standard deviation.

Immunophenotyping of T cells (including CD4+ and CD8+ T subsets), B cells, natural killer cells, monocytes and neutrophils was performed by flow cytometry on leucocytes isolated from the spleen of rats (FI cohort IA, week 13) in the EOGRTS (#100); however, a cohort for immunotoxicity was not included in this study. A decrease in absolute cell counts in the spleen was observed in both sexes at all dose levels for all previously analysed cell populations. The relative composition of lymphocyte subsets remained unchanged, which indicates no shift in CD4+/CD8+ ratio or other cellular distributions (see Table 16).

TABLE 16 Male and female rat spleen leucocytes count, white blood cells/spleen (FI cohort 1A, week 13; EOGRTS (#100)).

Dose in ppm	**0**	**120/60**	**600/300**	**3000/1500**
Dose in mg/kg bw per day	**0**	**8.65**	**44.3**	**223**
N	**10**	**10**	**10**	**10**
	Mean±5D	**Mean±SD(%**	**Mean±SD (%**	**Mean±SD (%)**
Male - spleen leucocytes (count)	12 E+07±4 E+07	10 E+07±4 E+07 (-12)	8 E+07 ± 2 E+07 (-27)	1 E+07±4 E+07 (-33)
Female - spleen leucocytes (count)	12 E+07±3 E+07	10 E+07±2 E+07 (-14)	9 E+07 ± 3 E+07 (-22)	8 E+07 ± 3 E+07 (-33)
Male - CD4+/CD8+ ratio	2.4±0.6	2.5±0.5	2.8±0.5	2.7±05
Female - CD4+/CD8+ ratio	2.7±0.4	2.4±0.7	2.4±0.6	2.5±0.7

Abbreviations: *N*, Number of animalsexamined; SD, standard deviation.

Note: No statistical analysis conducted.

%Effect size.

The observed decreases in absolute splenic immune cell numbers across doses suggest a potential immunomodulatory effect ofTFA, although their biological significance remains uncertain since immunophenotyping provides mechanistic insight into the steady-state lymphocyte subset distributions and absolute cell numbers in the spleen, but does not directly assess the immune function, such as increased susceptibility to infection or the ability to generate an effective immune response. While reductions in the number of splenic immune cells occurred in both sexes, lymphocyte subset distribution and the CD4+/CD8+ ratio were unchanged. In addition, no corroborating histopathological alterations were reported. However, the functional immune competence (e.g. the capacity to generate antibody response or resist infection) was not evaluated. In the absence of functional immunotoxicity testing, these findings introduced uncertainty regarding possible developmental immunotoxicity since effects were observed in animals where exposure has already happened in utero. This uncertainty was addressed through an additional database-related uncertainty factor, as the available data are insufficient to establish a NOAEL specifically for immune effects in the developing organism.

3.2.3.4.2 Neurotoxicity

No specific oral neurotoxicity studies with TFA have been submitted. Animals administered TFA in repeated dose studies (#8, #15, #16, #109, #124) and in the EOGRTS (#100) did not show clinical signs of neurotoxicity nor were the effects observed for exploratory motor activity, in open field observations, in sensory reactivity, or in grip strength (#8). There were also no treatment-related effects on brain weight (#8, #15, #16, #100, #109, #124) and histopathology (#8, #16, #100, #109, #124) in adults, where these were subject to investigation.

3.2.3.4.3 Endocrine disruption

As described above, evidence of endocrine disruption was observed in the EOGRTS (#100) and discussed in Section **3.2.3.3.1.**

3.2.4 Observations in humans

3.2.4.1 *Biomonitoring*

Three biomonitoring studies (#91, #94 and #96) measured TFA in several human matrices, with one of them reporting only qualitative results. All three were evaluated using the dedicated Risk of Bias (RoB) tool (see Appendix E). Based on this assessment, study #91 was assigned to Tier 1 and study #94 to Tier 2. Consequently, only these two studies (both measuring serum concentrations in non-European populations) were retained for further assessment (see Table 17).

Study #96, which analysed urine samples from a Belgian population, was classified as Tier 3 and excluded from further consideration due to a high RoB. This study relied on a non-targeted suspected-screening analytical approach, reported only presence/absence data without quantitative measurements, and did not employ a TFA reference standard. Compound identification was based on indirect MS/MS spectral confirmation, resulting in only tentative identification of TFA and low analytical confidence.

The two biomonitoring studies appraised as Tier 1 or Tier 2 following RoB analysis are summarised in Table 17. It should be noted that both cross-sectional studies, which measured TFA in serum, were conducted outside Europe.

TABLE 17 Summary of the biomonitoring studies measuring TFA levels.

Study reference	Population group	Biological matrix	Method	Median levels and detection rate	Study design
#91	Adults (n=252)	Serum	HPLC-MS/MS	Median: 8.46 ng/ml Detection rate: 97.0%	Cross-sectional
#94	Neonates (n=66)	Umbilical cord serum	UPLC-MS/MS	Median: 0.229 ng/ml Detection rate: 55%	Cross-sectional

Abbreviations: HPLC-MS/MS, High Performance Liquid Chromatography- Mass Spectrometry; UPLC-MS/MS, Ultra-high Performance Liquid Chromatography- Mass Spectrometry.

Overall, the available human biomonitoring evidence provides qualitative support for internal exposure to TFA in humans, indicating systemic absorption and background population levels, but is limited by methodological constraints and RoB considerations, precluding its use for quantitative hazard characterisation.

3.2.4.2 *Epidemiological studies*

Among the identified biomonitoring studies, only study #91 (summarised in Annex 5) included outcome data in addition to exposure measurements. For this reason, it was additionally evaluated as an epidemiological study using the National Toxicology Program (NTP) Office of Health Assessment and Translation (OHAT) tool (NTP, 2015, 2016) (see Annex 5), which includes criteria relevant to outcome assessment, confounding, attrition and statistical analysis. This study received an overall Tier 3 rating due to a 'probably high risk of bias' judgement in a key confounding domain (confounding bias) and was therefore not retained for further consideration.

3.2.5 Mode of action of TFA effects

Six in vitro mechanistic studies were identified as Tier 1 or Tier 2 following RoB analysis (**Annexes 6 and 7**) and summarised below:

- TFA did not show a concentration-response relationship on the differentiation of stem cells in the in vitro embryonic stem cell test (#10);
- TFA when exposed to DLD-1 human colorectal adenocarcinoma cells is transported by the human monocarboxylate transporter 1 (MCTl), assessed by quantifying intracellular uptake based on acidification of these cells with an EC50 of 12 mM. MCTl-mediated cellular uptake was evidenced by the inhibition of its uptake in the presence of the specific and potent MCTl inhibitor AR-C155858 (#114);
- TFA Na did not activate any of the stress response pathways measured in seven human reporter HepG2 cell lines (#131);
- Exposure to TFA Na had no significant impact on gene expression profiles or cellular morphology/function in human induced pluripotent stem cells differentiating into cardiomyocyte-, hepatocyte- and neuronal-like cells (#132);
- TFA was found to significantly decrease testosterone production in hormonally stimulated isolated rat Leydig cells following 5-h exposure (whereas no clear and consistent effect on testosterone production was observed following 24-h

exposure). TFA was also found to enhance lactate production by Sertoli cell-only cultures hormonally stimulated; the relevance of this single finding, in the absence of effect on the morphology of the cells and on effects on pyruvate production, is unknown according to the authors. Overall, TFA showed a slight effect on Leydig cell function and no clear effect on the viability and function of Sertoli cell-only cultures, and Sertoli-germ cell co-cultures derived from Sprague Dawley rats (#169).

- TFA suppressed antibody production (immunoglobulins IgG and IgM) in human peripheral blood mononuclear cells (PBMCs) following 7-day exposure (24-h pre-exposure, followed by 6-day stimulation of exposed cells) (Iulini et al., 2025). The WG noted that the concentration-related decrease was clearer for IgM release in female PBMCs compared to the decrease of IgG in female PBMCs or IgG or IgM releases in male PBMCs. The study showed some limitations in reporting: non-normalised data not shown, results for TFA are presented in a figure only (non-tabulated data) and it is not clear whether the statistical analysis has been performed in normalised or non-normalised data. The results pointed to a potential immunomodulatory mode of action for TFA.

Findings from a 14-day in vivo oral toxicity study (#15) suggest that TFA may act as a weak **peroxisome proliferator** in male rats, as evidenced by increased palmitoyl-CoA oxidation activity, a biomarker of peroxisomal □-oxidation and peroxisome proliferator-activated receptors (PPARs) activation. PPARs are nuclear receptors that play a central role in regulating lipid and glucose metabolism (Kim & Ahn, 2004; Kim et al., 2003), and their activation can influence energy homeostasis and metabolic adaptation. In addition, PPARs (particularly PPARy and PPARa) exert important immunomodulatory functions by linking cellular metabolism to immune cell differentiation and activity; for example, PPARy promotes anti-inflammatory macrophage polarisation and regulates T-cell responses, while PPARa can suppress pro-inflammatory signalling pathways such as NF-KB. Through these mechanisms, PPAR activation generally shifts immune responses towards a less inflammatory state in a context- and ligand-dependent manner (Christofides et al., 2021). In regulatory studies, TFA exposure was also associated with decreases in total bilirubin, cholesterol, triglycerides and glucose. In the absence of histopathological evidence of hepatocellular degeneration, this pattern is consistent with an adaptive hepatic response characterised by enhanced metabolic and clearance capacity rather than direct toxicity (Dekant & Dekant, 2023; Felter et al., 2018; Hall et al., 2012; Sugatani et al., 2001).

Evidence for PPARa involvement is limited, and TFA appears to act, at most, as a weak peroxisome proliferator in rodents and rodent-specific PPARa-mediated proliferative responses are considered of low human relevance (Felter et al., 2018; Dekant & Dekant, 2023;). Reductions in circulating bilirubin are more plausibly attributed to induction of hepatic conjugation and transport processes, including UDP-glucuronosyltransferase lAl (UGTlAl), which is primarily regulated via constitutive androstane receptor (CAR)-mediated pathways (Sugatani et al., 2001). Taken together, these receptor-mediated responses offer the most plausible explanation for the biochemical alterations reported, although they do not constitute definitive proof of a specific molecular target.

3.2.6 Consideration of non-critical effects

During the public consultation on this Scientific Report, numerous comments were received concerning the outputs of UBA (2020) and RIVM (2023), particularly with regard to the consideration of increased alanine aminotransferase (ALT) levels and decreased bilirubin levels observed in the available rat studies with TFA as relevant parameters for derivation of health-based guidance values. In addition, the EFSA WG on TFA organised a Technical Hearing with various stakeholders (including NGOs, industry representatives, UBA and RIVM) to explicitly gather their views on the selection of relevant parameters and reference points used by them and those to be applied in the current assessment ofTFA.

Following these activities, the WG carefully examined all arguments submitted. A comprehensive summary of the already available and newly submitted evidence, relevant to decide upon the relevance and benchmark for adversity of the following parameters has been prepared:

- Increase in alanine aminotransferase (ALT)

International guidelines for human safety assessment in evaluation of drugs consistently identify **ALT elevation** as a biomarker of liver toxicity in both non-clinical and clinical settings. Increases of two to three times the upper limit of normal (ULN) are generally considered indicative of hepatocellular damage in humans (EMA, 2010; FDA, 2009; HED, 2002), while in assessment of preclinical studies, increases of twofold to fourfold or higher compared with concurrent controls should raise concern unless an alternative explanation exists (Boone et al., 2005). A WoE approach recommends assessing ALT changes alongside other biomarkers of liver toxicity such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), y-glutamyltransferase (GGT) and glutamate dehydrogenase (GLDH), as well as clinical chemistry indicators like increase in bilirubin (Hall et al., 2012). In standard toxicity testing, mean ALT increases of twofold to fourfold in dogs or rats may raise concern and increases greater than threefold to fivefold are considered adverse even without histological changes (Boone et al., 2005; FDA, 2009). According to FDA guidance (2009), treatment discontinuation should be considered if ALT or AST exceeds eight times the ULN, remains above five times the ULN for more than 2 weeks or is greater than three times the ULN when accompanied by increased bilirubin or clinical symptoms. Health Canada (2012) applies similar thresholds, defining as one of the criteria for liver toxicity ALT values greater than two to three times ULN. JMPR (2015) notes that minor enzyme

fluctuations may be adaptive, with changes greater than 50 % in animal studies serving as a starting point for concern. In Corton et al. (2018), analysis of data indicates that increase in ALT above 141% was related to tumorigenic outcome in long-term rat studies; however, change in ALT was not observed as an isolated effect and histopathological information was not available in the primary Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System (TG-GATES) data set used for deriving the thresholds. Beyond its role as a marker of hepatocellular damage, increased intracellular ALT expression has also been linked to the administration of PPAR agonist drugs such as fibrates, suggesting that this mechanism may contribute to elevated serum ALT activity (Thulin et al., 2008). Overall, biological relevance and consistency across parameters are more important than statistical significance alone and although ALT is a relevant clinical pathology parameter for detecting chemically induced hepatocellular injury, the best approach remains an integrated evaluation of clinical pathology, histology and other study data (e.g. liver weight) (Boone et al., 2005; Hall et al., 2012; Ramaiah et al., 2017; Siska et al., 2022).

Consequently, in the absence of histopathological evidence of liver injury or sustained functional impairment, elevated ALT level is not considered a critical endpoint for the derivation of health-based guidance values.

- Decrease in bilirubin

The vast majority of references on **bilirubin changes indicate that increase, rather than decrease,** is generally associated with hepatic toxicity. Hall et al. (2012) notes that elevated bilirubin, especially when accompanied by increased bile acids, is a reliable indicator of impaired hepatic function, while decreases may be adaptive if linked to enhanced conjugation and excretion. ECETOC (2002) suggests that isolated changes beyond historical control values without histopathological correlation are typically non-adverse. EMA (2010) and FDA (2009) guidelines emphasise that concurrent increases in ALT and bilirubin should be given particular attention, as this pattern is associated with risk of liver failure in humans. Health Canada (2012) defines as one of criteria for liver toxicity conjugated bilirubin levels greater than two times ULN. In Corton et al. (2020), analysis of data indicates that increase in bilirubin above 115% was related to tumorigenic outcome in rat long-term studies; however, change in bilirubin was not observed as an isolated effect and histopathological information was not available in the primary TG-GATES data set used for deriving the thresholds. As regards decrease in bilirubin, JMPR (2015) states that low bilirubin levels are generally not of concern and often reflect microsomal enzyme induction. Mechanistically, this effect can be attributed to the upregulation of hepatic conjugation pathways, primarily by UGTIAl, and elimination via canalicular transporters such as MRP2 into the bile (Kock & Brouwer, 2012). There is some indication that PPARa agonists activate bilirubin metabolism in liver cells and its elimination by induction of PPARa-dependent UGTIAl (Bigo et al., 2014). In addition to the well-known origin of bilirubin as a natural yellow pigment produced during the breakdown of haemoglobin, there is evidence that bilirubin is a direct natural PPARa ligand that enhances fatty acid oxidation and alters lipoprotein metabolism (Kipp et al., 2025; Lee et al., 2025). Recent publications (Creeden et al., 2021; Kaur et al., 2025; Kipp et al., 2025; Lee et al., 2025) postulate bilirubin protective function stating that lower bilirubin level in humans can be related to higher risk of metabolic and cardiovascular diseases, obesity, type 2 diabetes, chronic kidney disease, systemic inflammation, ulcerative colitis, cognitive decline and sleep apnoea. However, the 'normal' reference ranges for bilirubin are debatable and need to be established across different subpopulations by taking into account potential confounders like sex, ethnicity, age, diet, smoking status, alcohol consumption, underlying liver disease, hemolytic disease, circadian rhythms, medication, physical activity, fasting status and other relevant variables (Vitek, 2019; Vitek et al., 2023). Because high variability in measured bilirubin levels is observed across analytical methods and automated clinical chemistry instruments, and bilirubin assays lack full methodological standardisation (Apperloo et al., 2005), these are considered among the least reliable clinical chemistry assays, resulting in poor comparability across epidemiological studies. Large, well-designed population studies are strongly recommended to overcome these limitations (Vitek, 2019 ; Vitek et al., 2023). No regulatory thresholds for decrease in bilirubin could be identified in regulatory guidance or relevant literature, either for observations in animals' toxicity studies or for clinical settings.

In context of cumulative risk assessment, clinical chemistry parameters and liver enzymes are ancillary and insufficiently specific to identify a liver specific toxicological effect, in contrast to liver-specific effects like histopathological lesions and increases in relative liver weight of<! 15% (EFSA, 2025).

Consequently, in the absence of histopathological evidence of liver injury or sustained functional impairment, elevated bilirubin level is not considered a critical endpoint for the derivation of health-based guidance values.

- Changes in cholesterol and glucose levels

In addition to ALT and bilirubin, the WG considered that observed changes in **cholesterol and glucose levels** in animal studies may be mechanistically linked to a mild nuclear receptor (e.g. PPARa) activation and subsequent peroxisome proliferation, processes known to modulate lipid and carbohydrate homeostasis in rodents. While these alterations were observed even at low doses tested, the absence of concurrent histopathological changes in key target tissues (such as the liver or endocrine pancreas) is considered indicative of an adaptive physiological response rather than an adverse effect. Although high-dose hepatocellular hypertrophy further supports a possible PPAR-mediated mode of action, the weight of evidence remains limited by the absence of robust, mechanistically targeted studies directly confirming PPAR involvement in TFA's biological activity. Overall, these clinical chemistry parameters are not considered critical endpoints for the derivation of health-based guidance values.

3.2.7 Consideration of critical effects and reference values

3.2.7.1 *Critical effects*

As described above (see **Section 3.2.3),** studies in rats have shown that the target organs ofTFA are thyroid (reproductive toxicity), liver (repeated dose toxicity) and fetal development (developmental studies). The effects seen at the lowest doses involve the thyroid (decrease in T4) and liver (increase weight and histopathology) (see Tables 2 and 5). Effects on development (eye malformations) have been reported at doses above 60 mg/kg bw per day (see Table 7.

3.2.7.2 *Dose-response analysis*

The dose response analysis of the critical effects identified in experimental animal studies was performed by means of the benchmark dose modelling (BMD). The EFSA guidance on the use of the benchmark dose modelling in risk assessment (EFSA Scientific Committee, 2022) was used. The full details of the BMD modelling are reported in Annex 11. The BMD modelling was considered and reported for all critical effects identified in Section **3.2.7.1.**

For the changes in thyroid, decreased T4 levels observed in adults and pups in the EOGRTS were modelled, considering a benchmark response (BMR) of 20% (see Appendix G).Independent analyses were performed for levels measured in adult rats (from parental and Fl generations) and pups (Fl generation, PND22). The BMD analyses resulted in the lowest benchmark dose lower confidence limit ($BMDL_{20}$) of 8.6 mg TFA Na/kg bw per day (adult male rats, F1), a value well in line with the NOAEL discussed in Section 3.2.3.3.1.

Increased relative liver weights and hepatocyte hypertrophy, observed upon subchronic exposure, were considered for BMD modelling of hepatic effects. For the analysis of the increased relative liver weight, a benchmark response (BMR) of 15% was selected as a relevant effect size, as recommended by JMPR (2015) and applied as relevant threshold in the European peer review for approval of pesticide active substances. The default BMR of 10% for quantal data was selected for the modelling of hepatocyte hypertrophy. Overall, for the selected hepatic effects, a lower $BMDL_{,0}$ of 18.6 mg TFA Na/kg bw per day was calculated for increased incidence of hepatocyte hypertrophy (male rats, study #8).

The combined incidences of eye malformations (absence of aqueous/vitreous humour and multiple folding of retina) in two developmental toxicity studies in rabbits (studies #88 and #103) were considered for BMD modelling of developmental effects. In view of the severity and low incidence of the effects, a protective BMR of 0.5% was selected. Separate BMD analyses of the individual data from the two studies indicated that in neither of them litter effects substantially affected the dose-response analysis (data not shown); therefore, aggregated analysis was performed, allowing the combined modelling of the two studies using the study as covariate. From this latter analysis, a lower $BMDL_{05}$ of 41.1 mg TFA Na/mg kg per day was calculated (study #88).

3.2.7.3 *Health-based guidance values*

The existing HBGVs for TFA were set during the peer review of the pesticide risk assessment of the active substance flurtamone (EFSA, 2017). In particular, an ADI of 0.05 mg/kg bw per day (expressed as TFA Na) was derived from the 90-day oral toxicity study in Wistar rats(# 8) based on a NOAEL of 9.9 mg/kg bw per day and applying the standard uncertainty factor of 100 plus an additional UF of 2 for the extrapolation from sub-chronic to long-term exposure. An ARfD was deemed unnecessary. Since that evaluation, new studies were made available.

The BMDL values identified for the critical effects ranged from 8.6 to 41.1 mg TFA Na/kg bw per day. The WG agreed that, based on the new evidence, the most appropriate reference point for setting the **ADI** is the $BMDL_{20}$ of 8.6 mg/kg bw per day, derived from changes in thyroid hormones (decreased T4 levels; adult male rats, Fl) in the EOGRTS (#100). The WG also agreed to apply the standard uncertainty factor of 100 to account for inter- and intra-species differences (EFSA Scientific Committee, 2012), together with an overall additional factor of 5 to address the uncertainties related to the absence of a long-term toxicity/carcinogenicity study and the possible developmental immunotoxicity, given the lack of functional immunotoxicity testing for TFA during the developmental phase. The overall additional uncertainty factor of 5 is based on expert's judgement, following the uncertainty analysis reported in **Section 4**. The resulting ADI is 0.014 mg/kg bw per day (expressed as TFA[25]).

For compounds having an effect on the hypothalamic-pituitary-thyroid axis, transitory decreased T4 levels during a certain period of pregnancy may have an impact on the development of the brain.Therefore, the effects on decreased circulating T4 levels are considered suitable for setting an **ARfD.** In the EORGTS (#100), $BMDL_{20}$ equal to 8.6 mg/kg bw per day is set based on T4 decrease in Fl males and is considered protective of all population in the EOGRTS in the absence of thyroid hormone analysis in the pregnant dams. Based on this $BMDL_{20}$ an ARfD of 0.07 mg/kg bw (expressed as TFA[16]) was established by applying a standard uncertainty factor of 100 to allow for inter- and intra-species differences.

[25]A conversion factor of 0.84 was applied to adjust for the difference in relative molecular weight between TFA Na and TFA, i.e., calculated from the ratio ofTFA (114 g/moll to TFA Na [136 g/mol].

4 UNCERTAINTY ANALYSIS

In the context of establishing consumer HBGVs, the WG discussed the several areas of assessment with the aim to identify potential sources of uncertainties that may underestimate the hazard and impact derivation of the HBGV (see Table 18).

Ordinal scales describe uncertainty using ordered categories like high, medium or low, so the analysis is qualitative for priority ranking (EFSA Scientific Committee, 2018). The WG considered that all uncertainties classified as of low impact would not underestimate the hazard and would not change the setting of HBGV (to a lower value) if they were resolved.[26]

TABLE 18 Identification and prioritisation of sources of uncertainty for the derivation of the HBGV.

Area of assessment	Sources of uncertainty	Description	Impact on the derivation of theHBGVs
Toxicokinetics (TK)	Information on absorption	No guideline-compliant ADME studies were submitted for TFA. The absence of mass-balance ADME studies precludes calculation of absolute oral bioavailability. Based on available TK investigations in rats and rabbits, TFA is absorbed after oral exposure and distributes systemically, including to the fetus and the eye.	Low
	TK parameters in humans	No guideline-compliant ADME studies were submitted for TFA. No direct human toxicokinetic data (such as plasma protein binding or elimination half-life) are available, limiting interspecies extrapolation and the translation of animal data to potential human risk.	Low
	Information of accumulation	No guideline-compliant ADME studies were submitted for TFA. Based on its physicochemical properties (low log Kaw, low bioconcentration factor and high polarity), TFA is expected to have a low potential for bioaccumulation.	Low
	Information on elimination	No guideline-compliant ADME studies were submitted for TFA. Based on its physicochemical properties (low log Kaw, low bioconcentration factor and high polarity), TFA is expected to have a low potential for bioaccumulation.	Low
	Information on the extent of metabolism	No guideline-compliant ADME studies were submitted for TFA. Under physiological pH, TFA and TFA salts dissociate, resulting in systemic exposure to the TFA anion.	Low
	Design of the TK investigations in experimental animals.	Analytical issues, including the possibility of contamination, introduce uncertainty around low-level measurements in control animals, which may affect baseline comparisons	Low
Genotoxicity	Information on in vivo genotoxicity	Lack of in vivo genotoxicity studies. Considering that TFA is unlikely to be genotoxic based on the in vitro tests, there is no need to perform in vivo tests, in line with EFSA Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011).	Low
	Design of chromosomal damage studies in vitro	Studies carried out with TFA Na report no induction of chromosomal damage, while inconsistency was observed among the studies performed with TFA showing a positive response in one chromosome aberration test and negative results in two in vitro micronucleus tests. Studies with TFA reported high variability in the level of cytotoxicity. No such variability was observed with TFA Na.The variability in cytotoxicity could be related to the physico-chemical characteristics ofTFA (a strong acid). Under physiological pH, TFA and TFA salts dissociate, resulting in systemic exposure to the TFA anion. Greater weight to the consistently negative results of chromosomal aberration studies conducted with the TFA salt than to those observed with the acid (see **Section 3.2.2**).	Low

[26]Self-task Mandate of the Plant Protection Products and their Residues (PPR) panel for a Scientific Opinion on waiving of the dog studies in the regulatory process of agrochemicals approval (EFSA-Q-2024-00199). https://open.efsa.europa.eu/question/EFSA-Q-2024-00199

TABLE 18 (Continued)

Area of assessment	Sources of uncertainty	Description	Impact on the derivation of theHBGVs
Toxicity studies in experimental animals: critical endpoints and critical study design	Design of studies in experimental animals	Lack of investigation of sperm/spermatid count at the low dose and mid dose in F0 and FI. Parental/offspring NOAEL considered protective by dividing the high dose (LOAEL) by a factor of 3 to extrapolate to a NOAEL (see **Section 3.2.3.3.1**).	Low
	Design of studies in experimental animals	Lack of investigation of thyroid toxicity (T4 levels and histopathology) in fetuses, in offspring before PND 22 and in pregnant dams. Uncertainty is covered by the standard uncertainty factor of 100 (see Appendix G)	Low
	Information on other effects that could be considered as critical	Lack of a developmental neurotoxicity study. Parental/offspring NOAEL considered protective based on T4 decrease.	Low
	Information on other effects that could be considered as critical	Lack of specific neurotoxicity studies. There are no indications of clinical signs of neurotoxicity in available toxicity studies (see **Section 3.2.3.4.2**).	Low
	Information on other effects that could be considered as critical	Lack of long-term and carcinogenicity studies. This uncertainty was taken into account by applying an additional uncertainty factor for the derivation of the ADI.	Medium
	Information on other effects that could be considered as critical	Lack of a developmental immunotoxicity cohort or an immunotoxicity testing using functional tests. This uncertainty was taken into account by applying an additional uncertainty factor for the derivation of the ADI.	Medium to High
	Information on non-rodent species.	Lack of dog studies The retrospective analysis (EFSA PPR Panel, 2026, in publishing) showed that, although dog studies were sometimes considered for HBGVs setting, genuine dog-specific sensitivity was rarely observed, as most differences in NOAELs in rodent and dog studies were due to experimental variability, study design or scaling rather than greater sensitivity of dogs.	Low
Observations in humans	Information in human biomonitoring studies	Evidence for human exposure is limited but indicates that humans are internally exposed to TFA.	Low
	Information in human epidemiological studies	Minimal information from epidemiological studies. However, it is already covered by the application of the standard uncertainty factor of 10.	Low
Mode of action	Uncertainties in the strength, consistency and specificity of the association of the key events and the critical effect.	Evidence for PPARa involvement is limited but tentatively postulated as the main mode of action.	Low
Identification of non-critical effects	Definition of adversity of some clinical chemistry findings.	Rats treated with TFA Na showed changes in some clinical chemistry parameters across all repeated dose studies with unclear adversity threshold and without clear dose-response (e.g. decrease in cholesterol, decrease in glucose and bilirubin levels in both sexes).	Low
Identification of target organ and critical effects	No uncertainty identified.		
Dose response analysis of critical endpoints	No uncertainty identified.		

Abbreviations: ADI, acceptable daily intake: ADME, absorption, distribution, metabolism and excretion: HBGVs, health-based guidance values: LOAEL, lowest observed adverse effect Level: NOAEL, no observed adverse effect level: TK, toxicokinetics.

Based on the uncertainty analysis done (see Table 18), two main areas of uncertainty were identified:

1. With respect to the lack of a long term (2-year)/carcinogenicity study on TFA, the WG considered that:

- In both the short-term (90-day) and chronic (52-week) toxicity studies in rats, there was no histopathological evidence of proliferative lesions, such as hyperplasia or other preneoplastic findings;
- TFA is unlikely to be genotoxic based on available data;

A peroxisome proliferator mode of action has been postulated; however, based on current scientific understanding, the human relevance to humans of this mode of action in relation to liver tumour formation is considered low; however, a robust mode of action study on TFA is not available.
Liver hypertrophy was reported in repeated dose toxicity studies (#15, #8, #124); it occurred at dose levels above 85 mg/kg bw per day.
In the chronic (52-week) rat study (#109), the maximum tolerated dose (MTD) was not achieved as the highest dose tested (37.8 mg/kg bw per day) was not properly selected. As a result, this study may have limited sensitivity to detect preneoplastic findings and does not fulfil the requirements of a full carcinogenicity assessment (see **Section 3.2.3.2**);

Overall, the WG concluded that an uncertainty remains due to the lack of a long-term (2-year)/carcinogenicity study and the limitations of the available chronic study. The WG categorised this uncertainty as medium.

2. With respect to immunophenotyping findings, the WG considered that:

A dose-related decrease in the total number of splenic immune cells was observed in both sexes at all dose levels in the EOGRTS in rats (FI, #100). For each individual cell type, the dose-response relationship was more evident in males than in females;
In the absence of functional immunotoxicity testing, these findings introduced uncertainty regarding possible developmental immunotoxicity;
Immunophenotyping results were not subjected to statistical analysis in the respective studies, limiting the interpretation of the findings;
A developmental immunotoxicity cohort or a functional test is not available for TFA, which could have elucidated potential immunotoxic consequences of the observed cellular changes;
Within the available in vivo toxicity data package on TFA or TFA Na, no consistent or dose-dependent effects on immunological endpoints were observed across studies in adult animals.
TFA suppressed antibody production (IgG and IgM) in human peripheral blood mononuclear cells (PBMCs) following 7-day exposure (Iulini et al., 2025).

Overall, the WG concluded that an uncertainty remains due to the lack of functional immunotoxicity testing for TFA following pre- and post-natal developmental exposure to follow up immunophenotyping findings and the results from the in vitro mechanistic study. The WG categorised this uncertainty as medium to high.

These uncertainties have been addressed by applying an additional overall uncertainty factor for the derivation of the ADI (see **Section 3.2.7.3**).

5 CONCLUSIONS

Based on a WoE of the available and reliable evidence, the WG concluded that TFA and its salt TFA Na are unlikely to be genotoxic. The WG agreed to set an ADI of 0.014 mg/kg bw per day and an ARfD of 0.07 mg/kg bw (both expressed as TFA).

ABBREVIATIONS

4NQO	4-Nitroquinoline 1-oxide
A/G ratio	Albumin Globulin ratio
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
APTT	Activated Partial Thromboplastin Time
ARfD	Acute Reference Dose
AST	Aspartate Transaminase
ATE	Acute Toxicity Estimate
AUC	Area Under the Curve
B(a)P	Benzo(a)pyrene
BCF	Beat Cross Frequency
BMD	Benchmark Mose Modelling
BMDL	Benchmark Dose Lower Confidence Limit
BMI	Body Mass Index
BMR	Benchmark Response
BROD	7-benzyloxyresorufin O-dealkylase
BW	Body Weight
BWG	Body Weight Gain
CA	Chromosomal Aberration

CAR	Constitutive Androstane Receptor
CAS	Chemical Abstract Service
CBPI	Cytokinesis-Block Proliferation Index
CHL	Chinese Hamster Lung cells
CHO	Chinese Hamster Ovary
CLH	Harmonised Classification and Labelling.
Cmax	Concentration Maximum
CoA	Coenzyme-A
CONTAM	EFSA Panel on Contaminants in the Food Chain.
CP	Cyclophosphamide
CTA	Comparative Thyroid Assessment
CV	Coefficient of Variation
CYP	Cytochrome
DIO	Iodothyronine Deiodinase
DMSO	Dimethyl sulfoxide
DNT	Developmental Neurotoxicity
ECHA	European Chemicals Agency
ED	Endocrine Disruptor
EMS	Ethyl methanesulfonate
EOGRTS	Extended One-Generation Reproductive Toxicity Study
EPA	US Environmental Protection Agency
EROD	Ethoxyresorufin O-deethylase
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GD	Gestation Day
GEF	Global Evaluation Factor
GGT	y-glutamyltransferase
GJIC	Gap Junctional Intercellular Communication
GLDH	Glutamate dehydrogenase
GLP	Good Laboratory Practice
HBGVs	Health-based guidance values
HCD	Historical Control Data
HCFCs	Hydrochlorofluorocarbons
HCG	Human Chorionic Gonadotropin
HFCs	Hydrofluorocarbons
hiPSCs	human-induced Pluripotent Stem Cells
HPLC-MS/MS	High Performance Liquid Chromatography - Mass Spectrometry
HPRT	human hypoxanthine-guanine phosphoribosyltransferase
HPT	Hypothalamic - Pituitary - Thyroid
IMF	Induced Mutant Frequency
InChiKey	International Chemical Identifier
IUPAC	International Union of Pure and Applied Chemistry
LC-HRMS	Liquid Chromatography - High Resolution Mass Spectrometry
LD	Lactation Day
LDS0	Median lethal dose
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCTI	Human Monocarboxylate Transporter 1
MCV	Mean Corpuscular Volume
MDL	Maximum Detection Limit
MI	Mitotic Index
MIH	Mitotic Inhibition
MMC	Mitomycin C
MMS	Methyl methanesulfonate
MNNG	Methynitronitrosoguanidine
MoA	Mode of Action
MOE	Margin of Exposure
MPV	Mean Platelet Volume
MTD	Maximum Tolerable Dose
n.a.	Not applicable

NEFA	Non-esterified Fatty Acids
NK	Natural killer
NOAEL	No Observed Adverse Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OHAT	Office of Health Assessment and Translation
PBMCs	human Peripheral Blood Mononuclear Cells
PFAS	Per- and polyfluoroalkyl substances
PFCAs	Perfluoroalkyl Carboxylic Acids
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFSAs	Perfluoroalkyl Sulfonic Acids
PMT	Persistent, Mobile and Toxic
PND	Postnatal Day
PPARs	Peroxisome Proliferator-activated Receptors
PPP	Plant Protection Product
PROD	Pentoxy-resorufin O-deethylase
PT	Prothrombin Time.
PTU	Propylthiouracil
QA/QC	Quality Assurance and Quality Control
RAC	Risk Assessment Committee
RBC	Red Blood Cells
REACH	Registration, Evaluation, Authorisation and restriction of Chemicals
RI	Replication Index
RICC	Relative increase in cell count
RoB	Risk of Bias
RPD	Relative Population Doubling
RS	Relative Survival
RSG	Relative Suspension Growth
RTG	Relative Total Growth
SCOC	Sertoli cell-only cultures
SD	Standard Deviation
SGCC	Sertoli-germ cell co-cultures
SMILES	Simplified Molecular Input Line Entry System
TDAR	T-cell Dependent Antibody Response
TFANa	Sodium Trifluoroacetate
TFA	Trifluoroacetic Acid
TFAK	Potassium Trifluoroacetate
TFSK	Potassium Trifluoromethanesulfinate
TG	Test Guideline
TG-GATES	Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System
TK	Toxicokinetic
Tmax	Time to maximum concentration
ToR	Terms of Reference
TPO	Thyroid Peroxidase
UF	Uncertainty Factor
ULN	Upper Limit of Normal
UPLC-MS/MS	Ultra-high Performance Liquid Chromatography - Mass Spectrometry
VAP	Average Path Velocity
VCL	Curvilinear Velocity
vPvM	very persistent and very mobile
WBC	White Blood Cells
WG	Working Group
WoE	Weight of Evidence

ACKNOWLEDGEMENTS

EFSA wishes to thank the following for the support provided to this scientific output: Wieneke Bil, Bas Bokkers (RIVM); Alexander Eckhardt, Helena Banning (UBA); Angeliki Lysimachou, Peter Clausing, Helmut Burtscher, Pauline Cervan (NGO representatives); Maura Karina Ferreira Emiliano, Anja Hueser, Antoinette Degroot, Lisa Bertomeu, Rich Bartlett, Stephanie Melching-Kollmuss (TFA Task force); Alexandra Fischer, Verena Haudek-Prinz, Lorenz Karl, Therese Weil (AT), Dirk Marien,

Liesbeth De Smet, Philippe Castelain, Kristel Brys (BE), Pernille Jacobsen, Peter Hammer Sorensen (DK), Tanja Heise, Franziska Kupprat (DE), Dimitra Nikolopoulou, Vasiliki Hatzi (EL), Manuel Sanz Bernal, Clara Aisa Sanchez, Veronica Campos Avedillo (ES), Francesca Metruccio (IT), Jessica Broeders (NL), Tanja Fatur (SI), Daniel Borg, Anneli Widenfalk, Anna Mentor (SE), Marianne Balmer, Christoph Geiser (CH) (Member States representatives).

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2024-00502

COPYRIGHT FOR NON-EFSA CONTENT

MAP DISCLAIMER

REFERENCES

Apperloo, J., van der Graaf, F., Scharnhorst, V., & Vader, H. L. (2005). Do we measure bilirubin correctly anno 2005? *Clinical Chemistry and Laboratory Medicine,* 43(5), 531-535. https://doi.org/10.1515/CCLM.2005.092

BAuA. (2025). Proposal for harmonised classification and labelling based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2: International chemical identification:Trifluoroacetic acid ... %. Version 3.0. https://echa.europa.eu/documents/10162/0dae2d2a-2b4c-b63e-c669-bd3a76197aa1.

Berridge, B. R., Mowat, V., Nagai, H., Nyska, A., Okazaki, Y., Clements, P.J., Rittinghausen, S., Tamaki, K., Vahle, J. L., & Kaufmann, W. (2016). Non-proliferative and proliferative lesions of the cardiovascular system of the rat and mouse. *Journal af Toxicalagic Pathology,* 29(3 Suppl), 15-475. https://doi.org/10.1293/tox.29.35-

Bigo, C., Kaeding, J., El Husseini, D., Rudkowska, I., Verreault, M., Vohl, M.-C., & Barbier, 0. (2014). PPARa: A master regulator of bilirubin homeostasis. *PPAR Research, 2014,* 747014. https://doi.org/10.1155/2014/747014

Bjiirnsdotter, M. K., Yeung, L. W. Y., Karrman, A., & Ericson Jogsten, I. (2020).Challenges in the analytical determination of ultra-short-chain perfluoroalkyl acids and implications for environmental and human health. *Analytical and Bioanalytical Chemistry, 472,* 4785-4796. https://doi.org/10.1007/s00216-020-02692-8

Boone, L., Meyer, D., Cusick, P., Ennulat, D., Provencher Bolliger, A., Everds, N., Meador, V., Elliott, G., Honor, D., Bounous, D., & Jordan, H. (2005). Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies. *Veterinary Clinical Pathology, 34,* 182-188. https://doi.org/10.1111/j.1939-165X.2005.tb00041.x

Chemicals Regulation Directorate. (2015). Retrospective analysis of the immunotoxic effects of plant protection products as reported in the Draft Assessment Reports for their peer review at EU level. *EFSA Supporting Publications,* EN-782.

Christofides, A., Konstantinidou, E., Jani, C., & Boussiotis, V. A. (2021). The role of peroxisome proliferator-activated receptors (PPAR) in immune responses. *Metabolism, 114,* 154338. https://doi.org/10.1016/j.metabol.2020.154338

Corton, C.J., Cunningham, M. L., Hummer, B.T., Lau, C., Meek, M.E., Peters, J.M., & Klaunig, J.E. (2020). Thresholds derived from common measures in rat studies are predictive of liver tumorigenic chemicals. *Toxicologic Pathology,* 48(7), 857-874. https://doi.org/10.1177/0192623320960412

Corton, C. J., Peters, J.M., & Klaunig, J.E.(2018). The PPARa-dependent rodent liver tumor response is not relevant to humans: Addressing misconceptions. *Archives of Toxicology, 92,* 83-119. https://doi.org/10.1007/s00204-017-2094-7

Creeden, J. F., Gordon, D. M., Stec, D. E., & Hinds, T. D., Jr. (2021). Bilirubin as a metabolic hormone: The physiological relevance of low levels. *American Journal of Physiology. Endocrinology and Metabolism, 320,* E191-E207. https://doi.org/10.1152/ajpendo.00405.2020

Danish Technical University, Nielsen, E., & Beltoft, V. (2021). Sundhedsm<Essig vurdering af trifluoreddikesyre og forslag til et sundhedsm<Essigt baseret kvalitetskriterium for drikkevand (No. 21/1025553).

Dekant, W., & Dekant, R. (2023). Mammalian toxicity of trifluoroacetate and assessment of human health risks due to environmental exposures. *Archives of Toxicalogy, 97,* 1069-1077. https://doi.org/10.1007/s00204-023-03454-y

Department Zorg. (2024). Diepte-analyse selectieprocedure gezondheidskundige advieswaarde voor trifluorazijnzuur (TFA) in drinkwater. https://assets.vlaanderen.be/image/upload/v1731657371/repositories-prd/PFAS_-_Gezondheidskundig_advies_voor_TFA_in_drinkwater_0_xlei0d.pdf

EFSA (European Food Safety Authority). (2017). Conclusion on the updated peer review of the pesticide risk assessment of the active substance flurtamone. *EFSA Journal,* 75(8), 4976. https://doi.org/10.2903/j.efsa.2017.4976

EFSA (European Food Safety Authority). (2020). Scientific opinion on the risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA Journal, 18(9),* 6223. https://doi.org/10.2903/j.efsa.2020.6223

EFSA (European Food Safety Authority). (2023a). Peer review of the pesticide risk assessment of the active substance tritosulfuron. *EFSA Journal,* 27(8), 8142. https://doi.org/10.2903/j.efsa.2023.8142

EFSA (European Food Safety Authority). (2023b). Harmonised approach for reporting reliability and relevance of genotoxicity studies. *EFSA Supporting Publications,* EN-8270. https://doi.org/10.2903/sp.efsa.2023.EN-8270

EFSA (European Food Safety Authority). (2024). Peer review of the pesticide risk assessment of the active substance flufenacet. *EFSA Journal,* 22(9), e8997. https://doi.org/10.2903/j.efsa.2024.8997

EFSA (European Food Safety Authority). (2025). Specific effects on liver relevant for performing a dietary cumulative risk assessment of pesticide residues. *EFSA Journal,* 23(5), e9409. https://doi.org/10.2903/j.efsa.2025.9409

EFSA Scientific Committee. (2011). Scientific Opinion on genotoxicitytesting strategies applicable to food and feed safety assessment. *EFSAJournal,* 9(9), 2379. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee. (2012). Guidance on selected default values to be used by the EFSA scientific committee, scientific panels and units in the absence of actual measured data. *EFSA Journal,* 70(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee. (2018). Scientific opinion on the principles and methods behind EFSA's guidance on uncertainty analysis in scientific assessment. *EFSA Journal,* 76(1), 5122. https://doi.org/10.2903/j.efsa.2018.5122

EFSA Scientific Committee. (2021). Scientific opinion on the guidance on aneugenicity assessment. *EFSA Journal, 19(8),* 6770. https://doi.org/10.2903/j.efsa.2021.6770

EFSA Scientific Committee, More, S. J., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T. I., Hernandez-Jerez, A. F., Bennekou, S. H., Koutsoumanis, K., Lambre, C., Machera, K., Mennes, W., Mullins, E., Nielsen, S.S., Schrenk, D., Turck, D., Younes, M., Aerts, M., Edler, L., ... Schlatter, J. (2022). Guidance on the use of the benchmark dose approach in risk assessment. *EFSA Journal,* 20(10), 7584. https://doi.org/10.2903/j.efsa.2022.7584

EFSA Scientific Committee. (2025). Risks to human and animal health from the presence of bromide in food and feed. *EFSA Journal,* 23(1), e9121. https://doi.org/10.2903/j.efsa.2025.9121

European Centre for Ecotoxicology and Toxicology of Chemicals. (2002). *Recognition of, and differentiation between, adverse and non-adverse effects in toxicology studies.* ECETOC.

European Chemicals Agency. (2023). Registry of restriction intentions until outcome: Restriction on the manufacture, placing on the market and use of per- and polyfluoroalkyl substances (PFAS): Annex XV restriction report (Version 2). https://echa.europa.eu/registry-of-restriction-intentions/-/dislist/details/0b0236el 8663449b

European Medicines Agency. (2010). Reflection paper on non-clinical evaluation of drug-induced liver injury (DILi). EMEA/CHMP/SWP/150115/2006.

Felter, S. P., Foreman, J.E., Boobis, A., Corton, J.C., Doi, A. **M.,** Flowers, L., Goodman, J., Haber, L. T., Jacobs, A., Klaunig, J. E., Lynch, A. **M.,** Maggs, J., & Pandiri, A. (2018). Human relevance of rodent liver tumors: Key insights from a toxicology forum workshop on nongenotoxic modes of action. *Regulatory Toxicology and Pharmacology, 92,* 1-7. https://doi.org/10.10l 6/j.yrtph.2017.11.003

Food and Drug Administration. (2009). In U.S. Food and Drug Administration (Ed.), *Guidance for industry: Drug-induced liver injury: Premarketing clinical evaluation.*

Gehen, S. C., Blacker, A. M., Boverhof, D.R., Hanley, T. R., Hastings, C. E., Ladies, G. S., & O'Neal, F. 0. (2014). Retrospective evaluation of the impact of functional immunotoxicity testing on pesticide hazard identification and risk assessment. *Critical Reviews in Toxicology, 44(5),* 407-419. https://doi.org/10.3109/10408444.2014.882291

Hall, A. P., Elcombe, C.R., Foster, J. R., Harada, T., Kaufmann, W., Knippel, A., Kuttler, K., Malarkey, D. E., Maronpot, R.R., Nishikawa, A., Nolte, T., Schulte, A., Strauss, V., & York, M. J. (2012). Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes-Conclusions from the 3rd international ESTP expert workshop. *Toxicologic Pathology, 40(7),* 971-994. https://doi.org/10.l 177/0192623312448935

Hassan, I., el-Masri, H., Kosian, P.A., Ford, J., Degitz, S. J., & Gilbert, M. E. (2017). Neurodevelopment and thyroid hormone synthesis inhibition in the rat: Quantitative understanding within the adverse outcome pathway framework. *Toxicological Sciences,* 760(1), 57-73. https://doi.org/10.1093/toxsci/kfx163

Health Canada. (2012). *Guidance document: Pre-market evaluation of hepototoxicity in health products.* Government of Canada.

HED. (2002). *Hepatocellular hypertrophy (HED Guidance Document#G2002.01).* U.S. Environmental Protection Agency.

Iulini, M., Galbiati, V., Marinovich, M., & Corsini, E. (2025). Decoding PFAS immunotoxicity: A NAMs-based comparison of short vs. long chains. *Frontiers in Toxicology,* 7, 1665163. https://doi.org/10.3389/ftox.2025.1665163

JMPR (Joint FAO/WHO Meeting on Pesticide Residues). (2015).*Guidance document for WHO monographers and reviewers: Pesticide residues in food.* World Health Organization.

JMPR (Joint FAO/WHO Meeting on Pesticide Residues). (2024). *Pesticide residues in food: Report 2024. Section 5.9: Cyclobutrifluram* (pp. 108-112). World Health Organization.

Kaur, A., Khadga, R., & Aran, R.(2025). Unraveling the dual role of bilirubin in neurological diseases: A comprehensive exploration of its neuroprotective and neurotoxic effects. *Brain Research, 1851,* 149472. https://doi.org/10.1016/j.brainres.2025.149472

Kim, H., Haluzik, M., Asghar, Z., Yau, D., Joseph, J. W., Fernandez, A. M., Reitman, M. L., Yakar, S., Stannard, B., Heron-Milhavet, L., Wheeler, M. B.,& LeRoith, D. (2003). Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes,* 52(7), 1770-1778. https://doi.org/10.2337/diabetes.52.7.1770

Kim, H. I., & Ahn, Y. H. (2004). Role of peroxisome proliferator-activated receptor-y in the glucose-sensing apparatus of liver and fl-cells. *Diabetes,* 53(Suppl. 1), 560-565. https://doi.org/10.2337/diabetes.53.2007.560

Kipp, Z. A., Pauss, S. N., Martinez, G. J., Hinds, T. D., Jr., & Lee, W. H. (2025). Bilirubin hepatic and intestinal transport and catabolism: Physiology, pathophysiology, and benefits. *Antioxidants,* 74(11), 1326. https://doi.org/10.3390/antioxl4111326

Klimisch, H.J., Andreae, M., & Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology,* 25(1), 1-5. https://doi.org/10.1006/rtph.1996.1076

Kock, K., & Brouwer, K. L. R. (2012). A perspective on efflux transport proteins in the liver. *Clinical Pharmacology & Therapeutics,* 92(5), 599-612. https://doi.org/10.1038/clpt.2012.79

Lee, W.-H., Kipp, Z. A., Pauss, S.N., Martinez, G. J., Bates, E. A., Badmus, 0. 0., Stec, D. E., & Hinds, T. D.,Jr.(2025). Heme oxygenase, biliverdin reductase, and bilirubin pathways regulate oxidative stress and insulin resistance: A focus on diabetes and therapeutics. *Clinical Science,* 739(2), 171-198. https://doi.org/10.1042/CS20242825

Lehmann, E. L. (1975). *Nonparametrics: Statistical methods based on ranks.* Holden-Day. https://doi.org/10.1002/zamm.19770570922

Morita, T., Watanabe, Y., Takeda, K., & Okumura, K. (1989). Effects of pH in the in vitro chromosomal aberration test. *Mutation Research,* 225(1-2), 55-60. https://doi.org/10.10l 6/0l 65-7992(89)90033-X

National Toxicology Program. (2015). *OHATrisk of bias rating tool for human and animal studies.* Office of Health Assessment and Translation.

National Toxicology Program. (2016). *Monograph on immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS).* National Institute of Environmental Health Sciences.

OECD (Organisation for Economic Co-operation and Development). (2021). *Reconciling terminology of the universe of per- and polyfluoroalkyl substances: Recommendations and practical guidance (OECD series on risk Management of Chemicals No. 61).* OECD Publishing. https://doi.org/10.1787/e458e796-en

Ramaiah, L., Tomlinson, L., Tripathi, N. K., Cregar, L. C., Vitsky, A., Beust, B. V., Barlow, V. G., Reagan, W. J., & Ennulat, D. (2017). Principles for assessing adversity in toxicologic clinical pathology. *Toxicologic Pathology,* 45(2), 260-266. https://doi.org/10.l 177/0192623316681646

RIVM. (2023). *RIVM-VSP Advice 14434A02: Drinking water guidance value for trifluoroacetic acid (TFA).* National Institute for Public Health and the Environment. https://www.rivm.nl/

Siska, W., Schultze, A. E., Ennulat, D., Biddle, K., Logan, M., Adedeji, A. 0., Arndt, T., & Aulbach, A. D. (2022). Scientific and regulatory policy committee points to consider: Integration of clinical pathology data with anatomic pathology data in nonclinical toxicology studies. *Toxicologic Pathology,* 50(6), 808-826. https://doi.org/10.l177/01926233221108887

Solomon, K. R., Velders, G. J., Wilson, S. R., Madronich, S., Longstreth, J., Aucamp, P. J., & Bornman, J. F. (2016). Sources, fates, toxicity, and risks oftrifluoroacetic acid and its salts: Relevance to substances regulated under the Montreal and Kyoto protocols. *Journal of Toxicology and Environmental Health, Part B, Critical Reviews, 19(7),* 289-304. https://doi.org/10.1080/10937404.2016.1175981

Sugatani, J., Kojima, H., Ueda, A., Kakizaki, S., Yoshinari, K., Gong, Q. H., Owens, I. S., Negishi, M., & Sueyoshi, T. (2001). The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase UGTlAl gene and regulation by the nuclear receptor CAR. *Hepatology,* 33(5), 1232-1238. https://doi.org/10.1053/jhep.2001.24172

Thakur, A. K. (1985). A FORTRAN program for testing trend and homogeneity in proportions. *Computer Programs in Biomedicine,* 79(2-3), 229-233. https://doi.org/10.1016/0010-468X(85)90015-7

Thulin, P., Rafter, I., Stockling, K., Tomkiewicz, C., Norjavaara, E., Aggerbeck, M., Hellmold, H., Ehrenborg, E., Andersson, U., Cotgreave, I., & Glinghammar, B. (2008). PPARa regulates the hepatotoxic biomarker alanine aminotransferase (ALTl) gene expression in human hepatocytes. *Toxicology and Applied Pharmacology,* 237(1), 1-9. https://doi.org/10.10l 6/j.taap.2008.03.007

UBA (Umweltbundesamt). (2020). Ableitung eines gesundheitlichen Leitwertes fur Trifluoressigsaure (TFA). https://www.umweltbundesamt.de/system/files/medien/5620/dokumente/ableitung_eines_gesund heitl ichen_leitwertes_f u er_trifluoressigsaeure_fuer_uba-homepage-20250731.pdf

United States Environmental Protection Agency.(2011). */ntegrated approaches to testing and assessment strategy: Use of new computational and molecular tools.* FIFRA Scientific Advisory Panel Consultation.

United States Environmental Protection Agency. (2013). A retrospective analysis of the immunotoxicity study (OCSPP Test Guideline No. 870.7800). https://www.epa.gov/

Vftek, L. (2019). Bilirubin as a predictor of diseases of civilization: Is it time to establish decision limits for serum bilirubin concentrations? *Archives of Biochemistry and Biophysics,* 672, 108062. https://doi.org/10.10l 6/j.abb.2019.108062

Vftek, L., Hinds, T. D., Jr., Stec, D. E., & Tiribelli, C. (2023). The physiology of bilirubin: Health and disease equilibrium. *Trends in Molecular Medicine,* 29(4), 315-328. https://doi.org/10.1016/j.molmed.2023.01.007

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: EFSA (European Food Safety Authority), Cavelier, A., Coja, T., Gall, A., Hernandez-Jerez, A., Vleminckx, C., Bergsma, E., Binaglia, M., Crivellente, F., Colas, M., Lanzoni, A., Louisse, J., De Jesus, R.R., Grillo, A., Mangas, I., & Parra Marte, J.M. (2026). Scientific Report on consumer health-based guidance values for trifluoroacetic acid. *EFSA Journal,* 24(7), el 0227. https://doi.org/10.2903/j.efsa.2026.10227