CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Trifluoroacetic Acid … %

EC Number: **200-929-3**

CAS Number: **76-05-1**

Index Number: **607-091-00-1**

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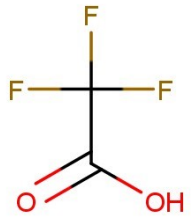
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the trifluoroacetic acid.

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Trifluoroacetic acid
Other names (usual name, trade name, abbreviation)	Perfluoroacetic acid Trifluoroethanoic acid TFA 2,2,2-trifluoroacetic acid
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	200-929-3
EC name (if available and appropriate)	Trifluoroacetic acid
CAS number (if available)	76-05-1
Other identity code (if available)	607-091-00-1
Molecular formula	$C_2HF_3O_2$
Structural formula	
SMILES notation (if available)	OC(=O)C(F)(F)F
Molecular weight or molecular weight range	114.02 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
-			

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
-				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
-					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current entry	607-091-00-1	trifluoroacetic acid … %	200-929-3	76-05-1	Acute Tox. 4* Skin Corr. 1A Aquatic Chronic 3	H332 H314 H412	GHS05 GHS07 Dgr	H332 H314 H412			B
Dossier submitters proposal					**Modify** Acute Tox. 3 **Add** Repr. 1B PMT vPvM	**Modify** H331 **Add** H360fD EUH450 EUH451	**Modify** GHS06 **Add** GHS08	**Modify** H331 **Add** H360fD EUH451	**Add** EUH071	**Add** inhalation: ATE = 5 mg/L (vapours)	B
Resulting Annex VI entry if agreed by RAC and COM					Repr. 1B Acute Tox. 3 Skin Corr. 1A Aquatic Chronic 3 PMT vPvM	H360fD H331 H314 H412 EUH450 EUH451	GHS08 GHS06 GHS05 Dgr	H360fD H331 H314 H412 EUH451	EUH071	inhalation: ATE = 5 mg/L (vapours)	B

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route	**Data conclusive but not sufficient for classification**	**Yes**
Acute toxicity via dermal route		
Acute toxicity via inhalation route	**Harmonised classification proposed**	
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity		
Carcinogenicity		
Reproductive toxicity	**Harmonised classification proposed**	**Yes**
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	**Data conclusive but not sufficient for classification**	**Yes**
Aspiration hazard	Hazard class not assessed in this dossier	No
Endocrine disruption for HH		
Hazardous to the aquatic environment		
Hazardous to the ozone layer		
Endocrine disruption for ENV		
PBT/vPVB		
PMT/vPvM	**Harmonised classification proposed**	**Yes**

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

TFA is registered under REACH (1907/2006/EC) and listed in Annex VI Table 3 of the Regulation (EC) No. 1272/2008 (CLP Regulation) with index number 607-091-00-1 and the following harmonised classification:

- Skin Corr. 1A H314
- Acute Tox. 4 * H332
- Aquatic Chronic 3 H412

The following self-classifications are notified in the C&L inventory for TFA:

- Met. Corr. 1 H290
- Skin Corr. 1A H314
- Acute Tox. 4 H302
- Eye Dam. 1 H318
- Aquatic Chronic 3 H412

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Commission Delegated Regulation (EU) 2023/707 introduced new hazard classes in CLP, thus there is a need for action at EU level due to changes in the CLP classification criteria.

5 IDENTIFIED USES

The following table gives an overview on registration information of TFA.

Table 8: Uses of TFA[1]

	Use(s)
Manufacture	ERC1: Manufacture of the substance - PROC 1, 2, 8a, 8b, 9, 15
Uses as intermediate	ERC 6a: Use of intermediate - PROC 3, 4, 8a, 8b, 9, 15 - SU 9: Manufacture of fine chemicals
Formulation	ERC2: Formulation into mixture - PROC 3, 4, 8a, 8b, 9, 15
Uses at industrial sites	ERC6a: Use of intermediate • PC 0: Other: Intermediate ERC6b: Use of reactive processing aid at industrial site (no inclusion into or onto article) • PROC 1, 2, 3, 4, 8a, 8b, 9, 13 ERC4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article) • PROC 1, 2, 3, 4, 8a, 8b, 15
Uses by professional workers	Laboratory professional use - ERC8b: Widespread use of reactive processing aid (no inclusion into or onto article, indoor) • PROC 1, 2, 3, 4, 8a, 8b, 9, 10, 15
Consumer uses	-
Article service life	-

Some per- and polyfluoroalkyl substances (PFASs) contain only a single $–CF_3$ group and because of their structure they are potential precursors to TFA. To this subgroup belong, amongst others, some

[1] according to ECHA's dissemination site: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5203, 21 February 2023

fluorinated gases and active ingredients in biocides, plant protection products and pharmaceuticals containing a $–CF_3$ group bound to an aromatic ring.

6 DATA SOURCES

The main source for studies were the IUCLID registration dossiers of TFA (UUID of registration dossier: c0bccced-2a93-4d3e-9cd7-2eda0a7239d1; last update by registrant: 08.03.2024), sodium trifluoroacetate (UUID of registration dossier: 6e7115c5-0703-4bf2-b83b-407b1bf1661b; last updated by registrant: 15.12.2023) and potassium trifluoroacetate (UUID of registration dossier: 7b2cc27d-1e51-4f0f-9418-cf4deaa2952c; last update by registrant: 08.08.2024).

The entries are also publicly available on ECHA's dissemination site

- TFA: https://chem.echa.europa.eu/100.000.846; last accessed 15.10.2024
- Sodium trifluoroacetate: https://chem.echa.europa.eu/100.018.982; last accessed 14.10.2024
- Potassium trifluoroacetate: https://chem.echa.europa.eu/100.018.980; last accessed 15.10.2024.

In addition, searches on PubMed, Web of Science, Embase and Science Direct were performed on March 24th 2022 and updated on August 25th 2023.

Search terms for substances were Trifluoroacetic acid, Trifluoracetic acid, Trifluoro acetic acid, Trifluoressigsäure as well as Trifluoroacetic acid sodium, and Sodium/Potassium/Ammonium/-Lithium/Cesium/Magnesium/Calcium trifluoroacetate (individual searches for each salt). Toxicologyspecific search terms were toxic, poison*, acute, severe, serious, extrem*, reproduct* toxicity, developmental, fecundity, fertil*, teratogen*, testes, testic*, ovar*, embryo*, fetus*, foetus*, feti, foetal, fetal, chronic, sub-acute, sub-chronic, longterm, repeat* dose, in vivo, in vitro

After removal of duplicates, the searches resulted in 682 entries and after screening of titles, 25 abstracts or if necessary full texts were screened further. Nine studies contained toxicological information for TFA or its salts but were not considered to add relevant information for this CLH dossier (Airaksinen et al., 1970; Airaksinen and Tammisto, 1968; Bartorelli, 2003; Han et al., 2001; Kowalska et al., 2023; Rosenberg and Wahlström, 1971; Upham et al., 1998; Waldron and Ratra, 1972; Walgren et al., 2004). All other relevant literature is described in detail in section 10 "Evaluation of health hazards".

Another source for studies (for the environment-related properties) was the Draft Renewal Assessment Report (DRAR) according to the Commission Regulation (EU) NO 1107/2009 FLUFENACET from November 2016.

7 PHYSICOCHEMICAL PROPERTIES

Table 9: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101.3 kPa	Liquid Colourless to pale yellow liquid, fuming, with pungent odour.	REACH registration data	Organoleptic observation
Melting/freezing point	-15.2 ± 0.2 °C	REACH registration data	Range of measured values and supporting literature data
Boiling point	72.05 ± 0.95 °C	REACH registration data	Range of measured values and supporting literature data
Relative density	1.52 (at 20 °C)	REACH registration data	Mean of measured value and supporting literature data
Vapour pressure	12.4 kPa (at 20 °C, interpolated) 139 kPa (at 80 °C, extrapolated)	REACH registration data	Measured, supported by literature data
Surface tension	72.5 mN/m (at 20 °C, 1 g/L)	REACH registration data	Measured
Water solubility	fully miscible (>10 g/L)	REACH registration data	Measured, supported by literature data
Partition coefficient n-octanol/water	log P_{ow} = -2.1	REACH registration data	Literature data (database)
Dissociation constant	pk_a = 0.3	REACH registration data	Measured, literature data O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 1725; Bowden D.J., Clegg S.L., Brimblecombe P., Chemosphere, Vol.32, No.2, pp.405-420, 1996
Viscosity	1.8 mPa s (at 20 °C, dynamic) 1.6 mPa s (at 40 °C, dynamic)	REACH registration data	Measured

The information in this table marked with „REACH registration data“ is based on information taken from the REACH registration dossier and ECHA’s public registration information as accessed on 26-04-2023.

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Toxicokinetic data are available for the acid TFA and three recent studies on the sodium salt NaTFA. TFA or its salts are expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, the TFA anion will be formed after absorption into blood.

Table 10: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
14-day repeated dose study Reliability: Klimisch 2 as reported by registrant (reliable with restriction), not GLP compliant Rabbit (New Zealand White) Females (nulliparous and non-pregnant) n/group = 6 NaTFA (99.9 %) Oral (gavage, vehicle: water), once daily 0, 750 mg/kg bw/day Dosing day 1 - 13	<u>Plasma TFA:</u> Mean C_{max} [individual C_{max}] / T_{max} / AUC_{0-24} • Day 1: 1680 mg/L (14.7 mM) [16.1 mM] / 3 h / 24800 h*mg/L • Day 13: 1890 mg/L [16.6 mM) [18.3 mM] / 2 h / 30700 h*mg/L No effects on Vitamin A No effects on blood pH **Blood lactate profiles** <u>[h post-dosing]:</u> Day 1 – Control • 1 h: 42.7 ± 38.72 mg/dL (n=6) • 3 h: 37.0 ± 18.90 mg/dL (n=6) • 8 h: 46.6 ± 24.78 mg/dL (n=5) • 24 h: 41.2 ± 25.96 mg/dL (n=6) Day 1 – 750 mg/kg bw/d • 1 h: 67.3 ± 30.85 mg/dL (n=6) • 3 h: 36.8 ± 14.76 mg/dL (n=6) • 8 h: 36.8 ± 17.42 mg/dL (n=6) • 24 h: 44.2 ± 18.78 mg/dL (n=5) Day 13 – Control • 1 h: 38.8 ± 14.05 mg/dL (n=6) • 3 h: 39.7 ± 23.65 mg/dL (n=6) • 8 h: 46.8 ± 14.22 mg/dL (n=6) • 24 h: 55.5 ± 33.49 mg/dL (n=6) Day 13 – 750 mg/kg bw/d • 1 h: 33.3 ± 38.23 mg/dL (n=6) • 3 h: 34.8 ± 7.68 mg/dL (n=5) • 8 h: 22.0* ± 9.49 mg/dL (n=4) • 24 h: 26.8 ± 12.88 mg/dL (n=6) **Blood glucose profile** <u>[h post doing]</u> Day 1 – Control • 1 h: 7.89 ± 0.686 mg/dL (n=6) • 3 h: 7.58 ± 0.282 mg/dL (n=6) • 8 h: 7.39 ± 0.354 mg/dL (n=6) • 24 h: 8.00 ± 0.517 mg/dL (n=6) Day 1 – 750 mg/kg bw/d • 1 h: 7.47 ± 0.287 mg/dL (n=6) • 3 h: 7.13* ± 0.309 mg/dL (n=6) • 8 h: 7.18 ± 0.360 mg/dL (n=6) • 24 h: 7.20* ± 0.482 mg/dL (n=5) Day 13 – Control • 1 h: 8.00 ± 0.700 mg/dL (n=6) • 3 h: 7.63 ± 0.378 mg/dL (n=6) • 8 h: 6.96 ± 0.385 mg/dL (n=6) • 24 h: 7.81 ± 0.446 mg/dL (n=6)		(Labcorp Laboratories, 2024a)

Method	Results	Remarks	Reference
	Day 13 – 750 mg/kg bw/d • 1 h: 7.85 ± 0.227 mg/dL (n=6) • 3 h: 7.95 ± 0.652 mg/dL (n=5) • 8 h: 7.76* ± 0.140 mg/dL (n=4) 24 h: 7.62 ± 0.275 mg/dL (n=6)		
Preliminary prenatal developmental toxicity study Reliability: Klimisch 2 as reported by registrant (reliable with restrictions), not GLP compliant NaTFA (99.9 %) Rabbit (New Zealand White), pregnant females Oral (gavage, vehicle: water), once daily GD 6-28 (23 days of treatment, Caesarean section on GD 29) 0, 10, 45, 180, 750 mg/kg bw/d n/group = 6 mated females Toxicokinetics: - Maternal TK sampling: Blood samples were collected from all animals on GD4/5 (pre-dose), on GD 6 and GD28, at 1, 2, 3, 5, 8 and 24 h after dosing. - Animals fasted: No - Blood sample site: Central auricular artery or the marginal ear vein - Anticoagulant: K2EDTA - Blood volume 0.2 mL. - Foetal TK sampling: Foetal blood samples were collected from the umbilical cord from all litter animals on GD 29 at necropsy. Each litter provided one pooled sample. - Analytical method: The plasma samples were analysed for concentrations of trifluoroacetate according to an adaptation of the method 01625, Krebber, R. and Ruttmann, F., Analytical method for the determination of trifluoroacetic acid in rat plasma by LC-MS/MS. The adaptation of the analytical method 01625 to rabbit samples was validated within the course of the study by running 5 recoveries at the limit of quantitation and 5 recoveries at 10 times the limit of quantitation. The quantitative determination is performed	<u>Summary mean TK parameters in plasma of dams:</u> GD 6 - C_{max} [T_{max} (h after application) / AUC_{0-24}] • 10 mg/kg bw/day: 59 mg/L (0.52 mM) [8 h / 1240 h*mg/L] • 45 mg/kg bw/day: 218 mg/L (1.93 mM) [5 h / 4730 h*mg/L] • 180 mg/kg bw/day: 679 mg/L (6.01 mM) [5 h / 14000 h*mg/L] • 750 mg/kg bw/day: 1860 mg/L (16.5 mM) [3 h / 26200 h*mg/L] GD 28 - C_{max} [T_{max} (h after application) / AUC_{0-24} / AUC ratio GD28/GD6] • 10 mg/kg bw/day: 170 mg/L (1.50 mM) [2 h / 3410 h*mg/L / 2.75] • 45 mg/kg bw/day: 445 mg/L (3.93 mM) [2 h / 8840 h*mg/L / 1.87] • 180 mg/kg bw/day: 839 mg/L (7.42 mM) [2 h / 12600 h*mg/L / 0.9] • 750 mg/kg bw/day: 1860 mg/L (16.5 mM) [3 h / 21800 h*mg/L / 0.83] <u>TFA plasma concentrations</u> Dams Mean Cmax • 10 mg/kg bw/day: 121 mg/L • 45 mg/kg bw/day: 311 mg/L • 180 mg/kg bw/day: 358 mg/L • 750 mg/kg bw/day: 425 mg/L Foetuses (pooled) Mean Cmax • 10 mg/kg bw/day: 120 mg/L • 45 mg/kg bw/day: 317 mg/L • 180 mg/kg bw/day: 331 mg/L • 750 mg/kg bw/day: 477 mg/L	The purpose of this study was to assess the TK-profile, glucose profile, vitamin A profile and clinical chemistry in pregnant rabbits. The results of this study were needed to decide on the best design of the mechanistic developmental study (including dose levels) to elucidate the mode of action for previously observed eye findings	(Labcorp Laboratories, 2024b)

Method	Results	Remarks	Reference
by HPLC with detection by tandem mass spectrometry in the negative mode. The quantification is performed using solvent standard solutions and internal standard. The lower limit of quantitation is 150 µg/L.	Ratio dams/foetuses • 10 mg/kg bw/day: 1.02 • 45 mg/kg bw/day: 0.983 • 180 mg/kg bw/day: 1.09 • 750 mg/kg bw/day: 1.15 Analysis of TFA in aqueous humour Dams (both eyes pooled): Mean C_{max} • 10 mg/kg bw/day: 58.7 mg/L (0.52 mM) • 45 mg/kg bw/day: 155 mg/L (1.36 mM) • 180 mg/kg bw/day: 152 mg/L (1.33 mM) • 750 mg/kg bw/day: 222 mg/L (1.95 mM) Foetuses (litter pooled): Mean C_{max} • 10 mg/kg bw/day: 96.7 mg/L (0.85 mM) • 45 mg/kg bw/day: 216 mg/L (1.89 mM) • 180 mg/kg bw/day: 268 mg/L (2.35 mM) • 750 mg/kg bw/day: 293 mg/L (2.57 mM) **Lactate in aqueous humour** Dams (GD 29, both eyes pooled): • Controls: 12 mM • 10 mg/kg bw/day: 11 mM • 45 mg/kg bw/day: 13 mM • 180 mg/kg bw/day: 9 mM • 750 mg/kg bw/day: 8.5 mM Foetuses (GD 29, litter pooled): • Controls: 21 mM • 10 mg/kg bw/day: 22 mM • 45 mg/kg bw/day: 20 mM • 180 mg/kg bw/day: 20.5 mM • 750 mg/kg bw/day: 22 mM No significant effects on blood glucose on GD 6 and GD 19 ↑ Blood lactate at hd on GD 6 (+216 %** at 2 h post-dosing, +228 %** at 3 h post-dosing), but not on GD 19 No significant effects on blood vitamin A levels No significant effects on blood pH		

Method	Results	Remarks	Reference
Prenatal developmental toxicity study, OECD TG 414 (main mechanistic developmental study) Reliability: Klimisch 1 as reported by registrant (reliable without restrictions), GLP compliance Rabbit (New Zealand White) mated females n/group = 30 n/group = 8 satellite females Sacrifice: All surviving study animals from the main phase were euthanised and subjected to necropsy and Caesarean section on GD 29. Satellite females were euthanised and subjected to necropsy on GD 20. NaTFA (99.9 %) Oral (gavage, vehicle: water), once daily 0, 30, 60, 250, 750 mg/kg bw/d GD 6 - 28 (main phase animals) Satellite females: GD 6 - 19	**TFA plasma concentrations** <u>Dams (Satellite) GD 6 [Ratio 24h/2h]:</u> • Controls 2h: 0.41 mg/L (0.004 (mM) • Controls 24 h: 0.455 mg/L (0.004 mM) [1.13] • 30 mg/kg bw/day 2 h: 103 mg/L (0.904 mM) • 30 mg/kg bw/day 24 h: 96.3 mg/L (0.845 mM) [0.945] • 60 mg/kg bw/day 2 h: 186 mg/L (1.632 mM) • 60 mg/kg bw/day 24 h: 174 mg/L (1.526 mM) [0.929] • 250 mg/kg bw/day 2 h: 674 mg/L (5.912 mM) • 250 mg/kg bw/day 24 h: 397 mg/L (3.483 mM) [0.584] • 750 mg/kg bw/day 2 h: 1140 mg/L (10.0 mM) • 750 mg/kg bw/day 24 h: 109 mg/L (3.588 mM) [0.361] <u>Dams (Satellite) GD 19 [Ratio 24h/2h]:</u> • Controls 2h: 15.1 mg/L (0.133 (mM) • Controls 24 h: 13.0 mg/L (0.114 mM) [0.836] • 30 mg/kg bw/day 2 h: 392 mg/L (3.439 mM) • 30 mg/kg bw/day 24 h: 316 mg/L (2.772 mM) [0.808] • 60 mg/kg bw/day 2 h: 445 mg/L (3.904 mM) • 60 mg/kg bw/day 24 h: 319 mg/L (2.798 mM) [0.719] • 250 mg/kg bw/day 2 h: 808 mg/L (7.088 mM) • 250 mg/kg bw/day 24 h: 342 mg/L (3.000 mM) [0.422] • 750 mg/kg bw/day 2 h: 978 mg/L (8.579 mM) • 750 mg/kg bw/day 24 h: 330 mg/L (2.895 mM) [0.343] <u>Dams (Main phase) GD 28:</u> • Controls: 0.987 mg/L (0.009 mM) • 30 mg/kg bw/day: 315 mg/L (2.763 mM) • 60 mg/kg bw/day: 445 mg/L (3.904 mM) • 250 mg/kg bw/day: 793 mg/L (6.956 mM)		(Labcorp Laboratories, 2024c)

Method	Results	Remarks	Reference
	• 750 mg/kg bw/day: 1120 mg/L (9.825 mM) Foetuses (post maternal euthanasia, GD 20): • Controls: 3.68 mg/L (0.032 mM) • 30 mg/kg bw/day: 166mg/L (1.456 mM) • 60 mg/kg bw/day: 193 mg/L (1.693 mM) • 250 mg/kg bw/day: 234 mg/L (2.053 mM) • 750 mg/kg bw/day: 251 mg/L (2.202 mM) **TFA in aqueous humour** Dams (GD 20, both eyes pooled): Mean C_{max} • 30 mg/kg bw/day: 186.1 mg/L (1.6 mM) • 60 mg/kg bw/day: 196.3 mg/L (1.7 mM) • 250 mg/kg bw/day: 199.6 mg/L (1.8 mM) • 750 mg/kg bw/day: 273.5 mg/L (2.4 mM) Foetuses (GD 29, litter pooled): Mean C_{max} • 30 mg/kg bw/day: 209.6 mg/L (1.8 mM) • 60 mg/kg bw/day: 254.0 mg/L (2.3 mM) • 250 mg/kg bw/day: 281.6 mg/L (2.5 mM) • 750 mg/kg bw/day: 322.5 mg/L (2.8 mM) **Lactate in aqueous humour** Dams (GD 20, both eyes pooled): • Controls: 14 mM • 30 mg/kg bw/day: 13.7 mM • 60 mg/kg bw/day: 13.3 mM • 250 mg/kg bw/day: 13 mM • 750 mg/kg bw/day: 12.5 mM Foetuses (GD 29, litter pooled): • Controls: 27 mM • 30 mg/kg bw/day: 24 mM • 60 mg/kg bw/day: 23 mM • 250 mg/kg bw/day: 26 mM • 750 mg/kg bw/day: 27 mM		

Method	Results	Remarks	Reference
	No effect on blood pH No effect on blood lactate on GD 19 (main phase animals) ↑ blood lactate in satellite females at hd on GD 6 (+57 %*) (not on GD 12) ↑ glucose at hmd and hd on GD 19 (mhd: +5 %**, hd: +6 %**) and at hd on GD 28 (+8 %)** (not for satellite females on GD 12)		
Non-guideline study on the extent of production of the aerobic metabolite of halothane, trifluoroacetic acid and the mode of its elimination Test material: TFA (CAS no. 76-05-1) Rabbit (strain not specified) n/group = 8 (m) for halothane inhalation n/group = 5 (m) for intravenous and enteral administration • Inhalation of halothane (Group 1) • Intravenous (TFA) with bile duct drainage (Group 2) • Intravenous (TFA) without bile duct drainage (Group 3) • Enteral via duodenal catheter (TFA) (Group 4) Inhalation: 1 % halothane Intravenous and enteral admin.: 100 µmol TFA	**Inhalation of halothane with bile duct drainage (group 1)** • TFA in bile immediately after halothane inhalation started • Amount of TFA reached its peak 12 h after inhalation was started (measured every hour) • TFA was detected in the bile 84 h after halothane administration was ceased. • The total amount of TFA excreted in the bile was (108 +/- 8.32) µmol, and that in the urine was (61.9 +/- 11.2) µmol. Volume of distribution (L) • 0.91 ± 0.13 (group 2) • 0.66 ± 0.05 (group 3) • 0.42 ± 0.12 (group 4) Distribution half-life (h) • 0.64 ± 0.23 (group 2) • 0.31 ± 0.05 (group 3) • 0.59 ± 0.15 (group 4) Elimination half-life (h) • 15.6 ± 2.13 (group 2) • 34.3 ± 7.44 (group 3) • 16.7 ± 4.32 (group 4) 1st order elimination coefficient • 0.16 ± 0.01 (group 2) • 0.04 ± 0.007 (group 3) • 0.13 ± 0.27 (group 4) Biliary excretion rate (%) • 51.8 ± 7.93 (group 2) • 0 (group 3) • 20.7 ± 4.30 (group 4) Urinary excretion rate (%) • 14.5 ± 3.09 (group 2) • 58.0 ± 3.98 (group 3) • 22.0 ± 4.94 (group 4) Metabolisation not measured **Group 2 and 3** • Elimination half-life longer without bile fistula than with bile fistula	From IUCLID dossier: "The present finding confirms that the bile was the major channel of elimination of TFA." "the most important mechanism of long-lasting excretion of TFA after halothane anaesthesia seems to be the enterohepatic circulation of TFA."	(Kinoshita, 1989) Klimisch 2 as reported by registrant (reliable with restrictions; well conducted although no guideline followed, only excretion studied)

Method	Results	Remarks	Reference
	Enteral TFA administration (group 4) • TFA appeared immediately in plasma • Plasma concentrations reached peak in one hour following enteral administration • Urinary excretion rate (22 %) slightly higher than biliary excretion rate (21 %)		
Trifluoroacetic acid and halothane were tested in the study in parallel. Mouse (C57BL) Inhalation for halothane (whole body, for 1 h on GD 18) Intravenous infusion of TFA (in saline solution) Halothane: 45, 100, 900, 1200, 3200 ppm (samples taken 4 h after end of exposure, at 3200 ppm additionally immediately after exposure, after 30 min, 1 h, 4 h and 24 h) TFA: 10 µmol, dams were killed 4 or 24 hrs after intravenous infusion Halothane: n/group = 7 - 8 (pregnant f, GD 18) TFA: n/group = 8 (pregnant f, GD 18) no control animal plasma and amniotic fluid, homogenised foetuses were used to measure TFA and bromide metabolites of halothane	**Inhalation of halothane:** 3200 ppm • at 0 h after end of exposure: 7000 µmol/L in blood, 600 µmol/L in amniotic fluid • at 30 min, decreased to 1/3 of the 0-h level (ca. 2333 µmol/L) in blood; 500 µmol/l in amniotic fluid • at 1 h 1/10 (ca. 700 µmol/L) in blood; 50 – 100 µmol/l in amniotic fluid • at 4 hrs 0.3 % of the 0 h level (ca. 21 µmol/L) in blood; same in amniotic fluid • at all survival times, the amniotic concentration was considerably lower, never exceeding 20 % of the blood levels 45 and 100 ppm • Dose-concentration relationship for TFA formation in all three samples 900, 1200 ppm • Only slightly higher than 100 ppm (saturation assumed) **Intravenous administration of TFA:** • similar pattern of TFA concentration in maternal plasma and amniotic fluid as after a halothane inhalation exposure • In this case, the ratio amniotic fluid/ maternal plasma was over 1 at 24 hrs.	The fact that infusion of TFA intravenously to the mother gives the same pattern of TFA accumulation in foetus and amniotic fluid as after halothane inhalation indicates that TFA is mainly formed by maternal metabolism.	(Ghantous et al., 1986) Klimisch 4 as reported by registrant (not assignable; study well conducted but no guideline followed and only few tissues examined for the distribution and metabolism endpoints)
These studies are described in the registration dossier, but are not relevant for this CLH dossier because TFA is not used as a test substance			(Danielsson et al., 1984; Divakaran et al., 1980; Fraser and Kaminsky, 1988)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Five animal studies related to the toxicokinetics of TFA are provided in the registration dossier for TFA, three of which only administered the precursor halothane (an important anaesthetic) and not TFA itself. In the two studies, which directly administered TFA, only intravenous and enteral administration of TFA were studied and oral, inhalation or dermal studies are not available. Furthermore, three studies on female rabbits (pregnant and non-pregnant) with toxicokinetic

information on NaTFA in rabbits are available from the registration dossier. Blood pH, vitamin A, blood lactate and glucose profiles were also studied in order to deepen the understanding of the mode of action of eye malformations in the rabbit studies (see discussion under 10.10.5).

Additionally, information on human ADME of TFA can be inferred from three epidemiological studies, and one *in silico* study.

Labcorp Laboratories (2024a)

In a 14-day repeated dose study, six female New Zealand White (NZW) rabbits were once daily orally dosed with 750 mg/kg bw/day NaTFA (gavage with water as vehicle). Six additional female rabbits were given vehicle only via gavage.

After dosing of 750 mg/kg bw/day, C_{max} plasma values of 14.7 or 16.6 mM were measured on day 1 and day 13 with corresponding median T_{max} values of 3 h or 2 h on day 1 and day 13, respectively. Mean C_{max} and AUC_{0-24} values were similar on day 1 and day 13, indicating no accumulation of NaTFA after multiple doses of 750 mg/kg bw/day in rabbits. According to the registration dossier "Mean accumulation ratio values ranged from 0.896 to 1.40 for C_{max} and from 1.05 to 1.34 for AUC_{0-24} at 750 mg/kg/day." (data not provided in registration dossier).

There were no effects on blood pH or vitamin A. The only statistically significant difference from control was observed in the blood lactate profiles 8 h post-doing on day 13 with a -53 % decrease of lactate levels (22.0 mg/dL at a dose of 750 mg/kg bw/day vs. 46.8 mg/dL in control group). Blood glucose was significantly lowered at 3 h (-6 %) and 24 h (-10 %) post-dosing on day 1 and significantly increased 8 h post-dosing on day 13 (+11 %). Overall, the effects of NaTFA dosing on glucose and lactate appear to be minor.

Labcorp Laboratories (2024b)

In a preliminary prenatal developmental toxicity study, six mated female NWZ rabbits each were orally dosed once daily via gavage (vehicle: water) with four different doses (10, 45, 180, 750 mg/kg bw/day) of NaTFA from gestation day (GD) 6--28 (dosing for 23 days). Six mated control females were dosed with water only. Foetuses were sampled after Caesarean section on GD 29. After administration of NaTFA, a plasma concentration of C_{max} = 16.5 mM was measured at the high dose. Mean C_{max} and AUC_{0-24} values increased with the increase in dose level from 10 to 750 mg/kg/day. The increases in mean C_{max} and AUC_{0-24} values were less than proportional to the dose. Accumulation of NaTFA was observed at the 10 and 45 mg/kg/day dose levels after multiple doses in pregnant rabbits (AUC ratio GD 28/GD 6 > 1), but not at 180 and 750 mg/kg bw/day (AUC ratio GD 28/GD 6 < 1).

Plasma concentrations of TFA were similar in pregnant female rabbits at 24 h post-dose and respective foetal rabbits at necropsy on GD 29. TFA was also measured in ocular samples of dams and foetuses. High TFA concentrations were determined in aqueous/vitreous humour samples of dams and foetuses at necropsy (i.e. ≥ 24 h after the last application). TFA levels in foetal samples were higher compared to the concentrations in eyes of the respective dams (see Table 10).

The only statistically significant difference from control was observed in the lactate profiles (i.e. an increase in the 750 mg/kg bw/day group on GD 6 2 h and 3 h post-dosing). There were no effects on lactate in ocular samples, blood pH, or vitamin A.

Labcorp Laboratories (2024c)

In a prenatal developmental toxicity study according to OECD TG 414, thirty mated female NWZ rabbits each were once daily dosed orally via gavage (vehicle: water) at four different doses (30, 60, 250, 750 mg/kg bw/day) of NaTFA from GD 6--28 (dosing for 23 days). Thirty mated control females were dosed with water only. Foetuses were sampled after Caesarean section on GD 29. Additionally, eight mated satellite females per dose group were dosed from GD 6 - 19 euthanised and subjected to necropsy on GD 20.

After administration of NaTFA, high plasma concentrations were measured, increasing with increasing dose. This increase was approximately proportional between 30 and 250 mg/kg/day, but less than proportional to the increase in dose between 250 and 750 mg/kg/day in the satellite dams, but the increase was overall not proportionate in the main study dams. Concentrations of TFA in foetuses on GD 20 increased with increasing dose administered to the dams, but less than proportional to the increase in dose received by the dams. The foetal (GD 20) to satellite dam (GD 19 at 24 h post-dose) ratio increased from 0.526 to 0.768 between 30 to 750 mg/kg/day. TFA concentrations in the main phase dams at 2 h post-dosing on GD 28 were generally similar to the concentrations at 2 h post-dosing on GD 19 in the satellite animals, suggesting that the greatest increase in TFA concentrations occurred during the organogenesis phase of pregnancy.

Notably, TFA was also measured in controls with individual TFA concentrations up to 57 mg/L at 24 h post-dosing on GD 19. It remains unclear whether contamination occurred (e.g. via drinking water). The registrant argued that TFA occurrence in controls did not have an impact on the conclusions of this study because the control levels are far below the concentrations measured in the treated groups.

TFA was also measured in ocular samples of dams and foetuses. High TFA concentrations were determined in aqueous/vitreous humour samples of dams and foetuses at necropsy (i.e. ≥ 24 h after the last dosing). TFA levels in foetal samples were slightly higher compared to the concentrations in eyes of the respective dams (see Table 10).

The only statistically significant difference from control consisted in the blood lactate profiles (i.e. a 57 % increase in the 750 mg/kg bw/day group on GD 6 in the satellite animals). There were no effects on lactate in ocular samples, blood pH, or vitamin A.

Kinoshita (1989)

The most reliable study (Klimisch 2 according to the registrant), assessed absorption, distribution, and excretion of halothane and TFA in rabbits (strain not specified). Metabolism of halothane to TFA was determined; metabolism of TFA was not measured. Halothane was administered via inhalation (group 1); TFA was administered intravenously with (group 2) or without (group 3) bile duct drainage/bile fistula. In group 4, TFA was administered enterally via duodenal catheter.

The distribution half-life after intravenous administration of TFA doubled with bile fistula (0.64 ± 0.23 h) when compared to the value obtained without bile fistula (0.31 ± 0.05 h). Elimination half-life doubled without bile fistula (34.3 ± 7.44 h) when compared to the group with bile fistula (15.6 ± 2.13 h). The results indicate that bile excretion is an important route of excretion for TFA, along with urinary excretion. Long-lasting excretion of TFA after halothane exposure resulted from enterohepatic circulation.

Ghantous et al. (1986)

Eight pregnant female mice (C57BL) received an intravenous infusion with 10 µmol TFA in saline solution on GD 18 over 1 h (total dose ca. 300 µmol/kg bw). There was no control group (the study compared intravenous TFA administration with halothane inhalation). Dams were killed 4 or 24 hrs after intravenous infusion of TFA. Only distribution was studied for TFA by measurements in plasma, amniotic fluid, and homogenised foetuses.

Four hours after a 1 h infusion of TFA into maternal blood, plasma concentrations of TFA were about 750 µmol/L, amniotic fluid concentrations were about 500 µmol/L and the concentration in the foetuses was 600 µmol/L. After 24 h, the TFA concentration was approximately half of the concentrations at 4 h in plasma (ca. 350 µmol/L) and in foetuses (ca. 290 µmol/L). Concentrations in amniotic fluid were higher than in plasma and foetuses and slightly higher than after 4 h (ca. 520 µmol/L).

Moreover, an equilibrium dialysis experiment in the study showed that TFA binds to macromolecules at a percentage of (28 ± 2.3) % in plasma and at a percentage of (27 ± 2.8) % in amniotic fluid.

Conclusion from animal studies

The three newer rabbit studies (Labcorp Laboratories, 2024a; Labcorp Laboratories, 2024b; Labcorp Laboratories, 2024c) indicate that NaTFA is rapidly absorbed and distributed in plasma as well as into the eyes. Some indications for accumulation could be seen at lower doses, but not at higher doses. TFA concentrations were similar between dams and foetuses, even after more than 24 h after the last administration.

There were no effects on blood pH or vitamin A, and only small or inconsistent effects on blood lactate profiles as well as blood glucose profiles.

The two older studies did not assess absorption, but measured distribution to liver, bile, plasma, amniotic fluid, and foetuses (Ghantous et al., 1986; Kinoshita, 1989). No metabolism of TFA was measured, but enterohepatic circulation and to a lesser extent urine were excretion routes (Kinoshita, 1989).

Other results from the two older animal studies described above (Ghantous et al., 1986; Kinoshita, 1989) as well as three other animal studies from the registration dossier (Danielsson et al., 1984; Divakaran et al., 1980; Fraser and Kaminsky, 1988) relate to inhalation of the anaesthetic halothane and resulting TFA formation, distribution and excretion. These results are partially listed in Table 10, but are not considered relevant for the classification of TFA.

Duan et al. (2020)

A cross-sectional epidemiological study measured 21 different PFASs, including TFA, in serum of the population of Tianjin, China (252 subjects). With 17.2 % of total known PFASs, TFA was the fourth most abundant PFAS (only after the legacy PFASs PFOS and PFOA, as well as 6:2 PFAES) in serum samples. TFA was detected in 97 % of the samples.

Median serum concentrations were 8.46 ng/mL (25^{th} percentile at 5.36 ng/mL and 75^{th} percentile at 12.55 ng/mL); the geometric mean was 7.21 ng/mL.

TFA concentrations in serum correlated positively with age (Pearson correlation: $r = 0.296$, $p < 0.001$). People older than 40 years had higher serum concentrations of TFA than people younger than 40 years.

Although the study cannot give clear information on TFA ADME, several points can be considered indicating absorption and distribution of TFA, but information or estimation of excretion or elimination half-lives cannot be extracted from this study.

Clearly, TFA is absorbed and distributed into the human blood, and is widely distributed throughout this studied population. The authors assume that uptake route of PFASs is mainly oral via food and drinking water. The study area is on the seashore and the population is discussed to have relatively high consumption of fish and seafood. The study area also appears to be affected by chemical industry, but no information is available whether TFA may also be absorbed via particles in the air (*"Tianjin is a seashore city along Bohai Bay, and it is also one of the China's four municipalities with heavy industry and dense population"*).

Because TFA can be measured in relatively high concentrations in serum and TFA concentrations increase with increasing age, the authors assume bioaccumulation of TFA despite high water solubility and low K_{ow} (log K_{ow} = -2.1). The authors refer to an earlier study (Boutonnet et al., 1999) and discuss that TFA has a similar structure to acetate which is a major biosynthetic molecule, and *"could be incorporated into biomolecule fractions such as protein, lipid, and so on, which results in the bioaccumulation of TFA in microorganisms, invertebrates, and plants"* (Duan et al., 2020).

Kim et al. (2022)

Persistent and mobile chemicals and PFASs, including TFA, were measured in urine samples of Flemish adolescents (n = 83). Spot urine sample were collected between September 2017 and June 2018 within the 4th cycle of the Flemish Environment and Health Study (FLEHS IV, 2016 - 2020).

TFA was detected in 30 - 63 % of the urine samples, depending on the confidence level of the analysis. Concentration levels are not reported by this study. Nevertheless, the study indicates that TFA can be excreted via urine by humans.

Jia et al. (2023)

Nineteen different PFASs, including TFA, were measured in cord serum samples of Chinese women. The samples were collected in January 2022 from "women who did not give birth to live foetuses with congenital malformations" in Shijiazhuang, China (n = 66). With 11.5 % of total target PFASs, TFA was the fourth most abundant PFAS (after PFOS, PFPrA, and PFPeA) in serum samples. TFA was detected in 55 % of the cord serum samples with concentrations ranging from 0.006 - 2.476 ng/mL (median: 0.229 ng/mL).

The presence of TFA in cord serum samples indicates distribution to the cord serum and potentially to the unborn child. Furthermore, the study indicates that the distribution to cord serum and the unborn child may be a route of excretion of TFA in pregnant women.

Yu et al. (2022)

Docking of 49 PFASs, including TFA, to 14 different types of human nuclear hormone receptors was simulated by the inverse docking tool Endocrine Disruptome[2]. Compared to other (longer chain) PFASs with higher binding affinities, TFA and four other short-chain PFAs exhibited moderate

[2] Kolšek K., Mavri J., Sollner Dolenc M., Gobec S. and Turk S. (2014): Endocrine Disruptome – An Open Source Prediction Tool for Assessing Endocrine Disruption Potential through Nuclear Receptor Binding. J Chem Inf Model 54 (4), 1254-1267. https://doi.org/10.1021/ci400649p

binding probabilities to androgen receptor (antagonist conformation), and low binding probabilities to all other nuclear hormone receptors.

Some binding of TFA to nuclear hormone receptors in humans can be inferred from this study, but the extent of receptor binding is likely rather low compared to other PFASs, where experimental data is available (e.g. PFOA, PFNA, PFOS).

10 EVALUATION OF HEALTH HAZARDS

Data on TFA and NaTFA are used as the basis for the evaluation of health hazards. TFA is a strong acid with a pK_a of 0.3, resulting in the corresponding base trifluoroacetate to be a weak base. Therefore, TFA or its salts are expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, the TFA anion trifluoroacetate will be formed after absorption into the blood. This justifies the use of TFA or its salts for the evaluation of all of the following health hazards.

Acute toxicity

10.1 Acute toxicity - oral route

Table 11: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity study, no guideline, no GLP, Klimisch 4 as reported by registrant (not assignable as only available as secondary literature)	Rat, strain not specified, n = 90 per sex per dose	TFA, purity not specified	Only LD values reported: LD_{100} = 1000 mg/kg bw LD_{10} = 500 mg/kg bw LD_0 = 250 mg/kg; single dose in aqueous solution to 90 rats followed by a 14-day observation period	1000 > LD_{50} > 500 mg/kg bw	(Institute of work health and safety of the USSR, 1964)
Acute Oral Toxicity, OECD TG 425, GLP compliant, Klimisch 1 as reported by registrant	Rat, Wistar, n = 5 females	NaTFA (purity: 95.1 %), vehicle: distilled water	2000 mg/kg bw single oral gavage dose in aqueous solution followed by a 14-day observation period	LD_{50} > 2000 mg/kg bw	(CiToxLAB Hungary, 2013)
Acute Oral toxicity - Acute Toxic Class Method), OECD TG 423, not GLP compliant,	Rat, Sprague-Dawley, n = 3 per sex	KTFA (purity: > 90 %), vehicle: 0.5 % carboxymethyl cellulose	Females: 500 mg/kg bw Males: 2000 mg/kg bw single oral gavage	Reversible piloerection in both doses LD_{50} > 2000 mg/kg bw	(RTC S.p.A., 2000)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Klimisch 2 as reported by registrant			dose followed by a 14-day observation period		

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

A study on acute toxicity after oral administration (gavage) of TFA is available (Institute of work health and safety of the USSR, 1964), the reliability of which cannot be determined as the results are only available as secondary literature. According to the registration dossier, in this study TFA was administered intragastrically (gavage) in a single dose in aqueous solution to 90 rats followed by a 14-day observation period, resulting in an LD_{100} = 1000 mg/kg bw, LD_{10} = 500 mg/kg bw, and LD_0 = 250 mg/kg. As a result described in the registration dossier, breathing disturbances similar to those that occur in acute inhalation poisoning were observed. In individual animals, motor activity was intensified immediately after administration of the substance, though more often the animals displayed less movement and motor activity. Varying degrees of haemorrhaging and necrosis in the mucous membranes of the stomach, dystrophic changes in the liver, kidneys and brain were detected in macro and micro-investigations of organs of dead rats. No effects were observed when the neutralised acid in the form of their salt was administered in a single dose into the stomach. A dose of 7000 mg/kg bw of the neutralised acid was not lethal for the animals, and furthermore no signs of damage to the tissues and organs were detected. Thus, the effects observed with the non-neutralised acid are most likely a result of corrosive action due to the low pH of the substance.

A second study on acute toxicity after oral administration (gavage) of the sodium salt NaTFA according to OECD TG 425 is available (CiToxLAB Hungary, 2013). In this study, an aqueous solution of NaTFA with a concentration of 2000 mg/kg bw was administered in a single dose to 5 female Wistar rats by oral gavage. No treatment-related adverse effects occurred during the 14-day observation period and the LD_{50} was therefore found to be above the administered dose of 2000 mg/kg bw. In another study according to OECD TG 423, the acute toxicity after oral administration (gavage) of the potassium salt KTFA was determined (RTC S.p.A., 2000). In this study KTFA in 0.5 % carboxymethyl cellulose was administered to three female and three male Sprague-Dawley rats. The doses were 500 and 2000 mg/kg bw for females and males, respectively. Piloerection occurred in both dose groups after treatment, but was reversible, hence the LD_{50} was found to be above the administered dose of 2000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

The available data for lethal doses in experimental animals by the Institute of work health and safety of the USSR (1964) are: LD_{100} = 1000 mg/kg bw and LD_{10} = 500 mg/kg bw, indicating that the LD_{50} for TFA is < 1000 and > 500 mg/kg bw. According to the Guidance for the application of the CLP criteria (ECHA, 2017), an LD_{50} (oral route) > 300 and ≤ 2000 mg/kg bw results in the acute toxicity hazard category 4. However, the reliability of these data cannot be determined as the results are only available as secondary literature. The neutralised TFA in the form of its salt was not lethal up to 7000 mg/kg bw, indicating that corrosivity is causing lethal effects after acute oral exposure to TFA. According to the Guidance on the Application of the CLP Criteria (ECHA, 2017) *"there are different hazard classes covering effects after single or brief exposure – 'Acute toxicity' and 'STOT-SE (Specific Target Organ Toxicity – Single Exposure)', skin irritation/corrosion and eye damage. These are independent of each other and may all be assigned to a substance or a mixture if the respective*

criteria are met. However, care should be taken not to assign each class for the same effect, essentially giving a multiple classification, even where the criteria for different classes are fulfilled. In such a case the most appropriate (the most severe hazard) class should be assigned."

The absence of lethal effects after neutralisation suggests that lethality after TFA exposure occurs as a result of corrosivity and therefore no acute toxicity classification is indicated because TFA is already classified as corrosive to the skin (Skin Corr. 1A, H314).

The studies with NaTFA (CiToxLAB Hungary, 2013) and KTFA (RTC S.p.A., 2000) resulted in an LD_{50} of > 2000 mg/kg bw, as no adverse effects after oral administration were observed. According to the Guidance for the application of the CLP criteria (ECHA, 2017), an LD_{50} > 2000 mg/kg bw results in no classification for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

The available oral acute toxicity study results in an LD_{50} (oral route) of < 1000 and > 500 mg/kg bw for TFA, which would warrant a classification as Acute Tox. 4, H302 for TFA. Because the results of this study indicate that the mode of action for acute toxicity of TFA is corrosivity, and the study for NaTFA resulted in an LD_{50} > 2000 mg/kg bw, no Acute Tox. classification is warranted for TFA.

10.2 Acute toxicity - dermal route

Table 12: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity study, no guideline, no GLP, Klimisch 4 as reported by registrant (not assignable as only available as secondary literature)	Rabbit, strain not specified, n not specified	TFA, purity not specified	Dose levels not reported	No lethality	(Institute of work health and safety of the USSR, 1964)
Acute Dermal Toxicity, OECD TG 402, GLP compliant, Klimisch 1 as reported by registrant	Rat, Wistar, n = 5 animals/sex/dose	KTFA/potassium trifluoromethanesulphinate (1:1), vehicle: water	5480 mg/kg bw (2000 mg/kg bw of active ingredients) 24 h semiocclusive exposure and observation for 14 days	LD_{50} > 2000 mg/kg bw	(CiToxLAB Hungary, 2012)

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

One study on acute toxicity after dermal administration of TFA is available (Institute of work health and safety of the USSR, 1964), the reliability of which cannot be determined as the results are only available as secondary literature. According to the registration dossier, in this study a single

application of TFA to the skin of rabbits produced a marked irritant and caustic action with formation of a dry ulcer extending as far as the muscular layer, without however becoming infected, followed by slow healing of the ulcer without moist granulations, with the formation of a soft delicate scar that was attached to the underlying tissue.

In another study with KTFA/potassium trifluoromethanesulphinate (1:1) according to OECD TG 402, the acute dermal toxicity was determined with five Wistar rats/sex. A semiocclusive dermal single application of KTFA/potassium trifluoromethanesulphinate (purity 36.5 %) without vehicle for 24 h with a concentration of 5480 mg/kg bw (2000 mg/kg bw of active ingredients) did not result in any adverse effects (CiToxLAB Hungary, 2012). Therefore, the LD_{50} was determined as > 2000 mg/kg bw. Therefore, the LD_{50} was determined as > 2000 mg/kg bw, corresponding in an LD_{50} of >1000 mg/kg bw for KTFA.

10.2.2 Comparison with the CLP criteria

No lethal dose after dermal exposure to TFA is reported and the LD_{50} was determined as > 1000 mg/kg bw in the study with KTFA. According to the Guidance for the application of the CLP criteria (ECHA, 2017), an LD_{50} between 1000 and 2000 mg/kg bw would justify a classification in Category 4, however the data in this report is not sufficient for classification.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

In accordance with Annexes VII and VIII of the REACH legislation, acute toxicity studies do not need to be conducted for TFA, because this substance is classified as corrosive to the skin (Skin Corr. 1A, H314). Therefore, no classification is warranted for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

Table 13: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity study, no guideline, no GLP, Klimisch 4 as reported by registrant (not assignable as only available as secondary literature)	Rat, strain not specified, n = 120 per sex per dose	TFA, purity not specified	Test concentrations not reported LC_{100}: 11.5 mg/L LC_{10}: 8.3 mg/L Maximum tolerated concentration: 7.2 mg/L Exposure duration: 2 h	10 mg/L (2 h) 5 mg/L (4 h, modified according to Haber's law)	(Institute of work health and safety of the USSR, 1964)
Acute inhalation toxicity study, no guideline, no GLP, Klimisch 4 as reported by registrant (not assignable as only available as	Mouse, strain not specified, n = 110 per sex per dose	TFA, purity not specified	Test concentrations not reported LC_{100}: 20.4 mg/L LC_{10}: 9.2 mg/L Maximum tolerated	13.5 mg/L (2 h) 6.75 mg/L (4 h, modified according to Haber's law)	(Institute of work health and safety of the USSR, 1964)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
secondary literature)			concentration: 8.4 mg/L Exposure duration: 2 h		
Acute inhalation toxicity study, OECD TG 403, GLP, Klimisch 2 as reported by registrant (designed to assess the irritant potential of TFA on the upper respiratory tract in rat rather than lethality)	Rat, Wistar, n = 10 per sex per group	TFA, 99.9 %, vapour	0; 30; 100 and 300 mg/m³ Nose-only 4 h exposure 14-days observation period	No lethality up to 300 mg/m³	(TNO, 2010)

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

One study on acute toxicity after inhalation of TFA is available (Institute of work health and safety of the USSR, 1964), the reliability of which cannot be determined as the results are only available as secondary literature. According to the registration dossier, this inhalation study was performed on rats (n = 120) and mice (n = 110). The observation period following administration was 14 days. The following lethal concentrations were determined for rats (2 hrs of exposure): LC_{100} = 11.5 mg/L, LC_{50} = 10.0 mg/L, LC_{10} = 8.3 mg/L, maximum tolerated concentration: 7.2 mg/L. The following lethal concentrations were determined for mice (2 hrs of exposure): LC_{100} = 20.4 mg/L, LC_{50} = 13.5 mg/L, LC_{10} = 9.2 mg/L, maximum tolerated concentration: 8.4 mg/L.

As the standard exposure time for acute toxicity studies is 4 h, the 2 h LC_{50}-values need to be modified according to Haber's law $C^n \times t = k$, with n = 1 for extrapolation to longer duration according to Guidance on IR&CSA, Section R.7.4.4.1[3], and hence divided by 2. This results in an $LC_{50,\,4h}$ for mice of 6.75 mg/L, and an $LC_{50,\,4h}$ for rats of 5 mg/L.

After 2 h exposure the experimental animals displayed symptoms of irritation of the mucous membranes of the respiratory pathways and eyes, motor activity being replaced by depression, adynamia and severe breathing disturbances. Widespread vascular changes with haemorrhaging into various organs were discovered on autopsy. According to the registration dossier, necrosis of the mucous membranes of the nose, trachea, bronchi and pulmonary tissues was found on microscopic examination. Pneumonia, pulmonary collapse and fatty degeneration of the liver cells, as well as protein and fatty dystrophy of the epithelium of individual groups of seminiferous tubules of the kidneys, were also apparent.

According to the registration dossier, no information on the method of exposure was provided.

[3] https://echa.europa.eu/documents/10162/17224/information_requirements_r7a_en.pdf/e4a2a18f-a2bd-4a04-ac6d-0ea425b2567f, last accessed on 15.10.2024

A second acute 4 h inhalation toxicity study performed according to OECD TG 403 with rats (Wistar), including additional examinations on broncho-alveolar lavage and upper respiratory tract histopathology is available (TNO, 2010). Test concentrations of TFA were: 0, 3, 10, 300 mg/m^3, n = 10 per dose group. Only males were tested, as males were more sensitive to the effects of TFA in a preliminary study. Five males of each dose group were necropsied one day after the end of exposure and five males of each dose group after 14 days.

Mortality and clinical signs of toxicity were not observed. Observed histopathological lesions consisted of very slight focal degeneration of the respiratory epithelium lining the dorsal part of the septum, only in animals of the high concentration. This effect was reversible as there was no irritation detectable in the recovery group 14 days after treatment. Based on effects on the respiratory epithelium, the NOAEC was determined at 300 mg/m^3.

10.3.2 Comparison with the CLP criteria

According to the Guidance for the application of the CLP criteria (Version 5.0, July 2017), an LC_{50} (inhalation route, vapour) > 2 and ≤ 10 mg/L (Institute of work health and safety of the USSR, 1964), would result in the Acute toxicity hazard category 3 and based on an adjusted 4 h-LC_{50} of 5 mg/L in rats an ATE of 5 mg/L is proposed.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

In accordance with Annexes VII and VIII of the REACH legislation, acute toxicity studies do not need to be conducted for TFA, because this substance is classified as corrosive to the skin (Skin Corr. 1A, H314). The available inhalation acute toxicity studies with TFA resulted in an LC_{50} (inhalation route, vapours, adjusted for 4 h) of 5 mg/L for rats and 6.75 mg/L for mice. Although limitations of the study reporting were noted, these LC_{50} values warrant classification as Acute Tox. 3, H331 (Toxic if inhaled). Because of the clinical signs of irritation and necrosis of the upper respiratory tract mucosa and because of the known corrosive nature of TFA, the supplemental hazard statement EUH071: 'corrosive to the respiratory tract' according to Annex I: 3.1.2.3.3 and Annex II, 1.2.6, CLP Regulation is proposed. Based on this assessment it is concluded that TFA warrants classification under CLP Regulation as **Acute Tox. Cat. 3 (H331; Toxic if inhaled); EUH071 — 'Corrosive to the respiratory tract'.**

10.4 Skin corrosion/irritation

Not assessed in this dossier, as the TFA (the acid) is already classified as Skin Corr. 1A.

10.5 Serious eye damage/eye irritation

Not assessed in this dossier

10.6 Respiratory sensitisation

Not assessed in this dossier

10.7 Skin sensitisation

Not assessed in this dossier

10.8 Germ cell mutagenicity

Not assessed in this dossier

10.9 Carcinogenicity

Not assessed in this dossier

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 14: Summary table of animal studies on adverse effects of TFA or its sodium salt on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Extended one generation reproductive toxicity study, OECD TG 443 Reliability: Klimisch 1 as reported by registrant (reliable without restriction) Rat (Wistar) P0 n/group = 25 (f) F1 n/sex/group/cohort: 20 (1 m and/or 1 f per litter) Scheduled termination P males: after weaning of F1 animals; P females: PPD28 F1: Unselected F1 offspring was sacrificed at culling on Day 4 and Day 22 of age. Selected Cohort 1A animals were sacrificed at approximately 13 weeks of age and Cohort 1B animals were sacrificed at approximately 14 weeks of age.	NaTFA (99.9 %) Oral (diet) 0, 120, 600, 3000 ppm, 10 weeks premating (9.71, 49.2 and 248 mg/kg/day for males and 10.26, 53.9 and 265 mg/kg/day for females), and throughout gestation period (8.65, 44.3 and 223 mg/kg/day for females) 0, 60, 300, 1500 ppm during lactation period, lactation day (LD) 1 - 21 (9.85, 47.5 and 233 mg/kg/day) 0, 60, 300, 1500 ppm, F1 from weaning (m offspring: 0, 9.37, 47.3, 242 mg/kg bw/d; f offspring: 0, 9.83, 49.4, 248 mg/kg bw/day)	No clinical signs or unscheduled deaths observed in parental animals **Maternal effects** No significant changes in overall bw gain during pre-mating period ↓ bw gain on GD 7 - 14 (-13 %**) at hd (but no significant change in overall bw gain during gestation period) ↓ bw on GD 14 (-5 %*) and on LD 1 (-5 %*) and LD 14 (-4 %*) at hd ↓ mean food consumption on GD 7 - 19 at hd and on GD 7 - 13 also at ld and md (all -5 - 10 %* - **) ↓ mean food consumption during lactation on LD 4 - 20 (-9 - 24 %* - **) No effects on oestrus cyclicity No effect on fertility or fecundity indices, cohabitation time, gestation length No effects on litter size **Paternal effects** ↓ bw gain in weeks 1, 4 and 13 of pre-mating period at md (-7-19 %* - **) and hd (-9-31 %* - **) ↓ overall bw gain during pre-mating period (-8 %**) at hd ↓ bw during pre-mating period day 50 - 120 at hd (-5-7 %*) and terminal bw (-6 %*) at hd ↓ mean food consumption in pre-mating week 5 (md and hd) and week 7 and 9 at hd (-5-6 %* - **)	(Labcorp Laboratories, 2021b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓ weight of cauda epididymis (-9 %*) at hd (only hd tested); hd value (0.231 g) within HCD range (0.230 - 0.233 g), but control value (0.253 g) above HCD range↓ total sperm in cauda epididymis (-18 %*) at hd (only hd tested); hd value (101 mio) within HCD range (74 - 104 mio), but control value (123 mio) above HCD range; no HCD time range provided ↓ normal % sperm (-1 %*) at hd (only hd tested); hd value (95.8 %) within HCD range (94.4 – 96.7 %), but control value above HCD range (97.1 %); no HCD time range provided ↑ total abnormal % sperm (+45 %*) at hd (only hd tested); hd value (4.2 %) within HCD range (3.3 – 5.6 %), but control value below HCD range (2.9 %); no HCD time range provided Further sperm effects in parental males (nature of effects similar to F1 males, but without statistical significance): • head abnormal +25 % (from 0.8 % in control to 1 % at hd; both values below HCD range (1.1 - 1.3 %)); no HCD time range provided • head flat +43 % (from 0.7 % in control to 1 % at hd; no HCD range provided); no HCD time range provided No effects on cohabitation time and fertility indices (mating index, fertility index) <u>Parental clinical chemistry:</u> ↑ ALP in males at md (+26 %**) and hd (+34 %**) ↓ plasma glucose at all doses in both sexes (f: -14 %* at ld, -11 %* at md, -14 %* at hd; m: -12 %* at ld, -18 %** at md, -13 %** at hd) ↓ non-esterified fatty acids at all doses in both sexes (f: -28 %** at ld, -21 %** at md, -32 %** at hd; m: -16 %** at ld, -24 %** at md, -31 %** at hd) ↓ triglycerides at all doses in males (-35 %** at ld, -33 %** at md, -38 %** at hd) ↑ A/G ratio at md (m: +13 %**) and hd (f: +9 %*; m: +17 %**)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		No adverse histopathological findings in the parental animals **Effects on F1 males** No clinical signs or unscheduled deaths observed in offspring ↓ abs. testes weight at hd (-8 % in Cohort 1A** and -7 % in Cohort 1B*) (no HCD range and time range provided) ↓ abs. epididymis weight at hd (-8 % in Cohort 1A*, n.s. in Cohort 1B) No effects on % motile and % progressive sperm at any dose (Cohort 1A) <u>Sperm analysis parameters only tested at hd (Cohort 1A):</u> ↓ abs. weight of left testis (-7 %**); control value (1.83 g) within HCD range (1.83 - 1.94 g), hd value (1.70 g) below HCD range; no HCD time range provided ↓ testis spermatid count per gram tissue (-16 %**); control (97 mio/g) and hd (81 mio/g) values within HCD range (72 – 99 mio/g); no HCD time range provided ↓ testis total spermatids (-24 %**); control (178 mio) and hd (136 mio) values within HCD range (133 -181 mio); no HCD time range provided <u>Sperm motility data tested at all doses (Cohort 1A):</u> ↓ motion value VAP at all doses; all dose values within HCD range (120 – 143 µm/s), control value slightly above HCD range • control: 146 µm/s • ld: 137 µm/s (-6 %)* • md: 138 µm/s (-5 %)*, • hd: 140 µm/s (-4 %)* ↓ motion value VCL at md and hd; md and hd values within HCD range (270 – 338 µm/s), control value above HCD range • control: 357 µm/s • md: 328 µm/s (-8 %)* • hd: 331 µm/s (-7 %)* ↓ motion value BCF at hd; hd values within	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		HCD range (32 – 35 hz), control value slightly above HCD range • control: 36 hz • hd: 35 hz (-3 %)* ↑ abnormal sperm (head abnormal) at hd; **hd value above HCD range** (1.2 – 2.1 %), control value below HCD range • control: 1.1 % • hd: 2.3 % (+109 %)* ↑ abnormal sperm (head flat) at hd; **hd value above HCD range** (0.9 – 1.5 %), control value within HCD range • control: 1.0 % • hd: 2.1 % (+110 %)* No HCD time ranges were provided. No adverse histopathological findings in F1 animals	
Combined repeated dose toxicity study with reproduction/developmental toxicity screening test, OECD TG 422, GLP Reliability: Klimisch 1 (reliable without restriction) Rat (Sprague-Dawley) n/sex/group = 10 (m & f)	KTFA/potassium trifluoromethanesulphinate (1:1) Oral (gavage) 0, 100, 300, 1000 mg/kg bw/day Vehicle: water Females: starting 2 weeks before pairing, during pairing, during gestation, during lactation, until sacrifice Males: starting 2 weeks before pairing, during pairing, until sacrifice	**Parental generation** No clinical effects observed. No effects on body weight observed. No effects on food consumption observed. <u>Haematology</u> In hd males: -10.2 % in haemoglobin content ($p < 0.01$) <u>Clinical chemistry</u> In hd males: +13.4 % in inorganic phosphorus ($p < 0.05$) and -4.9 % in calcium ($p < 0.05$) No effects observed on behaviour. <u>Organ weight</u> mean absolute and relative liver weights were increased in md and hd males (statistically significant) and in hd females (not statistically significant); correlation with microscopic changes observed in the liver mean absolute and relative kidney weights in hd females (statistically significant); no microscopic correlates No effects on gross pathology. <u>Histopathology</u> minimal centrilobular hypertrophy of hepatocytes in 4/5 hd males, 2/5 hd females and 1/5 md males, correlation with increased liver weights; not considered	(CIT BP, 2012)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		adverse because of absence of associated degenerative microscopic findings or change in enzyme activities males at all doses: haematopoiesis in the spleen minimally increased control: 4/4 Grade 1 ld: 2/5 Grade 1; 3/5 Grade 2 md: 3/5 Grade 1; 2/5 Grade 2 hd: 3/5 Grade 1; 2/5 Grade 2 <u>Mean number of implantation sites per female</u> Control: 17.2 ld: 15.7 md: 15.9 hd: 15.8 <u>Mean pre-implantation loss (%) per female</u> Control: 4.6 % ld: 6.6 % md: 8.8 % hd: 14.6 % <u>Mean number of pups delivered per female</u> Control: 15.0 ld: 13.7 md: 15.0 hd: 14.1 **Offspring** No clinical signs or mortality observed. No effects on body weight observed. No gross pathological findings. Sexual maturation, organ weight and histopathology not examined.	

From the STOT RE studies listed under 10.12, information directly relevant for classification of sexual function and fertility cannot be retrieved.

<u>Human Data on Reproductive Toxicity</u>

Human epidemiological studies on TFA are rare. The literature search did not result in epidemiological studies investigating the association of TFA in human blood and reproductive outcomes.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

EOGRTS in rats (Labcorp Laboratories, 2021b)

In an extended one-generation reproductive toxicity study (EOGRTS; according to OECD TG 443), NaTFA was orally administered via the diet to 25 male and 25 female Wistar rats per dose group. Doses were 0, 120, 600 and 3000 ppm in the period 10 weeks prior to mating for both sexes (equivalent to 9.71, 49.2 and 248 mg/kg/day for males and 10.26, 53.9 and 265 mg/kg/day for females), throughout the gestation period (8.65, 44.3 and 223 mg/kg/day for females), and during the lactation period (lactation day (LD) 1 - 21) 0, 60, 300, 1500 ppm (equivalent to 9.85, 47.5 and 233 mg/kg/day). In the offspring generation (F1), culling of litters was performed on postnatal day (PND) 4. For Cohorts 1A and 1B, 20 animals per sex per dose group (one male and/or one female per litter) were selected on PND 22. Offspring received 0, 60, 300, 1500 ppm from weaning (equivalent to 0, 9.37, 47.3, 242 mg/kg bw/day in male offspring and 0, 9.83, 49.4, 248 mg/kg bw/day in female offspring). Scheduled sacrifice for parental males and females was after weaning of F1 animals after confirmation that no further mating was required. F1 animals were sacrificed on PND 35.

For parental male and female rats, no significant effects were observed on cohabitation time or fertility indices. For parental females, no significant effects were observed on oestrus cyclicity, fecundity indices, or gestation length. In parental males, a significant decrease of the weight of the cauda epididymis and a decrease of total sperm were observed in the high dose group (3000 ppm, 248 mg/kg bw/day). A statistically significant decrease of the percentage of normal sperm (-1 %) and a significant increase of abnormal sperm (+45 %) at the high dose group was observed. Of note, only the high dose group of parental males was investigated for these effects.

Clinical chemistry parameters were affected in both, parental males and females. These effects included increased ALP in males in the mid (+26 %) and high dose (+34 %) group. In all dose groups of both parental sexes decreased plasma glucose (ca. -13 - 14 %, no clear dose-response in males or females), decreased non-esterified fatty acids (dose-response for males ranging from -16 % to -31 %), and decreased triglycerides in all dose groups of males (ca. -35 %, no dose-response) were reported. A/G ratio was increased in males of the mid dose (+13 %) and the high dose group (+17 %) and in females of the high dose group (+9 %). The relevance of these clinical chemistry parameters is considered unlikely to affect fertility parameters because a clear relationship between ALP and fertility has not been established, decreases of plasma glucose, non-esterified fatty acids, or triglycerides are not considered adverse, and the increase of A/G ratio was small.

In high dose F1 males of both, Cohort 1A and 1B, absolute weights of the testes were statistically significantly reduced by 7 – 8 %. Absolute epididymal weight was also significantly reduced in the high dose group by 8 % in Cohort 1A males, but not in Cohort 1B males. Percentages of motile or progressive sperm were not affected at any dose in Cohort 1A. In Cohort 1A, the left testis was homogenised and spermatid counts per gram were reduced by 16 % and total spermatids were reduced by 24 % (only testes of the high dose were examined); control and high dose values were in the range of historical control data (HCD). Several sperm motility parameters were significantly reduced in Cohort 1A males by 3 – 8 % (VAP at all doses, VCL at mid dose and high dose, BCF at high dose), but all values from treatment groups were within the HCD range. It should be noted that control values for these sperm motility parameters were outside the HCD range. Statistically significantly increased numbers of abnormal sperm (head abnormal and head flat) in the high dose group (+109 – 110 %) were above the given HCD range (head abnormal: 1.1 % at control, 2.3 % at high dose, HCD range: 1.2 – 2.1 %; head flat: 1.0 % at control, 2.1 % at high dose, HCD range: 0.9 – 1.5 %). However, HCD time ranges for fertility-relevant parameters were not provided by the registrant. HCD should be contemporary to the study (5 years), however, this information was not

available, therefore comparability is not given. Furthermore, HCD should only be used as additional evaluation, as the concurrent control is the most relevant comparator.

In conclusion, significant reduction of the weight of reproductive organs (testes, cauda epididymis), reduction of sperm (P)/spermatids (F1) as well as an increase of abnormal sperm among some other sperm parameters were observed in males of both generations. Because the reduction of sperm (P) or spermatids (F1) were only tested at the high dose, uncertainties remain regarding the dose-response of these effects and their dose thresholds. Studies on specific target organ toxicity with repeated exposure did not report effects on the weights or histopathology of reproductive organs, if examined (see 10.12). The fertility effects in this study did not lead to a significant effect on reproductive success of the parental generation.

Combined repeated dose toxicity study with reproduction/developmental toxicity screening test (CIT BP, 2012)

In a combined repeated dose toxicity study with reproduction/developmental toxicity screening test (according to OECD TG 422), KTFA/ potassium trifluoromethanesulphinate (1:1) was administered via oral gavage to 10 male and 10 female Sprague-Dawley rats per dose group. Doses were 0, 100, 300 and 1000 mg/kg bw/day starting 2 weeks before pairing, during pairing, during gestation (only f), during lactation (only f) and until sacrifice.

The mean number of implantation sites was reduced for all dose groups compared to the control (control: 17.2; ld: 15.7; md: 15.9; hd: 15.8) and the mean pre-implantation loss was increased with 4.6 %, 6.6 %, 8.8 % and 14.6 % for control, ld, md and hd, respectively. However, the overall reproductive success was not impaired.

10.10.3 Comparison with the CLP criteria

All data provided in the registration dossier as well as publicly available data were considered to conclude on the classification for toxicity to reproduction (sexual function and fertility effects). Human data on reproductive toxicity arising from exposure to TFA or its inorganic salts are not available. Data from one extended one-generation toxicity study with experimental animals are available (Table 14).

Category 1A: Known human reproductive toxicant
The classification of a substance in Category 1A is largely based on evidence from humans.

There is no information available which supports a known adverse effect of trifluoroacetic acid or its salts on reproduction in humans.

Category 1B: Presumed human reproductive toxicant
The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Some fertility effects in rats were identified but overall reproductive success was not impaired (Labcorp Laboratories, 2021b). Therefore, the evidence is considered not sufficiently convincing to place the substance in Category 1B.

Criteria for CATEGORY 2: Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Relevant fertility effects included decreased male fertility parameters in rats in the parental and offspring generation (reduced sperm/spermatids, increased percentage of abnormal sperm) or only in the offspring generation (sperm motility data) (Labcorp Laboratories, 2021b). The values of the parental male fertility effects were within the HCD range, but it should be noted that the respective control values were outside the HCD range. For the male offspring, all values on spermatid counts were within HCD range. For sperm motility, data of the offspring, values from dose groups were within HCD range but control values were outside the HCD range (above HCD range for decreasing effects, and below HCD range for increasing effects). The percentage of abnormal sperm (flat head) in the offspring was above HCD range. However, the concurrent controls are the most relevant comparator. Because male fertility parameters were only tested at the high dose, uncertainties remain regarding the dose-response of these effects and their dose thresholds. For parental females, significant effects were not observed on fertility.

Although some fertility effects were identified in rats, evidence is limited because overall reproductive success was not impaired (Labcorp Laboratories, 2021b). However, unaffected reproductive success despite impaired sperm quality is not uncommon for the rat as test species, as stated in the OECD Guidance document on Mammalian Reproductive Toxicity: "(…) the male rodent has a large excess of spermatozoa and therefore it takes a large reduction in sperm number to be reflected as a change in the fertility index."[4]. This has also been used in the argumentation of the RAC opinion on 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (DBMC): "Decreased fertility as revealed by effect on fertility index is a rather insensitive endpoint in rats. This may be explained by the rather high sperm reserve available in rats compared to humans"[5]. Hence, humans may be more sensitive with respect to fertility and reduced sperm quality than rodents. No marked parental systemic toxicity was observed which could lead to the observed fertility effects.

Regarding fertility effects, classification in category 2 is considered appropriate.

[4] https://one.oecd.org/document/ENV/JM/MONO(2008)16/en/pdf; p. 63; last access August 28th, 2023

[5] https://echa.europa.eu/documents/10162/f5a13a30-d11a-65b9-6730-592692c151f2; p. 7; last access August 28th, 2023

10.10.4 Adverse effects on development

Table 15: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity study, OECD TG 414 Reliability: Klimisch 1 as reported by registrant (reliable without restriction) Rabbit (New Zealand White) mated females no/group = 24 Sacrifice: All surviving study animals were euthanised and subjected to necropsy and caesarean section on GD 29.	NaTFA (99.9 %) 0, 180, 375, 750 mg/kg bw/d Oral (gavage; vehicle: water), once daily GD 6 - 28	**Maternal effects** <u>Clinical signs:</u> Hypoactivity and dilated pupils on GD 27 - 28 (1 female at md) Irregular breathing and unsteady gait on GD 7 (1 female at hd) <u>Mortality (not considered treatment related):</u> 1 control female on GD 27 (convulsion after dosing, post-mortem examination: perforated trachea, dosing trauma) 1 hd female on GD 27 (evidence of pregnancy loss, post-mortem examination: enlarged spleen, 8 corpora lutea, 2 resorbing implantation sites) <u>Body weight and food consumption:</u> No significant changes in unadjusted bw or bw gain No significant changes in adjusted bw on GD 29 ↓ maternal adj. negative bw gain (GD 6 - 29) at md (-48 %*) and hd (-56 %**; this means that body weight loss comparing GD 6 and GD 29 after caesarean section was less than in controls, i.e. no sign of toxicity) ↓ maternal food consumption on GD 6 - 20 at md (-3 - 32 %*-**) and hd (-3 - 31 %*-**) (no apparent time or dose relation) ↑ maternal food consumption on GD 26 and 28 at ld (+17 - 25 %), md (+18 - 24 %*-**) and hd (+14 - 22 %*-**) ↓ number of corpora lutea at hd (-20 %*) • control: 10.4 ± 2.06 • ld: 9.6 ± 2.29 • md: 9.6 ± 1.64 • hd: 8.3* ± 2.48 ↓ implantations at hd (-21 %*) • control: 9.1 ± 2.00 • ld: 8.5 ± 2.09 • md: 8.0 ± 2.71 • hd: 7.2* ± 2.67 ↓ litter size at hd (-26 %**) • control: 8.3 ± 2.47 • ld: 7.5 ± 1.78 • md: 7.2 ± 2.77 • hd: 6.1** ± 2.59	(Covance Laboratories, 2021b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓ gravid uterine weight at md (-18 %*) and hd (-29 %**) **Effects on offspring** ↓ mean foetal weight on GD 29 (combined sexes or f) at hd (-10 %*) ↓ mean foetal weight on GD 29 (m) at md (-12 %**) and hd (-11 %**) ↓ total litter weight at md (-22 %**) and hd (-34 %**) ↓ mean live litter size at hd (-27 %**) No effects on sex ratio **Malformations in offspring** Number of examined foetuses and litters for laboratory historical control data (HCD) range for all malformations: 5687 foetuses and 744 litters ↑ Skeletal malformations • multiple cervical/thoracic/lumbar/caudal vertebral and rib abnormalities with associated minor abnormalities affecting the vertebrae, ribs, sternum and costal cartilages in md and hd • fused/partially fused ribs (HCD range 0 - 4 foetuses, 0 – 3 litters) • 0/150 foetuses in 0/18 litters in control • 1/158 foetuses (0.6 %) in 1/21 litters (4.8 %) at ld • 4/173 foetuses (2.3 %) in 3/24 litters (12.5 %) at md • 8/140 foetuses (5.7 %) in 6/23 litters (26 %) at hd **↑ Eye malformations (dose-dependent in all groups)** • multiple folded retina (HCD range 0 – 1 foetus, 0 – 1 litter) • 0/150 foetuses in 0/18 litters in control • 1/158 foetuses (0.6 %) in 1/21 litters (4.8 %) at ld • 5/173 foetuses (2.9 %) in 4/24 litters (17 %) at md • 9/140 foetuses (6.4 %) in 8/23 litters (35 %) at hd • absent aqueous/vitreous humour (HCD range 0 – 1 foetus, 0 – 1 litter) • 0/150 foetuses in 0/18 litters in control	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		• 1/158 foetuses (0.6 %) in 1/21 litters (4.8 %) at ld • 6/173 foetuses (3.5 %) in 4/24 litters (17 %) at md • 8/140 foetuses (5.7 %) in 6/23 litters (26 %) at hd • retina ruptured into surrounding tissue (HCD range: 0 - 0) • 0/150 foetuses in control in 0/18 litters • 0/158 foetuses in 0/21 litters at ld • 1/173 foetuses (0.58 %) in 1/24 litters (4.2 %) at md • 1/140 foetuses (0.71 %) in 1/23 litters (4.3 %) at hd • microphthalmia (HCD range: 0 – 1 foetus in 0 – 1 litter) - 0/150 foetuses in control in 0/18 litters - 1/158 foetuses (0.6 %) in 1/21 (4.8 %) litters at ld - 1/173 foetuses (0.58 %) in 1/24 litters (4.2 %) at md - 1/140 foetuses (0.71 %) in 1/23 litters (4.3 %) at hd • absent lens (HCD range 0 – 1 foetus in 0 – 1 litter) - 0/150 foetuses in control in 0/18 litters - 0/158 foetuses in 0/21 litters at ld - 0/173 foetuses in 9/24 litters at md - 1/140 foetuses (0.71 %) in 1/23 litters (4.3 %) in hd <u>Cardiovascular abnormalities</u> • transposition of ascending aorta/pulmonary trunk in control (1/150 foetuses, 1/18 litters) and all doses (2/158 at ld, 1/173 at md, 2/140 at hd); (HCD range 0 - 1) • double outlet ventricle(s) at ld (1/158) and md (1/173) (HCD range: 0 - 0) <u>Kidney malformations</u> • displaced kidney at ld (1/158) (HCD range 0 - 0), • fused/caudally displaced kidney at hd (1/140) (HCD range 0 - 0) <u>↑ Few external malformations</u>	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		• omphalocele: 2/173 foetuses in 2/24 litters at md (Control: 0/150 foetuses, HCD range 0 - 2) • gastroschisis: 1/173 foetuses in 1/24 litters at md and 1/140 foetuses in 1/23 litters at hd (Control: 0/150 foetuses, HCD range 0 - 1) • cleft palate: 1/140 foetuses in 1/23 litters (Control: 0 foetuses, HCD range 0 – 1 foetus, 0 – 1 litter) <u>Eye histopathology</u> • slight to marked retinal folds o 1 foetus in ld o 5 of foetuses in md (number of examined eyes: 8) o 11 foetuses at hd (number of examined eyes: 11) • lens degeneration in 6/20 foetuses with retinal folds • haemorrhage in vitreous chamber in 2/20 foetuses that had retinal folds • absence of aqueous and/or vitreous humour/body (13/20 that had retinal folds) Microscopic, structural (anatomical) disorganisation of the eyes at md (1 m, 1 f) and at hd (1 m, 1 f)	
Preliminary study to prenatal developmental toxicity study Reliability: Klimisch 1 as reported by registrant (reliable without restriction) Rabbit (New Zealand White) mated females n/group = 7 Sacrifice: All surviving study animals were euthanised and subjected to necropsy and caesarean section on GD 29.	NaTFA (99.9 %) 0, 250, 500, 1000 - 750 mg/kg bw/day (hd reduced after second dose on GD 7) Oral (gavage; vehicle: water), once daily GD 6 - 28	**Maternal effects** <u>Clinical signs:</u> Unsteady gait and/or hypoactivity • at hd on GD 6 (4 females) and GD 7 (all females) • at md on GD 8 (4 females) and on GD 8, 9, 12 (1 female) • at ld on GD 8 or 9 (2 females) Impaired locomotion on GD 7 at hd (limited use of limbs, 2 females) Rapid breathing, reduced body tone and flattened posture on GD 7 at hd (1 female) High dose was lowered to 750 mg/kg bw/day from GD 8 and clinical signs were not present any more by GD 12 <u>Body weight and food consumption (data not available from registration dossier):</u> ↓ maternal bw gain at ld and hd during treatment GD 6 - 28 No changes in maternal adj. bw on GD 29 and adjusted bw gain GD 6 - 28	(Covance Laboratories, 2021a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No statistical analysis was performed for this study		↓ maternal gravid uterus on GD 29 at ld and hd ↓ maternal food consumption GD 6 - 16 at ld and GD 6 - 18 at md and hd (thereafter comparable or higher than control food consumption) No effects on pregnancy rate No effects on number of litters ↓ live foetuses/dam in all doses (-33 % at ld, -5 % at md, –27 % at hd) No changes in mean placental weights **Effects on offspring** ↑ total resorptions in all doses (+157 % at ld, +114 % at md, +143 % at hd) ↑ early resorptions/dam in all doses (+267 % at ld, +100 % at md, +333 % at hd) ↑ late resorptions/dam at ld and md (+75 % at ld and md, no change at hd) No total litter losses by resorption No effects on number of abortions ↑ post-implantation loss per litter in all doses (+301 % at ld, +104 % at md, +235 % at hd) ↓ foetal weight (-5 % at ld, –7 % at md, -9 % at hd) ↓ mean total litter weight (-37 % at ld and hd, -11 % at md) ↓ sex ratio (%m) (-34 % at ld, -5 % at md, -28 % at hd) <u>Single incidences of eye malformations:</u> • 1 foetus at ld with dark areas in right eye (HCD range NA) • 1 foetus at hd with small right eye (HCD range 0 - 0) • 1 foetus at hd with opaque right eye (HCD range NA) <u>Variations of the respiratory tract:</u> • absence of accessory lung lobe (1/54 foetuses and 1/7 litters at md, and 4/41 and 2/7 litters at hd) • incidences of ascending aorta/pulmonary trunk transposition (1/54 foetuses at md and 1/41 foetuses at hd); within HCD range (0 - 1)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity study, OECD TG 414 (main mechanistic developmental study) Reliability: Klimisch 1 as reported by registrant (reliable without restrictions), GLP Rabbit (New Zealand White) Mated females n/group = 30 n/group = 8 satellite females Sacrifice: All surviving study animals from the main phase were euthanised and subjected to necropsy and Caesarean section on GD 29. Satellite females: were euthanised and subjected to necropsy on GD 20.	NaTFA (99.9 %) Oral (gavage, vehicle: water), once daily 0, 30 (ld), 60 (lmd), 250 (hmd), 750 (hd) mg/kg bw/d GD 6 - 28 (main phase animals) Satellite females: GD 6 - 19	**Maternal effects** Maternal clinical signs and mortality: Decreased faecal output and small faecal pellets at hmd and hd 1 unscheduled euthanisation in mhd on GD 27 (unexpected dosing event) 1 unscheduled euthanisation in hd on GD 27 (evidence of abortion) <u>Maternal body weight (gains) and food consumption</u> ↓ bw on GD 6 - 7 at hmd and hd ↓ bw gain GD 6 - 14 at all doses (-45 % at ld**, -45 % at lmd**, -36 % at hmd**, -36 % at hd**; mainly resulting from reduced bw gain on GD 13 - 14, no dose-response) No effects on overall bw gain ↑ adj. bw at hd on GD 29 (+6 %)** ↓ weight of gravid uterine at hd (-18 %)* ↓ food consumption at hmd and hd GD 6 - 15 (-12 - 22 %)*-** ↑ food consumption in all dose groups during late gestation GD 25 - 29 (+7 - 21 %)*-** <u>Maternal clinical chemistry (satellite females sampled on GD 5 and GD 12):</u> ↑ cholesterol at hd on GD 19 (+35 %)** and GD 28 (+33 %)** ↓ non-esterified fatty acids at hmd and hd on GD 19 (hmd: -28 %*, hd: -31 %*) and at lmd, hmd, and hd on GD 28 (lmd: -33 %**, hmd: -33 %**, hd: -36 %**) (satellite females at GD 12 -53 %**) ↑ triglycerides in all dose groups on GD 19 (ld: +33 %*, lmd: +30 %*, hmd: +26 %*, hd: +25 %*), and at hmd and hd on GD 28 (hmd: +37 %**, hd: +57 %**) (satellite females at hd on GD 12: +81 %**) ↓ bile acids in all dose groups on GD 19 (ld: -46 %**, lmd: -48 %**, hmd: -62 %**, hd: -67 %**), and at lmd, hmd, and hd on GD 28 (lmd: -25 %*, hmd: -43 %**, hd: -51 %**) (satellite females in all dose groups on GD 12: ld: -33 %**, lmd: -49 %**, hmd: -43 %**, hd: -53 %**) <u>Maternal Organ weights</u> ↑ abs. liver weights higher in all dose groups (ld: +12 %*, lmd: +16 %**, hmd: +26 %**, hd: +28 %**, histopathological correlations) ↑ abs. kidney weights higher in all dose groups (ld: +17 %**, lmd: +15 %**, hmd: +27 %**, hd: +31 %**, no macroscopic or microscopic correlations) <u>Maternal histopathology:</u>	(Labcorp Laboratories, 2024c)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Liver: Minimal hepatocellular hypertrophy at lmd (3/28), hmd (3/28), and hd (12/28) Minimal to moderate bile duct hyperplasia at lmd (1/28 minimal + 1/28 mild), hmd (4/28 mild), hd (8/28 minimal + 2/28 mild + 1/28 moderate) Minimal to moderate portal/bile duct fibrosis at hmd (1/28 minimal + 1/28 mild) and hd (2/28 minimal +1/28 mild + 1/28 moderate) minimal to slight periportal lymphotic inflammatory cell infiltrate in control (3/28 minimal), at ld (6/29 minimal), at lmd (1/28 slight/mild), at hmd (2/28 minimal + 2/28 slight/mild), and at hd (9/28 minimal + 2/28 slight/mild) <u>Reproductive outcomes:</u> 1 abortion at hd on GD 27 (early euthanasia, 5 late resorptions, 3 live young) No effect on corpora lutea No effect on implantation sites No changes in pregnancy duration No effect on placenta weight No effect on number of pregnant females or total number of litters No effect on pre-implantation loss No effect on litter size (live foetuses/dam) No total litter losses No early or late resorptions No litters with total resorptions **Offspring** ↑ post-implantation loss at hd (+139 %, from 8.1 % in control to 19.4 % at hd)* ↓ mean foetal weight at hd (-9 %)** ↓ mean litter weight at hd (-22 %)** No dead foetuses No effect on sex ratio No changes in postnatal survival **<u>Foetal eye malformations (all above HCD range):</u>** • Multiple folded retina (HCD range 0/2128 foetuses, 0/270 litters) o 0/209 foetuses in 0/28 litters in control o 0/244 foetuses in 0/29 litters at ld o 0/219 foetuses in 0/28 litters at lmd o 2/193 foetuses (1.0 %) in 2/28 litters (7.1 %) at hmd (1 foetus also had absent aqueous humour) o 12/178 foetuses (6.7 %) in 9/28 litters (32 %) at hd • Absent aqueous/vitreous humour (HCD range 0/2128 foetuses, 0/270 litters) o 0/209 foetuses in 0/28 litters in	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		control o 0/244 foetuses in 0/29 litters at ld o 0/219 foetuses in 0/28 litters at lmd o 1/193 foetus (0.5 %) in 1/28 litter (3.6 %) at hmd o 8/178 foetuses (4.5 %) in 7/28 litters (25 %) at hd • Haemorrhage(s) aqueous/vitreous humour (HCD range 0/2128 foetuses, 0/270 litters) o 0/209 foetuses in 0/28 litters in control o 0/244 foetuses in 0/29 litters at ld o 0/219 foetuses in 0/28 litters at lmd o 0/193 foetuses in 0/28 litters at hmd o 5/178 foetuses (2.8 %) in 5/28 litters (17.9 %) at hd • Misshapen lens (HCD range 0/2128 foetuses, 0/270 litters) o 0/209 foetuses in 0/28 litters in control o 0/244 foetuses in 0/29 litters at ld o 0/219 foetuses in 0/28 litters at lmd o 0/193 foetuses in 0/28 litters at hmd o 2/178 foetuses (1.1 %) in 2/28 litters (7.1 %) at hd • Folded retina (HCD range 0/2128 foetuses, 0/270 litters) o 0/209 foetuses in 0/28 litters in control o 0/244 foetuses in 0/29 litters at ld o 0/219 foetuses in 0/28 litters at lmd o 0/193 foetuses in 0/28 litters at hmd o 1/178 foetuses (0.6 %) in 1/28 litter (3.6 %) at hd <u>Foetal visceral variations/malformations:</u> • Retroesophageal right subclavian artery (HCD range 0 - 2.0/2128 foetuses, 0 - 8.7/270 litters) o 1/209 foetuses (0.5 %) 1/28 litter (3.6 %) in control o 0/244 foetuses in 0/29 litters at ld o 1/219 foetuses (0.5 %) in 1/28 litter (3.6 %) at lmd o 5/193 foetuses (2.6 %) in 4/28 litters (14.2 %) at hmd o 13/178 foetuses (7.3 %) in 8/28 litters (28.6 %) at hd <u>Foetal skeletal variations/malformations:</u> • Fused/partially fused ribs (HCD range 0 - 0.5/2128 foetuses, 0 - 4.6/270 litters)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		○ 0/209 foetuses in 0/28 litters in control ○ 1/244 foetus (0.4 %) in 1/29 litter (3.4 %) at ld ○ 0/219 foetuses in 0/28 litters at lmd ○ 1/193 foetus (0.5 %) in 1/28 litter (3.6 %) at hmd ○ 6/178 foetuses (3.4 %) in 3/28 litters (10.7 %) at hd • Sternebrae fused/partially fused (HCD range 0 - 3.1/2128 foetuses, 0 - 18.3/270 litters) ○ 1/209 foetuses (0.5 %) in 1/28 litters (3.6 %) control ○ 0/244 in 0/28 litters at ld ○ 3/219 foetuses (1.4 %) in 2/28 litters (7.1 %) at lmd ○ 3/193 foetuses (1.6 %) in 3/28 litters (10.7 %) at hmd ○ 3/178 foetuses (1.7 %) in 3/28 litters (10.7 %) at hd • Sternebrae bipartite ossified at hd (3/178 foetuses from 3/28 litters); HCD range 0 - 1.1/2128 foetuses, 0 - 5.0/270 litters • Sternebrae misaligned ossification sites at hd (2/178 foetuses from 2/28 litters); HCD range 0 - 1.7/2128 foetuses, 0 - 10/270 litters Sternebrae misaligned hemicentres at hd (3/178 foetuses from 3/28 litters); controls 1/209 foetuses from 1/28 litter; HCD range 0 - 1.5/2128 foetuses, 0 - 9.5/270 litters	
Preliminary prenatal developmental toxicity study Reliability: Klimisch 2 as reported by registrant (reliable with restrictions), no GLP Rabbit (New Zealand White) Mated females n/group = 6 Sacrifice: All surviving study animals were euthanised and	NaTFA (99.9 %) Oral (gavage, vehicle: water), once daily 0, 10, 45, 180, 750 mg/kg bw/d GD 6 - 28	**Maternal effects** <u>Maternal clinical effects:</u> ↑ abnormal gait and rapid breathing during the first three days of treatment at hd ↓ activity at hd ↓ faecal output at hd (2 animals with loose faeces) <u>Maternal body weight (gains) and food consumption:</u> No significant effects on bw (gain) ↓ food consumption at hd GD 10-11 (-28 %*), GD 11 - 12 (-47 %**), GD 12 - 13 (-41 %*) ↑ food consumption in all doses on GD 20 - 21 (ld: +33 %*, md: +34 %*, hd: +22 %*) Maternal clinical chemistry: ↑ cholesterol at hd (+55 %*) ↑ triglycerides at hd (+74 %*) ↓ bile acids at hd (-60 %*) ↑ urea at hd (+28 %*) ↓ number of females pregnant and total number of litters (6/6 in control and ld, 5/6 at lmd and hmd, 4/6	(Labcorp Laboratories, 2024b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
subjected to necropsy and caesarean section on GD 29.		at hd) Similar number of corpora lutea across groups ↓ implantations/dam at hd (-21 %, not statistically significant) ↑ pre-implantation loss/litter at hd (+150 %, not statistically significant) No effect on litter size (live foetuses/dam) **Developmental effects** No effect on live foetuses/dam No effects on early or late resorptions No significant effect on placenta weight or total litter weight or foetal weight No effects on pre- or post-implantation loss per litter	
Prenatal developmental toxicity study, OECD TG 414 Reliability: Klimisch 1 as reported by registrant (reliable without restriction) Rat (SD) N = 27 (mated females)	KTFA/potassium trifluoromethanesulphinate (51 % KTFA) Oral (gavage; vehicle: water) GD 5 – 19 0, 100, 300, 1000 mg/kg bw/d	**Maternal effects** No clinical signs or mortality observed. No difference in body weight observed. No difference in food consumption. No effects on number of abortions. <u>Pre- and post-implantation loss</u> Decreased in hd, only significant in hd for post-implantation No difference in mean numbers of corpora lutea, implantation sites, total placental weights, and gravid-uterine weights. **Effects on offspring** No effects on foetal body weight observed. Significant increase in percentage of live foetuses in at hd (97.2 % vs. control 93.8 %). No difference in sex ratio observed. No effects observed on litter size and weights No effects observed in external or skeletal or visceral malformations. **Embryotoxic/teratogenic effects** 1 md pup: narrow tail 1 hd pup: absent tail 2 control pup: small brain 1 pup from control, 1 from md and 3 from hd: signs of uronephrosis or small kidney in unilateral or bilateral kidneys 5 pups from control, 5 from md and 4 from hd: signs of parietal incomplete ossification or signs of wavy rib No significant adverse effects compared to control observed.	(Safety Evaluation Center of Shenyang Research Institue of Chemical Industry, 2020)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity study, OECD TG 414 Reliability: Klimisch 1 as reported by registrant (reliable without restriction) Rat (SD) N = 22 (mated females) Sacrifice: Scheduled sacrifice and caesarean section on GD 20.	TFA, Purity 99 % (w/w) Oral (gavage; vehicle: water) GD 6 - 19 0, 37.5, 75, 150 mg/kg bw/d	No effects on number of abortions, No changes in pregnancy duration, No changes in number of pregnant females (one female not pregnant each in control and high dose group) No effects observed on number of live offspring or postnatal survival No effects observed No effects observed on litter size and weights No effects observed with regard to changes in sex ratio No effects observed in external or skeletal or visceral malformations Ophthalmological effects not examined	(Huntingdon Life Sciences, 2010)
Developmental toxicity study No guideline Reliability: Klimisch 2 as reported by registrant (reliable with restriction) Rat (SD) n/group (dams) = 43 - 45 (additional 5 – 6 satellite dams in each treatment group were euthanised before term to assess maternal hepatic and renal function with urine collection over 17 h on GD 20) n/group/sex (pups; PND 3 – PND 12) = 4 males and 4 females (where possible) from each of 8 - 11 litters per treatment group (urine	TFA (analytical purity: 99.7 %) Oral (gavage; vehicle: distilled water) GD 10 – GD 20 0, 75, 150 mg/kg bw/d	**Maternal effects** No treatment related mortality ↓ bw change at hd (-18 % GD 10 – 15)* No change in bw on GD 21 (exp. dams) or on GD 20 (satellite dams) ↑ abs. liver weight at both doses (+22 - 23 %)* ↑ rel. liver weight at both doses (+32 - 33 %)* No effects on length of gestation **Effects on offspring** No significant effects on survival, pup bw or litter size No effects on sex ratio No external malformations <u>Some changes of liver and kidney function in offspring (mainly on PND 3, but not on PND 12 or 49)</u> ↑ serum GLDH activity on PND 3 at both doses (+306 % at ld*, +419 % at hd*), but not on PND 12 ↑ serum AST activity on PND 3 at both doses (+27 % at ld*, +53 % at hd*), but not on PND 12 ↑ serum urea at hd (+45 %)* on PND 3, but not on PND 12 ↓ urinary GGT excretion at hd (–25 %)* on PND 3, but not on PND 12 ↑ urinary excretion of beta2-microglobulin at hd on PND 3 (+142 %) on PND 3, but not on PND 12	(Saillenfait et al., 1997)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
collection after 6.5 h isolation from fluid intake) n/sex/group (PND 49) = 1 from each of five litters per treatment group (24 h in metabolic chamber) Offspring examined on PND 3, 12, or 49 for hepatic and renal biochemistry and/or function (through serum and urinary parameters) GLDH (glutamate dehydrogenase) AST (aspartate aminotransferase) GGT (gamma glutamyl transferase)		No effects on urinary ALP on PND 3 or PND 12 No effects on glomerular filtration rate (abs. creatinine clearance) on PND 3 or PND 12 ↑ urinary excretion of beta2-microglobulin at ld on PND 49 (+55 %)* (hd at same level as control, i.e. no dose-response) No effects on liver or kidney weight, serum parameters (GLDH, AST, urea, creatinine), urinary parameters (volume, GGT, ALP), or histochemical examination of liver G-6-Pase or renal ALP activity on PND 49 Ophthalmological effects not examined	
Extended one generation reproductive toxicity study, OECD TG 443 Reliability: Klimisch 1 as reported by registrant (reliable without restriction) Rat (Wistar) P0 n/group = 25 (f) F1 n/sex/group/cohort: 20 (1 m and/or 1 f per litter) Scheduled termination P males: after weaning of F1 animals;	NaTFA (99.9 %) Oral (diet) 0, 120, 600, 3000 ppm, 10 weeks premating (9.71, 49.2 and 248 mg/kg/day for males and 10.26, 53.9 and 265 mg/kg/day for females), and throughout gestation period (8.65, 44.3 and 223 mg/kg/day for females) 0, 60, 300, 1500 ppm during lactation period, LD 1 - 21 (9.85, 47.5 and 233 mg/kg/day) 0, 60, 300, 1500 ppm, F1 from weaning (m offspring: 0, 9.37, 47.3, 242 mg/kg bw/day; f offspring: 0, 9.83, 49.4, 248 mg/kg bw/day)	<u>Maternal body weight parameters and food consumption:</u> No significant changes in overall bw gain during pre-mating period ↓ bw gain on GD 7 - 14 (-13 %**) at hd (but no significant change in overall bw gain during gestation period) ↓ bw on GD 14 (-5 %*) and on LD 1 (-5 %*) and LD 14 (-4 %*) at hd ↓ mean food consumption on GD 7 - 19 at hd and on GD 7 - 13 also at ld and md (all -5 - 10 %* - **) ↓ mean food consumption during lactation on LD 4 - 20 (-9 - 24 %* - **) <u>Paternal body weight parameters and food consumption:</u> ↓ bw gain in week 1, 4 and 13 of pre-mating period at md (-7 - 19 %* - **) and hd (-9-31 %* - **) ↓ overall bw gain during pre-mating period (-8 %**) at hd ↓ bw during pre-mating period day 50 - 120 at hd	(Labcorp Laboratories, 2021b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
P females: PPD 28 F1: Unselected F1 offspring was sacrificed at culling on day 4 and day 22 of age. Selected Cohort 1A animals were sacrificed at approximately 13 weeks of age and Cohort 1B animals were sacrificed at approximately 14 weeks of age.		(-5-7 %*) and terminal bw (-6 %*) at hd ↓ mean food consumption in pre-mating week 5 (md and hd) and week 7 and 9 at hd (-5 - 6 %* - **) <u>Parental haematology:</u> ↓ haematocrit in females at hd (-3 %*) ↓ haemoglobin at hd (f: -6 %**; m: -5 %*) ↑ platelet counts in females at hd (+24 %**) ↓ monocyte count in males at hd (-33 %*) <u>Parental clinical chemistry:</u> ↑ ALP in males at md (+26 %**) and hd (+34 %**) ↓ plasma glucose at all doses in both sexes (f: -14 %* at ld, -11 %* at md, -14 %* at hd; m: -12 %* at ld, -18 %** at md, -13 %** at hd) ↓ non-esterified fatty acids at all doses in both sexes (f: -28 %** at ld, -21 %** at md, -32 %** at hd; m: -16 %** at ld, -24 %** at md, -31 %** at hd) ↓ triglycerides at all doses in males (-35 %** at ld, -33 %** at md, -38 %** at hd) ↑ A/G ratio at md (m: +13 %**) and hd (f: +9 %*; m: +17 %**) <u>Parental thyroid hormones:</u> ↓ total T4 at hd (f: -32 %**; m: (-41 %**); all values are within HCD range <u>Parental macroscopic findings:</u> ↑ abs. kidney weights at md (m: +3 %**) and hd (f & m: +4 %**) ↑ rel. kidney weights in males at md (+5 %**) and hd (+11 %**) ↑ rel. liver weights at hd (f: +14 %**; m: +16 %**) ↑ abs. and rel. thyroid and parathyroid weights at hd in females (abs.: +19 %*, rel.: +21 %**) No adverse histopathological findings in the parental animals **Effects in F1 males and females** ↑ post-implantation loss at hd (+185 %*, from 3.4 % in control to 9.7 % at hd, within HCD range 4.5 - 10.6 %)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Body weights (F1) in lactation period (PND 1, 4, 7, 14, 21) No differences in bw at PND 1, 4 (before and after culling, f & m) ↓ bw on PND 7/14/21 at hd (f: -7 %*/-8 %**/-7 %*; m: -8 %**/-7 %**/-7 %*) ↓ bw gain PND 4 - 14 at hd (f: -1 %**; m: -8 %*) ↓ bw gain PND 1 - 21 at hd (f & m: -7 %*) Body weights (F1) on postweaning day 0 - 39 (age: 21 - 60 d) ↓ bw preweaning on PND 7, 14, 21 (m and f: -7 %*) and on all days past weaning (m: -5 - 9 %*-**; f: -3 - 9 %*-**) at hd ↓ bw gain during lactation PND 1 - 21 at hd (m and f: -7 %*) Food consumption (F1) ↓ food consumption during week 1, 8, 10 (f: -0 - 7 %*) and week 4 (m: -5 %*) at hd ↑ food consumption during week 5 at md (f: +7 %) No test substance-related effects on litter size, sex ratio, pup survival at birth Haematology F1: ↓ haematocrit at md (f: -4 %)* and at hd (f & m: -4 %)** ↓ haemoglobin at md (f: -3 %)*and at hd (f: -4 %**; m: -5 %**) ↑ platelet counts at md (+19 %)* and at hd (+16 %)* (f only) ↓ monocyte count at hd (-42 %)* (m only) ↓ lymphocyte count at hd (-37 %)* (m only) Clinical chemistry F1: ↑ ALT at hd (f: +14 %*; m: +40 %**) ↑ ALP (+34 %)* and AST (+13 %)* at hd (m only) ↓ plasma glucose at all doses (f: -18 % at ld**, -18 % at md**, -15 % at hd**; m: -19 % at ld**, -24 % at md**, -23 % at hd**) ↓ triglycerides in males at all doses (-26 % at ld*, -23 % at md*, -18 % at hd*) ↓ non-esterified fatty acids at ld (m: -11 %)*, at md	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		(m: -21 %)*, and at hd (f: -25 %**; m: -27 %**) ↓ bilirubin at md (m: -100 %**) and at hd (f & m: -100 %)** (from 1 to 0) ↑ A/G ratio at md (f: +6 %)* and at hd (f: +11 %**; m: +17 %**) <u>Macroscopic findings (F1):</u> ↑ rel. liver weight at hd (f: +16 %**; m: +20 %** in Cohort 1A) ↓ abs. kidney weight at hd (f: -7 %*; m: -6 %* in Cohort 1A) ↑ rel. weight of thyroid with parathyroids at md (only males: +16 %) and at hd (f: +12 %*; m: +16 %* in Cohort 1A) ↓ abs. adrenal weight at hd (only measured in Cohort 1A males: -15 %**) ↓ abs. pituitary weight at hd (f: -15 % in Cohort 1A*, n.s. in Cohort 1B, males of Cohort 1A or 1B, and rel. pituitary weights also n.s.) ↓ terminal bw at hd (f: -6 % in Cohort 1A*, -5 % in Cohort 1B*; m: -10 % in Cohort 1A**, -5 % in Cohort 1B*) <u>Developmental immunotoxicity effects (F1)</u> ↓ number of cells/spleen for T cells, B cells, NK cells, CD4+, CD8+, monocytes, neutrophils in males and females at all doses No effects on remaining immunophenotyping parameters in males or females (no further details reported) ↓ CD4+ T-cell % at ld (females) ↑ CD8+ T-cell % at ld (females) <u>Thyroid hormones (F1)</u> ↑ TSH at all doses at PND 22 in females (+34/11/80 % at ld/md/hd); no statistics possible because SD is given as NA (too many values below LOQ) ↑ TSH for males (control and ld: all values < LOQ, from md to hd: +29 %); no statistics possible because SD is given as NA (too many values below LOQ) ↓ **total T4** for F1 at PND 22 at md (m: -17 %*) and hd (f & m: -37 %**); all values are within HCD range ↓ **total T4** for Cohort 1A at all doses in **males** (-15 %* at ld, -32 %** at md, -52 %** at hd); **hd**	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		value is not within HCD range; n.s. change for females (all values are within HCD range) Eye samples were fixated, but no results are reported in the registration dossier (either not examined or examined, but not reported). No adverse histopathological findings in F1 animals	
Preliminary study of reproductive performance No guideline because of preliminary nature of study Reliability: Klimisch 1 as reported by registrant (reliable without restriction) Rat (Wistar) P0 n/group = 6 (f) F1 n/sex/group: 6 Scheduled termination of P females: day 21 of lactation (without litters: day 25 after mating) Unselected F1 were sacrificed at culling on PND 4, or at scheduled sacrifice on LD 21 Selected F1 were sacrificed on day 35 of age (complete macroscopic examination)	NaTFA (99.9 %) Oral (diet) 0, 1400, 3400, 8400 ppm (0, 97.5, 230, 547 mg/kg bw/day), P from day 6 after mating (GD 6) throughout gestation period, and F1 from weaning until day 35 of age (0, 202, 476, 1290 mg/kg bw/day for males and 0, 228, 557, 1369 mg/kg bw/day for females), 0, 700, 1700, 4200 ppm, P during lactation period until weaning of offspring (0, 110.2, 262, 578 mg/kg bw/day), and F1 from mid lactation until weaning (no internal doses reported)	**Maternal effects** No clinical signs or unscheduled deaths observed in maternal animals ↓ bw gain GD 6 - 10 in all treatments with dose-response (-31 % at ld*, -38 % at md*, -69 % at hd**) ↓ abs bw on GD 14 at hd (-6 %)* and on LD 1 at hd (-9 %)* ↓ food consumption from GD 6 - 19 at hd (-12 %) and during lactation at hd (-13 %) no effects on duration of gestation or gestation index ↓ live birth index [(number of live offspring on day 1 after littering) / (total number of offspring born)*100] (decrease not further specified) ↓ mean litter size prior to litter standardisation on day 4 at hd (decrease not further specified) No test substance-related effects on pup survival from day 4 or sex ratio, at any dose according to study authors. **Effects in F1 males and females** ↓ live birth index [(number of live offspring on day 1 after littering) / (total number of offspring born)*100] <u>Lactation period F1 (PND = LD)</u> ↓ bw on PND 1 at hd (f: -12 %*; m: -13 %*) ↓ bw on PND 7 at md (m: -12 %*) and at hd (f: -18 %**; m: -16 %*) ↓ bw on PND 11, 14, 17, 21 at hd (f: -13-14 %*-**; m: -12-13 %*-**) No difference in bw gain from PND 1 - 4 (f & m) ↓ bw gain PND 4 - 7 at all doses (f: -17 % at ld*, -12 % at md*, -22 % at hd**; m: -21 % at ld* and md*, -26 % at hd*) ↓ bw gain PND 11 - 14 at hd (f: -21 %*; m: -15 %*)	(Labcorp Laboratories, 2021a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓ bw gain PND 1 - 21 at hd (f & m: -13 %*) <u>Postweaning day 0 - 39 (age: 21 - 60 d)</u> ↓ bw on PND 21 (at weaning) at hd (f & m: ca. -11 %*) ↓ bw gain PND 24 - 28 in females at md* and hd*; effect size N/A ↓ bw gain PND 21 - 35 at md (f: ca. -15 %) and at hd (m: ca. -21 %); statistics N/A ↓ food consumption PND 21 - 34 (-11 at md, -10 % at hd; no information on significance or sex differences) ↓ mean litter size prior to litter standardisation on day 4 at hd ↑ abs. liver weight of F1 f & m at all doses (f: +18 % at ld(n.s.), +11 % at md(n.s.), +20 % hd*; m: +21 % at ld*, +20 % at md*, +17 % at hd*) ↑ rel. liver weight of F1 f & m at all doses (f: +25 % at ld**, +26 % at md**, +33 % at hd**; m: +26 % at ld**, +28 % at md**, +44 % at hd**)) No test substance-related effects on pup survival from day 4 or sex ratio, at any dose according to study authors. Sexual maturation or F1 adult developmental landmarks not examined. Ophthalmological effects not examined.	

* statistically significant with $p \leq 0.05$
** statistically significant with $p \leq 0.01$
n.s. – not statistically significant
bw – body weight
abs. / rel. – absolute / relative
GD / LD / PND – gestational day / lactational day / postnatal day
ld / lmd / md / hmd / hd – low dose / lower mid dose / mid dose / higher mid dose / high dose
↑ / ↓ – increase / decrease (relative to control if not noted otherwise)
d / week – day / week
f / m – female /male

Table 16: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vitro/*in utero* (similar to ECVAM Embryotoxicity testing in post-implantation embryo culture; mice instead of rats; 24 – 26 h of incubation instead of 48 h) Reliability 3 as reported by registrant (not reliable; significant methodological deficiencies) Mouse (CD-1) 4 embryos were cultured in 4 mL of medium per 30 mL culture bottle Non-exposed male and female mice were mated (2 hours of co-habitation of 1 male and 2 females). Pregnant females were killed on GD 9 and the uterus was removed for exposure of the embryos (early somite stage; 3 – 6 somites) to the test item. Method of embryo culture previously described by Sadler (1979) Additional to experiments with acetic acids and various	TFA (no purity indicated) Exposure in culture medium of whole embryo culture (vehicle: water), 24 – 26 h incubation n = 35 at 0 µM TFA n = 6 at 1000 µM TFA n = 23 at 5000 µM TFA n = 25 at 6000 µM TFA n = 15 at 7500 µM TFA n = 11 at 10000 µM TFA n = 9 at 12500 µM TFA	The pH of the culture media decreased with increasing TFA concentration (from 8.35 in controls down to 7.2 in the highest tested concentration of 12.5 mM). Additional experiments with lowered pH (pH 7.5 and 7.0) adjusted by addition of HCl without TFA were performed to estimate the effects and effect size from lowered pH. Initial pH of culture medium: 8.35 in control medium 8.27 at 1 mM 7.96 at 5 mM 7.89 at 6 mM 7.74 at 7.5 mM 7.41 at 10 mM 7.2 with 12.5 mM TFA The sample sizes varied considerably in-between treatment groups (n = 35 in control, n = 6 - 25 in treatments). No explanation is provided why the sample sizes are so different. Four embryos were cultured in one culture flask. However, the sample sizes in each group cannot always be divided by 4. Hence, some culture flasks must have had less embryos in it. The influence of the number of embryos in one culture flask remains uncertain. It appears that statistically, embryos are taken as replicates and not culture flasks. Embryos should be considered pseudo replicates. Additional to experiments with acetic acids and various haloacetic acids (di- and trifluoracetic acid; mono-, di-, and trichloroacetic acid; mono-, di-, and tribromoacetic acid), **two additional experiments with lowered pH** without (halo-) acetic	↑ percentage of any malformation at all doses (dose-dependent increase, significant at ≥ 5 mM) • 5.7 % in control • 33.3 % at 1 mM (ns) • 39.1 % at 5 mM* • 44 % at 6 mM* • 60 % at 7.5 mM* • 90.9 % at 10 mM* • 100 % at 12.5 mM* ↑ eye defects at concentrations ≥ 5 mM • 0 % in control • 16.7 % at 1 mM (ns) • 34.8 % at 5 mM* • 28 % at 6 mM* • 40 % at 7.5 mM* • 45.5 % at 10 mM* • 44.4 % at 12.5 mM* ↑ pharyngeal arch defects at all doses, except 5 mM and 7.5 mM • 0 % in control • 33.3 % at 1 mM*, • 13.1 % at 5 mM (ns) • 40 % at 6 mM*, • 6.7 % at 7.5 mM (ns), • 45.5 % at 10 mM*, • 100 % at 12.5 mM* ↑ neural tube defects at concentrations ≥ 6 mM • 0 % at control • 0 % at 1 mM • 8.7 % at 5 mM (ns) • 28 % at 6 mM*, • 40 % at 7.5 mM*, • 90.9 % at 10 mM*, • 100 % at 12.5 mM*	(Hunter et al., 1996)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
haloacetic acids (di- and trifluoracetic acid; mono-, di-, and trichloroacetic acid; mono-, di-, and tribromoacetic acid), **two additional experiments with lowered pH** without (halo-) acetic acids were prepared by addition of HCl (pH 7.5 or 7.0) to estimate effects of lowered pH apart from the presence of (halo-) acetic acids		acids were prepared by addition of HCl (pH 7.5 or 7.0) to estimate effects of lowered pH apart from the presence of (halo-) acetic acids		

Human Data on Reproductive Toxicity

Human epidemiological studies on TFA are rare. The literature search identified no epidemiological studies investigating the association of TFA in human blood and reproductive outcomes.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

PNDTS in rabbits (Covance Laboratories, 2021b)

In a prenatal developmental toxicity study according to OECD TG 414 (Klimisch score: 1, reliable without restriction), NaTFA (vehicle: water) was administered once daily by oral gavage to 24 mated female New Zealand White rabbits per dose group. Doses were 180, 375, and 750 mg/kg bw/day on GD 6 – 28. The control group received vehicle only via oral gavage. Pregnant females were sacrificed on GD 29 and the gravid uterine removed by Caesarean section.

In females of the high dose group, a significant reduction of the number of corpora lutea (-20 %), implantations (-20 %) and litter size (-26 %) was observed. Additionally, the weight of the gravid uterine was lower in the mid dose group (-18 %) and the high dose group (-29 %) as compared to the control group. The maternal body weight gain adjusted by the weight of the gravid uterine (GD 6 – GD $29_{adj.}$) was negative in all dose groups and was significantly reduced in the mid (-48 %) and high dose group (-56 %), meaning that body weight loss comparing GD 6 and GD 29 after Caesarean section was less than in controls. Hence, this is not considered of toxicological relevance. Food consumption was significantly reduced from GD 6 – 20 in the mid and high dose group (varying between -3 - 32 % without time- or dose-response relation) and may be related to the reduced numbers

of implantations and litter size. On the last days of gestation (GD 26 and GD 28), however, maternal food consumption was increased at all doses (+14 – 25 %).

The significantly reduced litter size in the high dose group is considered as an adverse effect and could not be related to maternal toxicity. The effect can indicate fertility effects or developmental effects, depending on start of the treatment. The reduced implantations, reduced litter size and resulting lower weight of gravid uterine may be secondary to the reduced number of corpora lutea. Because treatment started on GD 6 after initial formation of corpora lutea, it remains unclear whether this effect can be related to NaTFA treatment.

Foetal body weight was significantly reduced at the high dose by 10 % for female foetuses or when sexes were combined, and at the mid and high dose group by 12 or 11 % for male foetuses. Total litter weight was significantly reduced by 22 % at the mid dose and by 34 % in the high dose group. Live litter size was significantly reduced by 27 % in the high dose group. There were no effects on sex ratio.

In the foetal heads, visceral malformations were observed in the development of the eyes. Multiple folded retinas were observed in all doses in a dose-response manner (0/150 foetuses in 0/18 litters in control; 1/158 foetuses in 1/21 litters at low dose; 5/173 foetuses in 4/24 litters at mid dose; 9/140 in 8/23 litters at high dose). Moreover, absence of the aqueous/vitreous humour was observed in all dose groups (0/150 foetuses in 0/18 litters in control; 1/158 foetuses in 1/21 litters at low dose; 6/173 foetuses in 4/24 litters at mid dose; 8/140 in 6/23 litters at high dose). HCD range of each multiple folded retinae and absent aqueous/vitreous humour was given as 0 – 1 foetus (5687 examined) and 0 – 1 litter (744 examined) by the test laboratory. In one foetus each of the mid- and the high dose group, the retina had ruptured into the surrounding tissue which was not observed in any foetus in the historical control data. Small/misshapen lens was observed in one foetus each of the mid and high dose group, absent lens in one foetus of the high dose group, microphthalmia in one foetus of each dose group, and a cleft palate in one foetus of the high dose group; HCD range is given as 0 – 1 foetus (5687 examined) in 0 – 1 litter (744 examined) for each of these observations.

In the foetal thorax, fused/partially fused ribs were observed in 1/158 foetuses in the low dose group, (0.006 %; 1/21 litters) 4/173 foetuses in the mid dose group (0.02 %; 3/24 litters), and 8/140 foetuses in the high dose group (0.06 %; 6/23 litters). No fused/partially fused ribs were observed in the control group (150 foetuses, 18 litters) and HCD range was given as 0 – 4/5687 foetuses and 0 – 3/744 litters by the registrant. The incidences of fused/partially fused ribs are thus above the HCD range. Single incidences of other skeletal malformations, e.g. thoracic scoliosis (1/140 foetuses at high dose), multiple thoracic vertebral/rib abnormalities (2/140 foetuses at high dose) and others were within HCD range.

A transposition of the ascending aorta with pulmonary trunk was observed in one foetus each of the control and the mid dose group and in two foetuses each of the low- and high dose group (2 foetuses of 2 litters at low dose and in the same litter at high dose); HCD range is given as 0 – 1 foetus and 0 – 1 litter. Double outlet ventricle(s) were observed in one foetus of the low and the mid dose group, which was not observed in the test controls nor in the historical control data. Fused/caudally displaced kidney(s) were observed in one foetus in the high dose group, which was not observed in HCD.

Anogenital distance was not examined.

Table 14.1: Most important developmental effects on the eyes of NZW rabbit foetuses (excerpt from Table 14)

LOAEL effect	Affected level	0 mg/kg	180 mg/kg	375 mg/kg	750 mg/kg
Multiple folded retina*	Foetuses	0/150 (0 %)	1/158 (0.6 %)	5/173 (2.9 %)	9/140 (6.4 %)
	Litters	0/18 (0 %)	1/21 (4.8 %)	4/24 (17 %)	8/23 (35 %)
Absent aqueous/vitreous humour*	Foetuses	0/150 (0 %)	1/158 (0.6 %)	6/173 (3.5 %)	8/140 (5.7 %)
	Litters	0/18 (0 %)	1/21 (4.8 %)	4/24 (17 %)	6/23 (26 %)

*Laboratory HCD range for each effect: 0 - 1/5687 foetuses and 0 - 1/744 litters

In conclusion, adverse effects in the development of the eyes (folding of retina, formation of aqueous/vitreous humour) were observed in NZW rabbit foetuses. These types of malformations are rarely found in this species (see laboratory HCD range). The single incidence at the low dose of 180 mg/kg bw/day were indicated as LOAEL by the registrant. Lower doses were not tested and a NOAEL cannot be determined.

Preliminary study to PNDTS in rabbits (Covance Laboratories, 2021a)

Prior to the main PNDTS, a preliminary prenatal developmental toxicity study was performed, similar to OECD TG 414 but with less mated females, higher doses, less analysed parameters (e.g. no skeletal examination and no histological analysis of heads) and without statistical analysis (Klimisch score 1, reliable without restriction). Seven mated female New Zealand White rabbits were administered NaTFA via oral gavage once daily on GD 6 - 28 at doses of 0, 250, 500, 1000 mg/kg bw/d. The high dose was reduced to 750 mg/kg bw/day after the first two doses on GD 6 and 7 because of pronounced clinical signs in maternal animals of the high dose group (unsteady gait, decreased activity and impaired locomotion in several females, and one female on GD 7 with rapid breathing, reduced body tone and flattened posture). Unsteady gait and hypoactivity were also present to a lesser extent in few females in the low and mid dose group. Clinical signs were not present any more by GD 12. Pregnant females were sacrificed on GD 29 and the gravid uterine removed by Caesarean section.

Maternal body weight gain was reduced in the low and the high dose during treatment (GD 6 - 28). However, this reduction can be explained by a reduction of the weight of the gravid uterus on (measured on GD 29). There were no changes in adjusted maternal body weight on GD 29 and adjusted body weight gain during treatment (GD 6 – 28). Maternal food consumption was lower than in controls until GD 16 in the low dose group and until GD 18 in the mid and high dose group; after these time points, food consumption was comparable to or higher than food consumption in the controls. The data on body weight on GD 29, body weight gain, food consumption and weight of the gravid uterine are not available and this information relies on the written result descriptions of the registrant. It remains unclear whether statistical analysis was performed for these data.

The pregnancy rate was not affected by any dose (7/7 females pregnant). There was no change in the number of litters (7 litters in each group). The number of live foetuses per dam was reduced in all groups, particularly pronounced in the low and high dose group (-335 % at low dose, -27 % at high dose; -5 % at mid dose). No changes were observed for the mean placental weights. Statistical analyses for these effects were not performed by the authors and data were not available for statistical review by the dossier submitter.

Reduced number of live foetuses per dam in the low and high dose groups can be considered as impaired pregnancy outcome and thus an adverse effect. It cannot be fully excluded that the reduced number of foetuses resulted from reduced feed intake during the beginning of gestation (until GD 16 in the low dose and until GD 18 in the mid and high dose group, quantification not possible because

data are not available). However, adjusted body weights and body weight gains were not affected by treatment and therefore, the reduced unadjusted body weight gain is likely a result from fewer implantations and the resulting lower weight of the gravid uterine. Adverse developmental effects can therefore not be excluded. The reduced number of live foetuses can indicate a fertility effect or a developmental effect, depending on start of the treatment.

Post-implantation loss per litter was higher in all doses, particularly pronounced in the low dose and the high dose group (+301 % at low dose, +235 % at high dose; +104 % at mid dose). In all dose groups, a reduction of mean foetal weight (combined sexes) by 5 - 9 % from low dose to high dose and a reduction of mean total litter weight (-37 % in the low dose and high dose group; -11 % in the mid dose group) was observed. The sex ratio (percentage males) was significantly reduced in all dose groups but without a clear dose-dependent response (-34 % at low dose, -5 % at mid dose, -28 % at high dose). Single incidences of eye abnormalities were observed in the low dose group (dark area in right eye) or high dose group (small or opaque right eye). Missing accessory lung lobe in 1/54 foetuses and 1/7 litters were observed in the mid dose group; incidences increased in the high dose group with 4/41 foetuses in 2/7 litters. This effect is considered as a treatment-related variation that could not be explained by the lowered body weight of foetuses (no HCD range available).

In conclusion, few incidences of eye abnormalities are considered as supportive evidence for developmental toxicity that are not expected to occur secondary to limited reductions in body weight gains. With regard to the reduced foetal weight, it cannot be fully excluded that these developmental effects may be related to reduced feed intake in the beginning of gestation (until GD 16 in the low dose and until GD 18 in the mid and high dose group). However, adjusted body weight and body weight gain was not affected by treatment and therefore, the reduced unadjusted body weight gain is likely a result from fewer implantations and the resulting lower weight of the gravid uterine. Maternal influence may therefore not be substantial for the described developmental effects. The observed post-implantation loss and changes in sex ratio (without a clear dose-relationship) remain unclear as no consistent finding were seen in other studies. In comparison to the main PNDTS (Covance Laboratories, 2021b), the strength of evidence is lower for this study.

Second PNDTS in rabbits (Labcorp Laboratories, 2024c)

This PNDTS largely repeats the PNDTS performed by Covance Laboratories (2021b) and will not be described in detail here (methods and results listed in Table 14).

Cholesterol in dams was statistically significantly elevated during late gestation in the high dose group (up to +35 %) and triglycerides were statistically significantly increased in all dose groups on GD 19 and in the two highest doses on GD 28 (up to +57 %). Non-esterified fatty acids were decreased in the two highest dose groups on GD 19 and in the three highest dose groups on GD 28 (down to -36 %). Bile acids were statistically significantly reduced in all dose groups on GD 19 (down to -67 % reduction in the high dose group) and in the three highest dose groups on GD 28 (down to -53 % reduction in the high dose group).

The results on developmental parameters mainly confirm the developmental findings from the first PNDTS, especially the foetal eye malformations (excerpt from Table 14 below).

Table 14.2: Most important developmental effects on the eyes of NZW rabbit foetuses in second PNDTS (excerpt from Table 14)

LOAEL effect	Affected level	0 mg/kg bw/day	30 mg/kg bw/day	60 mg/kg bw/day	250 mg/kg bw/day	750 mg/kg bw/day
Multiple folded retina*	Foetuses	0/209 (0 %)	0/244 (0 %)	0/219 (0 %)	2/193 (1.0 %)	12/178 (6.7 %)
	Litters	0/28 (0 %)	0/29 (0 %)	0/28 (0 %)	2/28 (7.1 %)	9/28 (32 %)
Absent aqueous/vitreous humour*	Foetuses	0/209 (0 %)	0/244 (0 %)	0/219 (0 %)	1/193 (0.5 %)	8/178 (4.5 %)
	Litters	0/28 (0 %)	0/29 (0 %)	0/28 (0 %)	1/28 (3.6 %)	7/28 (25 %)

*Laboratory HCD range for each effect: 0/2128 foetuses and 0/270 litters

Skeletal malformation as fused/partially fused ribs with incidences above the HCD range were also confirmed by this PNDTS. Additionally, fused/partially fused sternebrae were reported, but within the provided HCD range.

Retroesophageal right subclavian artery (which has not been reported in the earlier rabbit PNDTS) was reported, but is considered a visceral variation by the registrant because it does not adversely affect health or survival.

Post-implantation loss was significantly increased by 139 % in the high dose group (from 8.1 % in control to 19.4 %). This confirms the increased post-implantation loss observed in the previous preliminary PNDTS in rabbits (Covance Laboratories, 2021a) and in the main EOGRTS in rats (Labcorp Laboratories, 2021b).

Second preliminary PNDTS in rabbits (Labcorp Laboratories, 2024b)

This preliminary PNDTS largely repeats the preliminary PNDTS performed by Covance Laboratories (2021a) and will not be described in detail here (methods and results listed in Table 14).

Cholesterol and triglycerides in dams were statistically significantly elevated by +55 % or +74 %, respectively, in the high dose group (750 mg/kg bw/day). Bile acids were statistically significantly reduced by -60 % in the high dose group.

The number of females pregnant and total number of litters decreased in a dose-dependent manner with increasing dose (6/6 in control and in the low dose group, 5/6 in the two mid dose groups, and 4/6 in the high dose group). It remains uncertain whether this reduction of pregnant females was related to the effects in clinical chemistry in the high dose group. There was a notable reduction of implantations per dam (-21 %) and an increase in pre-implantation loss per litter at hd (+150 %) in the high dose group, but without statistical significance. There were no effects on number of corpora lutea or litter size.

There were no statistically significant effects on developmental parameters in pups.

PNDTS in rats (Safety Evaluation Center of Shenyang Research Institue of Chemical Industry, 2020)

In a prenatal developmental toxicity study (OECD TG 414), KTFA/potassium trifluoromethane-sulphinate (51 % KTFA) was administered via oral gavage (vehicle: water) to 27 mated female Sprague Dawley rats from GD 5 – 19. Daily doses were 0, 100, 300 and 1000 mg/kg bw/d. Pregnant females were sacrificed on GD 20 and the gravid uterine removed by Caesarean section.

There were no effects on the number of abortions, mean numbers of corpora lutea, implantation sites, total placental weights, and gravid-uterine weights. In the high dose group, pre- and post-implantation

loss were decreased, but only significant for post-implantation. Some embryotoxic/teratogenic effects were observed; however, they occurred in all groups, including the control group and were therefore not significant. The NOAEL was determined to be 1000 mg/kg bw/day, corresponding to 510 mg KTFA/kg/day.

PNDTS in rats (Huntingdon Life Sciences, 2010)

In a prenatal developmental toxicity study (OECD TG 414), TFA was administered via oral gavage (vehicle: water) two 22 mated female Sprague Dawley rats from GD 6 – 19. Daily doses were 0, 37.5, 75, 150 mg/kg bw/d. Pregnant females were sacrificed on GD 20 and the gravid uterine removed by Caesarean section.

There were no changes in pregnancy duration, and no significant changes in number of pregnant females. Of note, one female was not pregnant each in the control and high dose group.

No effects were observed on number of live offspring or postnatal survival, on litter size and foetal weights, on sex ratio. No external, skeletal, or visceral malformations were observed. Hence, no developmental toxicity is suggested by this study. Effects on development were not observed in this study.

Non-guideline developmental toxicity study (Saillenfait et al., 1997)

In a non-guideline developmental toxicity study, TFA was administered by oral gavage (vehicle: distilled water) to mated female SD rats from GD 10 – 20 in doses of 0, 75, 150 mg/kg bw/d. Each group had 43 – 45 dams. Additional 5 – 6 satellite dams in each treatment group were euthanised before term to assess maternal hepatic and renal function with urine collection over 17 h on GD 20. Offspring were delivered naturally and were examined for hepatic and renal biochemistry and/or function through serum and urinary parameters. Four males and four females (where possible) from each of 8 – 11 litters per treatment group were examined. On PND 3 and PND 12 urine was collected after 6.5 h isolation from fluid intake and offspring were sacrificed 6.5 h after urine collection. On PND 49, one male and one female (where possible) from each of five litters per treatment group were examined by 24–h urine collection in a metabolic chamber and sacrificed afterwards.

Body weight gain of dams was significantly reduced (-18 %) in the higher dose from GD 10 to GD 15. Body weights on GD 6, 10, 15, and 21 (dams) or GD 20 (satellite dams) were not significantly different. Absolute and relative liver weights were significantly increased at both doses (abs.: +22 – 23 %; rel.: +32 – 33 %). There were no changes in kidney weight, gross pathology, serum parameters glutamate dehydrogenase (GLDH), aspartate aminotransferase (AST), urea nitrogen, creatinine or urinary parameters volume, alkaline phosphatase (ALP), β_2-microglobulin (β_2-m). Urinary gamma glutamyl transferase (GGT) was significantly reduced at both doses (-55 % at both doses). No effects on gestational length in maternal females were observed in this study.

No effects on survival, pup body weight or litter size were observed in any dose group until PND 3. There were no effects on pup survival, sex ratio and no external malformations. Some changes of liver and kidney function in offspring were observed, such as increased activities of GLDH (+306 % at low dose, +419 % at high dose) and AST (+27 % at low dose, +53 % at high dose) at both doses on PND 3, but not on PND 12. Moreover, serum urea was increased (+45 %), GGT excretion reduced (-25 %) in the high dose group on PND 3, but not on PND 12.

Urinary excretion of β_2-microglobulin (β_2-m) was increased (+142 %) in the high dose group on PND 3, but not on PND 12. No effects were observed on glomerular filtration rate (i.e. abs. creatinine clearance) on PND 3 and PND 12.

On PND 49, only urinary excretion of β_2-m was increased in the low dose group (+55 %), but in the higher dose the excretion was at control level. Serum parameters (GLDH, AST, urea, creatinine) and urinary parameters (volume, GGT, ALP) were not affected on PND 49. No changes were observed in histochemical examination of liver G-6-Pase activity or renal ALP activity of offspring sampled on PND 49.

In conclusion, moderately increased AST may be indicative of some liver dysfunction, increased serum urea concentration and lower GGT excretion rate indicate reduced kidney filtration capacity (no histopathology available on organs of the offspring). The marked increase of glutamate dehydrogenase (GLDH) activity (+306 % at low dose, +419 % at high dose) may be of interest, as elevated enzyme activity is indicative of (liver) cell necrosis and mitochondrial toxicity (e.g., Church and Watkins, 2017, Jaeschke and McGill 2013). GLDH was not examined in other studies. These effects were observed in the early postnatal period (PND 3), but appeared to be reversible (almost no effects on PND 12 and PND 49). Because of its occurrence only on PND 3, the observed effects are possibly related to the in-utero exposure.

EOGRTS in rats (Labcorp Laboratories, 2021b)

In an extended one-generation reproductive toxicity study according to OECD TG 443), NaTFA was administered orally via the diet to 25 male and 25 female Wistar rats per dose group. Doses were 0, 120, 600 and 3000 ppm in the period 10 weeks prior to mating for both sexes (equivalent to 9.71, 49.2 and 248 mg/kg/day for males and 10.26, 53.9 and 265 mg/kg/day for females), throughout the gestation period (8.65, 44.3 and 223 mg/kg/day for females), and during the lactation period (LD 1 - 21) 0, 60, 300, 1500 ppm (equivalent to 9.85, 47.5 and 233 mg/kg/day). In the offspring generation (F1), culling of litters was performed on PND 4. For cohorts 1A and 1B, 20 animals per sex per dose group (one male and/or one female per litter) were selected on PND 22. Offspring received 0, 60, 300, 1500 ppm from weaning (equivalent to 0, 9.37, 47.3, 242 mg/kg bw/day in male offspring and 0, 9.83, 49.4, 248 mg/kg bw/day in female offspring). Scheduled sacrifice was after weaning of F1 animals for parental males and females after confirmation that no further mating was required. F1 animals were sacrificed on PND 35.

Clinical chemistry parameters were affected in both, parental males and females. These effects included increased ALP in males in the mid (+26 %) and high dose (+34 %) group, in all dose groups of both parental sexes decreased plasma glucose (ca. -13-14 %, no clear dose-response in males or females) and non-esterified fatty acids (dose-response for males ranging from -16 % to -31 %) and decreased triglycerides in all dose groups of males (ca. -35 %, no dose-response). A/G ratio was increased in males in the mid dose (+13 %) and the high dose group (+17 %) and in females in the high dose group (+9 %). The relevance of these clinical chemistry parameters is considered as unlikely for development of the offspring because no clear relation of ALP and development is established, a decreases of plasma glucose, non-esterified fatty acids, or triglycerides are not considered adverse, and the increase of A/G ratio was small.

In the high dose group (3000 ppm, 265 mg/kg bw/day), a significant increase of post-implantation loss was observed in females (from 3.4 % in control to 9.7 % in the high dose group), but within the range of historical control data (4.5 - 10.6 %). No test substance-related effects on litter size, sex ratio, or pup survival at birth were recorded according to the registrant.

Effects in F1 generation are listed in Table 15 and include effects on

- body weight and body weight gain (both ≤ 8 %, starting from PND 7 throughout lactation and whole postweaning period in the high dose group in males and females)
- haematology (e.g. reduced monocyte (-42 %) and lymphocyte (-37 %) counts in the high dose group in males),

- the immune system (e.g. reduced white cell numbers/spleen in males and females at all doses, no information on dose-response available),
- clinical chemistry (e.g., increased ALT in the high dose group in females, increased AST and ALT in the high dose group in males, +14 - 40 %),
- organ weights (e.g., increased rel. liver weight in the high dose group in males and females, increased rel. weight of thyroids incl. parathyroids in all doses in males and in the high dose group in females),
- thyroid hormones (increased TSH at all doses in females, reduced total T4 in the mid and high dose group in males and in the high dose group in females),
- male reproductive parameters (reduced weight of the testes and epididymes in the high dose group of Cohort 1A and 1B), and
- male sperm quality (e.g. reduced number of spermatids in the high dose group (only high dose tested) and reduced sperm motion value VAP in all dose groups, increased number of abnormal sperm in the high dose group; all sperm parameters only tested in Cohort 1A).

Samples of the F1 eyes were taken, but no histopathology data are available.

Various parameters were impaired in the development of the F1 generation. Many effects were also observed in the parental generation (e.g. reduced body weight and body weight gain, reduced haemoglobin, increased ALP in males, and reduced plasma glucose) and may result from prolonged repeated exposure to NaTFA (weaning and post-weaning) in the F1 generation. Effects that only occurred in the F1 generation but not in the parental generation included reduced blood lymphocyte counts in males in the high dose group, reduced absolute adrenal weight in males in the high dose group and reduced absolute pituitary weight in females in the high dose group. Because of a lack of information on effects on the immune system in F1 animals, these effects cannot be assessed further (no information on effect sizes or dose-response available).

Preliminary EOGRTS (Labcorp Laboratories, 2021a)

In a preliminary study of reproductive performance (no guideline because of the preliminary nature of the study), NaTFA was administered orally via diet to six female Wistar rats per dose group. Daily doses for parental females were 0, 1400, 3400, 8400 ppm (0, 97.5, 230, 547 mg/kg bw/day) from GD 6 throughout gestation period. During lactation parental females were dosed at 0, 700, 1700, 4200 ppm until weaning of offspring (corresponding to 0, 110.2, 262, 578 mg/kg bw/day); the offspring generation (F1) received the same doses from mid lactation until weaning (no internal doses reported). Six F1 pups per sex per dose group received 0, 1400, 3400, or 8400 ppm (0, 202, 476, 1290 mg/kg bw/day for males and 0, 228, 557, 1369 mg/kg bw/day for females) from weaning until day 35 of age. Scheduled termination of parental females was on LD 21. Females without litters were sacrificed on day 25 after mating. Unselected F1 were sacrificed on PND 4 (culling), or at scheduled kill on LD 21 (establishment of Cohort 1A and 1B). Selected F1 were sacrificed on day 35 of age for complete macroscopic examination.

Maternal body weight gain from GD 6 – 10 was significantly reduced in all treatment groups in a dose-response manner (-31 % at low dose, -38 % at mid dose, -69 % at high dose). Absolute body weights were significantly reduced on GD 14 (-6 %) and on LD 1 (-9 %) in the high dose group. Food consumption was significantly reduced from GD 6 – 19 (-12 %) and during lactation (-13 %) in the high dose group.

There were no effects on the duration of gestation or the gestation index. In the high dose group, the live birth index (number of live offspring on PND 1/total number of offspring born) and the mean litter size prior to litter standardisation on PND 4 were significantly reduced; however, the reduction was not further specified and no original data were available. Numbers of live offspring, total number

of offspring born, and effect sizes were not reported. No test substance-related effects on pup survival from PND 4 or sex ratio were observed in any dose group according to registrant.

The reduced live birth index and reduced litter size in the high dose group can be considered as an adverse effect, but interpretation is difficult because effect sizes and statistical significance cannot be assessed without original data, which were not available. The parameters "live birth index" as well as "litter size prior to standardisation on PND 4" can be indicative of fertility effects or postnatal survival, i.e. developmental effects. The reduced live birth index and reduced litter size in the high dose group may be related to reduced maternal body weight gain early in gestation and low body weight on the first lactational day. Maternal food consumption was lower than in controls throughout gestation and lactation in the high dose group (-12 - 13 %) but to a lesser extent when compared to the reduced body weight gain in the high dose group (-69 %).

Pup body weights were statistically significantly reduced by 12 – 18 % on PND 1, 4, 7, 14 and 21 in males and females at the high dose group (4200 ppm, no internal doses reported). Additionally, body weight of F1 males was also significantly reduced in the mid dose group on PND 7.

Pup body weight gain was not affected from PND 1 – 4. However, from PND 4 – 7, body weight gain was significantly reduced at all doses in females (-12 % to -22 %) and males (-21 % to -26 %). From PND 11 - 14, body weight gain was statistically significantly reduced only in the high dose group (-21 % in females, and -15 % in males). From PND 1 – 21, body weight gain was significantly reduced in females and males of the high dose group (-13 % in both sexes).

Relative weight of the liver was significantly increased at all doses in F1 males (+26 - 44 %) and females (+25 - 33 %). Absolute liver weights were increased at all doses with statistical significance at all doses in males (+21 % at low dose, +20 % at mid dose and +17 % at high dose); in females the increase was only statistically significant at the high dose (+20 %). Histological samples of the liver were not examined.

Sexual maturation or adult developmental landmarks of the F1 generation were not examined in this preliminary study.

In conclusion, offspring development in this study was impaired in terms of increased postnatal mortality (reduced live birth index and reduced litter size prior to standardisation), reduced body weight (gain) and increased liver weight in males and females in this preliminary EOGRTS. As neither histology of the liver nor developmental landmarks were examined in this preliminary study, no conclusion on adversity of the observed effects can be made. The increase of postnatal mortality as well as the reduced offspring body weight parameters in the high dose group may be related to reduced maternal body weight gain early in gestation and low body weight on the first lactational day; maternal food consumption was lower than in controls throughout gestation and lactation. From mid-lactation until weaning, mixed exposure via milk and food consumption has to be assumed for the F1 generation. It is unlikely that the adverse developmental effects observed are a result of maternal toxicity as no marked maternal toxicity was observed (e.g., absolute body weights of dams were significantly reduced on GD 14 (-6 %) and on LD 1 (-9 %) in the high dose group).

In vitro/in utero developmental study (Hunter et al., 1996)

In an *in vitro/in utero* study, the gravid uteri of untreated parental CD-1 mice were excised and transferred to culture bottles with four embryos in each culture flask with 4 mL each (flask volume: 30 mL) for 24 – 26 h. TFA (free acid) concentrations were 0, 1, 5, 6, 7.5, 10, and 12 mM (vehicle: water) with initial pH of culture media gradually decreasing from 8.35 in control media down to 7.2 at 12.5 mM. Embryos were exposed to the TFA treatment during the early somite stage (3 – 6 somites). Sample sizes varied considerably throughout the dose groups (n = 35 at 0 mM, n = 6 at

1 mM, n = 23 at 5 mM, n = 25 at 6 mM, n = 15 at 7.5 mM, n = 11 at 1 mM, n = 9 at 12.5 mM). The method was previously described by Sadler (1979).

The percentage of any malformation increased at all doses in a dose-dependent manner reaching statistical significance at ≥ 5 mM (e.g., 6 % in control, 39 % at 5 mM, 100 % at 12.5 mM). Relative incidences of eye defects were elevated at all concentrations ≥ 5 mM (e.g., 35 % at 5 mM up to 46 % at 10 mM) compared to controls where no eye defects were observed.

Significantly elevated relative incidences of pharyngeal arch defects were observed in all TFA doses except at 5 mM and 7.5 mM (33.3 % at 1 mM, 13 % at 5 mM, 40 % at 6 mM, 7 % at 7.5 mM, 46 % at 10 mM, 100 % at 12.5 mM), leaving some uncertainties regarding dose-response relationship of this developmental effect. Rotational defects, heart and somite dysmorphology were only significantly elevated at the highest tested concentration of 12.5 mM. The number of somite pairs (21.9 ± 0.1 in controls) was decreased at all doses reaching statistical significance at 6 mM (20.0 ± 0.1) and at 12.5 mM (17.6 ± 0.1).

Relative incidence of neural tube defects increased with increasing TFA concentrations at concentrations ≥ 5 mM (8.7 %) with statistically significant differences ≥ 6 mM (28 % at 6 mM up to 100 % at 12.5 mM).

In order to estimate the effects of lowered pH apart from the presence of TFA, two additional experiments with lowered pH without addition of TFA were prepared by addition of HCl (pH 7.5 or 7.0). At pH 7.5, no decrease in number of somites and no malformations occurred. At pH 7.0, however, abnormal development was observed in 24.1 % (7/29) of embryos, including non-closure of the neural tube in the rhombencephalon. No further information on observed malformations in these separate experiments with lowered pH is provided in the publication. Thus, it remains unclear whether other developmental parameters (eye defects, pharyngeal arch defects, rotational defects, heart dysmorphology, somite dysmorphology, number of somites) were affected at pH 7.0 adjusted by HCl without addition of TFA. Effects at TFA concentrations at or below 7.5 mM are thus probably not linked to lowered pH, but for effects that occur at 10 mM (pH 7.41) and/or especially at 12.5 mM TFA (pH 7.2), a partial contribution of lowered pH to the observed malformations can be assumed. The authors argue that lowered pH is probably not the major contributor for malformations because only 24 % of the embryos displayed neural tube defects at pH 7.0, whereas for example at TFA concentrations of 10 mM (pH 7.41) 90.9 %, and at TFA concentrations of 12.5 mM (pH 7.2) 100 % of the embryos had neural tube defects.

In conclusion, the effects in this study cannot clearly be related to TFA exposure because the pH of the culture medium dropped considerably with increasing TFA concentrations from pH 8.35 in the control to pH 7.2 at the highest dose. The observed developmental effects may thus result from TFA exposure, the lowered pH in the culture medium, or a combination of both. By additional experiments where pH was lowered without addition of TFA, the authors conclude that lowered pH may have influenced the observed malformations at a pH < 7.5, i.e. at the two highest tested TFA concentrations, although lower pH is argued not to be the major contributor for malformations. Another weakness of the study is the considerable variation of number of cultured embryos in-between exposure groups. The results are therefore considered as additional information. Particularly the occurrences of eye defects, pharyngeal arch defects as well as neural tube defects (all three affected already at lower concentrations with pH > 7.5) serve as supporting evidence in the context of the observed eye malformations in the *in vivo* rabbit studies (Covance Laboratories, 2021a; Covance Laboratories, 2021b).

Discussion on the mode of action of developmental eye effects in rabbits

Regarding the MoA of the eye effects reported under 10.10.5, the registrant provided information on toxicokinetics and prenatal developmental toxicity studies (Labcorp Laboratories, 2024a; Labcorp Laboratories, 2024b; Labcorp Laboratories, 2024c) as well as in vitro studies (not included in this dossier). The registrant concluded in a written statement to the German CA that NaTFA does not seem to interfere with the retinoic acid pathway, because it did not bind to CYP26 (a central enzyme in retinoic acid pathway) nor had any effects on vitamin A up to the highest tested dose of 750 mg/kg bw/day (see 9.1).

Furthermore, the registrant concluded based on an MCT1-assay that TFA is a substrate for monocarboxylate transporters (MCTs), which is (among other functions) important for the lactate and water homeostasis in the eyes (EC_{50} of 12 mM). The observed eye malformations in the offspring of NaTFA-treated dams occurred at dosing concentrations of 180, 250, 375 and 750 mg/kg bw/day (Labcorp Laboratories, 2024b; Labcorp Laboratories, 2024c). The internal exposures in plasma and eyes of dams and foetuses are reported in Table 10 and only exceeded the EC_{50} for MCTs in plasma of dams treated with 750 mg/kg bw/day with a maximum mean C_{max} of 10 – 16.5 mM (Labcorp Laboratories, 2024a; Labcorp Laboratories, 2024b; Labcorp Laboratories, 2024c). Internal plasma exposure of dams treated with 180 or 250 mg/kg bw/day was around 7 mM (Labcorp Laboratories, 2024a; Labcorp Laboratories, 2024b; Labcorp Laboratories, 2024c). Internal foetal exposures of dams treated with 250 or 750 mg/kg bw/day were 2.1 – 2.2 mM (Labcorp Laboratories, 2024c). Internal exposure in the eyes of dams and foetuses treated with 180 mg/kg bw/day were 1.33 mM and 2.35 mM, respectively (Labcorp Laboratories, 2024b). Internal exposure in the eyes of dams and foetuses treated with 250 mg/kg bw/day were 1.8 mM and 2.5 mM, respectively (Labcorp Laboratories, 2024c). Internal exposure in the eyes of dams and foetuses treated with 750 mg/kg bw/day were 1.95 – 2.4 mM and 2.57 – 2.8 mM, respectively (Labcorp Laboratories, 2024b; Labcorp Laboratories, 2024c). It should be noted that for the internal ocular exposure, samples were taken at least 24 h after the last dosing. Overall, the eye malformations in the PNDTSs were not limited to internal concentrations above the EC_{50} for MCTs (i.e. 12 mM). The registrant also points out that TFA is a chaotropic salt, which can affect protein integrity. This in turn can lead to impairment of the aqueous/vitreous humour.

10.10.6 Comparison with the CLP criteria

In a weight of evidence approach all data provided in the registration dossier and publicly available were considered to conclude on the classification for reproductive toxicity. Human data on developmental toxicity arising from exposure to TFA or its salts are not available. Data from extended one-generation toxicity studies, prenatal developmental toxicity studies and non-guideline developmental toxicity studies with experimental animals are available (Table 15).

Category 1A: Known human reproductive toxicant
The classification of a substance in Category 1A is largely based on evidence from humans.

There is no information available which supports a known adverse effect of TFA on reproduction in humans. Assignment to classification category 1A is therefore not appropriate.

Category 1B: Presumed human reproductive toxicant
The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other

toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Clear evidence of developmental toxicity, i.e. malformations in the eye is provided (Covance Laboratories, 2021b; Labcorp Laboratories, 2024c). Classification in category 1B is therefore considered appropriate.

Supportive evidence is provided by eye abnormalities in a preliminary study in rabbits (Covance Laboratories, 2021a). Eye defects and neural tube defects were also seen in an *in vitro/utero* study in mice, noting that this artificial test design has limitations as described under 10.10.5 (Hunter et al., 1996). Further supportive evidence is indicated by post-implantation loss in rats and rabbits (Labcorp Laboratories, 2021b; Labcorp Laboratories, 2024c) and reduced pup body weight (gains) in rats (Labcorp Laboratories, 2021a; Labcorp Laboratories, 2021b), with absence of maternal toxicity for the main EOGRTS (Labcorp Laboratories, 2021b). Skeletal malformations such as fused ribs in rabbits (Covance Laboratories, 2021b), or impaired liver and kidney function in early development in rats (Labcorp Laboratories, 2021b; Saillenfait et al., 1997) can further be considered supportive evidence.

Of note, in the specific target organ toxicity studies after repeated doses (described in 10.12), ophthalmological findings (e.g. cornea opacity), were reported for rats after oral administration of the sodium salt (Bayer CropScience, 2007; WuXi AppTech, 2019). In the one-year repeated-dose toxicity study (WuXi AppTech, 2019), the registrant stated that "Cornea opacity, cataract, ocular discharge, periorbital swelling, iris synechia, retinal vessel tortuosity, fundus hyperreflectivity and incomplete pupil dilation post-mydriatic were observed in some animals during the dosing and recovery phase exams." The study data can be interpreted as the eye being a target organ also in adult rats after chronic oral exposure. However, the detailed data of this study are not reported. Eye effects in these repeated dose toxicity studies are considered supportive of the developmental effects observed in the OECD TG 414 studies with rats.

Observed developmental effects in the described studies occur together with other toxic effects in maternal rats and rabbits (mainly reduced body weight (gain)). According to the Guidance on the Application of CLP Criteria (2024), "developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity". The observed eye malformations in rabbits and other developmental effects cannot be demonstrated to be secondary to maternal toxicity.

Overall, evidence is sufficiently convincing to place TFA in Category 1B.

Criteria for CATEGORY 2: Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Evidence is sufficiently convincing to place TFA in Category 1B. Classification in Category 2 is therefore considered not appropriate.

10.10.7 Adverse effects on or via lactation

There is no information available indicating adverse effects of TFA or its inorganic salts on or via lactation, neither from human nor from animal studies. Therefore, classification on or via lactation is not warranted.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

TFA and its inorganic salts have the potential to cause adverse effects in animal models. In a weight of evidence approach, the data are presented, summarised and compared against the criteria for classification for reproductive toxicity under the CLP Regulation. Based on this assessment it is concluded that TFA are most appropriately classified under CLP Regulation as:

Repr. 1B (H360fD; Suspected of damaging fertility; may damage the unborn child).

10.11 Specific target organ toxicity-single exposure

Not assessed in this dossier

10.12 Specific target organ toxicity-repeated exposure

Table 17: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
1-year repeated-dose toxicity study, OECD TG 452, GLP Reliability: Klimisch 1 as reported by registrant (reliable without restriction) Rat (SD) n/sex/dose = 20 (recovery 10; TK 3/sex for C and 9/sex for ld, md, and hd)	NaTFA Oral (drinking water), 52 weeks + 6 weeks recovery 0, 30, 120, 600 ppm (mg/L) in drinking water (0, 1.8, 7.6, 37.8 mg/kg bw/day in males and 3.3, 12.2 and 64.0 mg/kg bw/day in females	No effects on mortality No effects on body weight No clinical signs No relevant effects on weights or histopathology of testes and epididymis “Cornea opacity, cataract, ocular discharge, periorbital swelling, iris synechia, retinal vessel tortuosity, fundus hyperreflectivity and incomplete pupil dilation post-mydriatic were observed in some animals during the dosing and recovery phase exams. Because there was no dose-response correlation, these ocular findings were not considered as test article-related.” (Data are not reported and can hence not be assessed.) TK: Plasma concentrations increased proportionally with dosing without marked differences between males and females Overall NOAEL: 600 ppm (males: 37.8 mg/kg bw/d; females: 64.0 mg/kg bw/day)	(WuXi AppTech, 2019)
90-d repeated dose toxicity study, OECD TG 408, GLP Reliability: Klimisch 1 as reported by registrant	NaTFA (99 %) Oral (diet) 0, 160, 1600, 16000 ppm (0, 9.9, 98, 1043 mg/kg bw/day for	No effects on mortality (m & f) <u>Body weight and body weight gain</u> ↓ male mean bw at hd (−5 to −11 % from study day 15 onwards, $p \leq 0.01$) ↓ male mean bw gain at hd (−17 % on day 92), statistically significant at most time points with $p \leq 0.01$ or $p \leq 0.05$ ↓ female mean bw at hd (up to −6 %, n.s.)	(Bayer CropScience, 2007)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
(reliable without restriction) Rat (Wistar Rj:WI (IOPS HAN)) n/sex/group = 10	males; 0, 12.2, 123, 1216 mg/kg bw/day for females; this corresponds to following TFA doses for males: 0, 8.4, 82.3, 876 mg/kg bw/day and for females: 10.1, 103.3, 1021 mg/kg bw/day)	↓ female bw gain at hd (−14 % on day 92), statistically significant on a number of time points with $p \leq 0.01$ or $p \leq 0.05$ <u>Haematology in females (no effects in males)</u> ↓ haemoglobin concentration at hd (−8 %, $p \leq 0.01$) ↓ mean corpuscular volume at hd (−6 %, $p \leq 0.01$) ↓ mean corpuscular haemoglobin at hd (−7 %, $p \leq 0.01$) ↓ mean corpuscular haematocrit at hd (−6 %, $p \leq 0.01$) <u>Urinalysis</u> ↑ ketone levels at md and hd in both sexes (effect sizes not reported, statistically significant) <u>Clinical chemistry</u> ↓ female total bilirubin at md (−52 %, $p \leq 0.01$) and hd (−76 %, $p \leq 0.01$) ↓ female glucose at md (−25 %, $p \leq 0.01$) and hd (−17 %, $p \leq 0.05$) ↓ male total bilirubin at md (−69 %, $p \leq 0.01$) and hd (−81 %, $p < 0.01$) ↓ male glucose at md (−28 %, $p \leq 0.01$) and hd (−29 %, $p < 0.01$) ↑ male ALP at hd (+95 %, $p \leq 0.01$) ↑ male ALT at hd (+38 %, $p \leq 0.05$); +85 % in md but n.s. <u>Liver weights and histopathology</u> ↑ male abs. liver weight +9 % in md ($p \leq 0.05$); +19 % in hd ($p \leq 0.01$) ↑ male rel. liver weight +14 % in md ($p \leq 0.01$); +33 % in hd ($p \leq 0.01$) ↑ male liver weight rel. to brain weight: +8 % in md ($p \leq 0.05$); +24 % in hd ($p \leq 0.01$) ↑ male centrilobular to panlobular hepatocellular hypertrophy (diffuse) in md (5/10, minimal to slight) and hd (10/10, slight to moderate) ↑ incidence of hepatocellular necrotic foci in males in hd (out of 10 animals examined 5 with minimal, 1 with slight and 1 with moderate necrotic foci versus 3/10 in C (1 x minimal, 1 x slight, 1 x moderate)) ↑ female abs. liver weight +13 % in md ($p \leq 0.05$); +23 % in hd ($p \leq 0.01$) ↑ female rel. liver weight +12 % in md ($p \leq 0.01$); +28 % in hd ($p \leq 0.01$) ↑ female liver weight rel. to brain weight +9 % in md ($p > 0.05$); +24 % in hd ($p \leq 0.01$) ↑ female centrilobular to panlobular hepatocellular hypertrophy (diffuse) in hd (9/10, minimal to slight) <u>Ophthalmological findings in males (no effects in females)</u> •corneal opacity in the left eye of one male in hd	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		•anterior synechia in the iris of the left eye of another male in hd No effects on weights or histopathology of testes and epididymis	
28-day repeated dose toxicity study, OECD TG 407, GLP Reliability: Klimisch 4 (not assignable as study report was not available) Rat (Wistar) n/sex/group = 5 (m & f)	TFA (99.1 %, trifluoroacetate) Oral (diet) 0, 600, 1800, 5400, 16000 ppm (m: 0, 50, 149, 436, 1315 mg/kg bw/d; f: 0, 52, 157, 457, 1344 mg/kg bw/day)	No effects on mortality No effects on body weight <u>Clinical chemistry</u>: ↑ ALT (m: +37 %; f: +23 %; NOEL: 436/457 mg/kg bw/day) ↓ Cholesterol (m, ≈ -30 %; NOEL: 149/1344 mg/kg bw/day) ↓ Glucose (m & f, ≈ -30 %; LOEL: 50/52 mg/kg bw/day) ↑ Urinary ketone (m & f; LOEL: 50/52 mg/kg bw/day) ↑ Urinary volume (m, +65 %; NOEL: 436 mg/kg bw/day) <u>Anatomic pathology of the liver:</u> ↑ Rel. Liver weight (m: +15 %; f: +13 %; NOEL: 50/157 mg/kg bw/day) No histopathological effects Testes and epididymes not examined	(BayerCropScience, 2014)
28-day combined repeated dose toxicity study with reproduction/developmental toxicity screening test, OECD TG 422, GLP Reliability: Klimisch 1 (reliable without restriction) Rat (Sprague-Dawley) n/sex/group = 10 (m & f)	KTFA/potassium trifluoromethane sulphinate (1:1) Oral (gavage) 0, 100, 300, 1000 mg/kg bw/day Vehicle: water Females: starting 2 weeks before pairing, during pairing, during gestation, during lactation until sacrifice Males: starting 2 weeks before pairing, during pairing, until sacrifice	No effects on mortality. No effects on body weight. No effects on food consumption. <u>Haematology</u> In hd males: -10.2 % in haemoglobin content ($p < 0.01$) <u>Clinical chemistry</u> In hd males: +13.4 % in inorganic phosphorus ($p < 0.05$) and -4.9 % in calcium ($p < 0.05$) No effects observed on behaviour. <u>Organ weight</u> mean absolute and relative liver weights were increased in md and hd males (statistically significant) and in hd females (not statistically significant); correlation with microscopic changes observed in the liver mean absolute and relative kidney weights in hd females increased (statistically significant); no microscopic correlates No effects on gross pathology. <u>Histopathology</u> minimal centrilobular hypertrophy of hepatocytes in 4/5 hd males, 2/5 hd females and 1/5 md males, correlation with	(CIT BP, 2012)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		increased liver weights; not considered adverse because of absence of associated degenerative microscopic findings or change in enzyme activities haematopoiesis in the spleen minimally increased males: control: 4/5 Grade 1 ld: 2/5 Grade 1; 3/5 Grade 2 md: 3/5 Grade 1; 2/5 Grade 2 hd: 3/5 Grade 1; 2/5 Grade 2 females: control: 4/5 Grade 2; 1/5 Grade 3 ld & md not assessed hd: 1/5 Grade 1; 4/5 Grade 2	
14-day repeated dose toxicity study, non-guideline, GLP Reliability: Klimisch 4 (not assignable as study report was not available) Rat (Wistar) n/sex/group = 5	TFA (98.7 %, trifluoroacetate) Oral (diet) 0, 600, 1200, 2400 ppm (m: 0, 43, 85, 170 mg/kg bw/d; f: 0, 45, 91, 190 mg/kg bw/day)	No effects on mortality No effects on body weight <u>Liver:</u> ↑ Rel. Liver weight (m only, + 23 % in hd; NOEL: 43/190 mg/kg bw/day) ↑ hepatocellular mitoses in all males and 2/3 of females in hd ↑ slight diffuse centrilobular hepatocellular hypertrophy (m only, 40 % of males in MD, 20 % of males in hd ↑ Hepatic cytochrome P-450 content (+19 % in m, +14 % in f; NOEL: 85/91 mg/kg bw/day) ↑ Peroxisome proliferation (m only; NOEL: 43/190 mg/kg bw/day) --> TFA appears to be a weak peroxisome proliferator in male rats <u>Clinical chemistry:</u> ↑ specific and total palmitoyl-CoA oxidation activities, m only, +84 % and +92 % in hd respectively; NOEL: 43/190 mg/kg bw/d <u>Haematology:</u> ↓ White blood cell count; significant effects for f only; −30 % in hd, NOAEL: 170/91 mg/kg bw/d ↓ Lymphocyte count, significant effects in f only; −38 % in hd; NOAEL: 170/91 mg/kg bw/d ↑ % of neutrophils, significant effects in f only; +65 % in hd; NOAEL: 170/91 mg/kg bw/d Overall NOAEL: 43 mg/kg bw/day in males (based on liver findings: ↑ rel. liver weights in combination with ↑	(BayerCropScience, 2014)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		hepatocellular hypertrophy and ↑ palmitoyl-CoA oxidation activities) and 91 mg/kg bw/day in females (based on ↓ white blood cell count and ↓ lymphocyte cell count) Testes and epididymes not examined	
14-day Reliability: Klimisch 2 as reported by registrant (reliable with restriction), no GLP Rabbit (New Zealand White) Females (nulliparous and non-pregnant) n/group = 6	NaTFA (99.9 %) Oral (gavage, vehicle: water), once daily 0, 750 mg/kg bw/day Dosing day 1 - 13	No clinical signs No mortality ↓ body weight gain (-89 %*; 750 mg/kg bw/day: -80 g, control: +90 g) ↓ food consumption throughout dosing period (-26 %* on day one up to -51 %** on day 11), overall food consumption -41 %, but not significant Haematology not examined Clinical chemistry: ↓ ALP (-34 %)** ↓ bile acids (-77 %) ↑ cholesterol (+44 %)* ↑ triglycerides (+68 %)* ↓ Ca^{2+} (-4 %)** ↓ albumin (-9 %)** ↓ A/G ratio (-17 %)* (↑ D3 hydroxy butyrate (appr. +50 %, control < 0.01 mmol/L, 750 mg/kg bw/day: 0.15 mmol/L)) Organ weights and histopathology not examined (no abnormalities at macroscopic examination after 13 days of treatment) Remark: The purpose of this study was to assess the TK-profile, glucose profile, vitamin A profile and clinical chemistry in pregnant rabbits. The results of this study were needed to decide on the best design of the mechanistic developmental study (including dose levels) to elucidate the mode of action for previously observed eye findings.	(Labcorp Laboratories, 2024a)
5 - 14-day repeated-dose toxicity study, no guideline, no GLP Reliability: Klimisch 4 as reported by registrant (reliable with restrictions:	NaTFA (CAS 2923-18-4) Oral (diet, 0.5 %) For serum and liver lipid analyses, TFA was applied i.p. at a dose of 23 µmoles per 100 g bw/d	No effects on mortality No effects on body weight No clinical signs Anatomic pathology of the liver (microscopic): Morphological examination of the liver lobule: moderate increase in cell size, in the number of 3,3'-diaminobenzidine (DAB)-positive particles (indicating the presence of H_2O_2), particularly in the centrilobular zone, indicating the occurrence of increased peroxisome proliferation, as well as an increase in the total volume of peroxisomes per cell	(Just et al., 1989)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
limited parameters assessed) Rat (Wistar) n/dose group = NA (m only)		Testes and epididymes not examined	
5 - 6 day repeated-dose toxicity study, no guideline no GLP Reliability: Klimisch 2 (reliable with restrictions: limited parameters assessed) Rat (Wistar), n = 25 for control, n = 4 - 10 for dose groups	TFA (neutralised with NaOH) Oral (drinking water) Dose corresponds to approximately 130 µmoles TFA/100 g bw/day or 148 mg/kg bw/d	No effects on mortality No effects on body weight No clinical signs <u>Anatomic pathology of the liver (macroscopic):</u> ↑ rel. liver weight (+43 %) Testes and epididymes not examined	(Stier et al., 1972)
10-day repeated- dose toxicity study, no guideline Reliability: Klimisch 4 as reported by registrant (not assignable, limited reporting in this short communication) Rat (SD) n/dose group = 5 - 6 (m only)	TFA (acid, and Na+ salt) Oral (drinking water) 0, 1 N	No effects on mortality ↓ body weight (−30 - 40 %, not clearly documented) <u>Anatomic pathology of the liver (macroscopic):</u> ↑ absolute liver weight, not clearly documented Average percentage liver/body weight on the 10th day: • control (distilled water): 6.5 +/- 0.58 • trifluoroacetic acid (1 N): 8.5 +/- 0.41 • sodium trifluoroacetate (1 N): 7.5 +/- 0.45 • acetic acid (1 N): 6.1 +/- 0.29 • sodium acetate (1 N): 6.3 +/- 0.51 General comment: Test animals exposed to drinking water with TFA and NaTFA rejected drinking water leading to dehydration and potentially explaining increased relative liver weights. Body weights and liver weights were 30 - 40 % less in these groups than in the distilled water, acetic acid, and sodium acetate control groups. Testes and epididymes not examined	(Blake et al., 1970)
8-day repeated-dose toxicity	NaTFA	No effects on mortality	(Blake et al., 1970)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
study, no guideline Reliability: Klimisch 4 as reported by registrant (not assignable, limited reporting in this short communication) Rat (SD) n/dose group = 5 - 6 (m only)	Oral (gavage) 0, 0.5 N, 1 N	No effects on body weight <u>Anatomic pathology of the liver (macroscopic):</u> No effect on liver weight • control (distilled water): 5.1 +/- 0.56 % • Sodium trifluoroacetate (0.5 N): 5.9 +/- 0.58 % • Sodium trifluoroacetate (1 N): 5.5 +/- 0.56 % General comment: Administration via gavage did not lead to dehydration, nor reduced body weight or increased liver weight and no significant effects on liver/body weight ratio. Testes and epididymes not examined	
2 - 10-day repeated-dose toxicity study, no guideline Reliability: Klimisch 3 as reported by registrant (not assignable, limited reporting) Mouse (C57BL) n/dose group = not reported (m only)	NaTFA (98 %) EC 220-879-6; CAS 2923-18-4 Oral (diet) 0, 0.02 % w/w of diet	No effects on mortality No effects on body weight <u>Clinical chemistry:</u> • protein content of hepatic mitochondrial fraction: C: (6.6 +/- 1.3) mg/g liver (wet weight); 0.02 %: (9.9 +/- 1.5) mg/g liver (wet weight); ↑+50 % ($p < 0.05$) • protein content of hepatic microsomal fraction: C: (6.2 +/- 2.1) mg/g liver (wet weight); 0.02 %: (7.7 +/- 1.3) mg/g liver (wet weight) • protein content of hepatic cytosolic fraction: C: (26.4 +/- 1.6) mg/g liver (wet weight); 0.02 %: (26.8 +/- 2.0) mg/g liver (wet weight) • no significant effects on peroxisomal catalase and lauroyl-CoA oxidase activity; palmitoyl-CoA oxidation activities: C: (20.1 +/- 4.0) nmol NADH reduced/min per mg mitochondrial protein; 0.02 %: (25.4 +/- 1.5) nmol NADH reduced/min per mg mitochondrial protein ↑+26.4 % ($p < 0.05$) <u>Anatomic pathology of the liver (macroscopic):</u> No effects on abs. and rel. liver weight after ten days Testes and epididymes not examined	(Permadi et al., 1993)
5-months repeated-dose toxicity study, no guideline	TFA EC 200-929-3 / CAS 76-05-1	no effects on mortality <u>Clinical signs:</u> anxiety of the animals, breath holding, lachrymation, hyperaemia of the conjunctiva, and sanious and sanious-	(Institute of work health and safety of

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Reliability: Klimisch 4 as reported by registrant (not assignable because of limited reporting) Rat (strain not specified) n = 12 (control) n = 36 (treatment)	Inhalation (vapours and aerosols) 4 h/d, 6 d/week 0.4 - 0.7 mg/L	suppurative nasal discharge most likely caused by irritant property of TFA Haematology: ↑ total leukocyte count and blood sugar level Urinanalysis: ↑ protein in the urines Ophthalmoscopic examination: hyperaemia of the conjunctiva and lachrymation; “severe signs of irritation of the respiratory pathway and of the eyes” (from Effect levels table in dossier) Anatomic pathology (microscopic): signs of irritation in the upper respiratory pathways (rhinitis, tracheitis, bronchitis) and lungs (thickening of the alveolar septa, peribronchitis, emphysema, pulmonary collapse). Dystrophy of the liver and kidneys. LOAEL: < 0.05 mg/L air (severe signs of irritation of the respiratory pathway and of the eyes, effects in the liver and kidney and body weight loss) Testes and epididymes not examined	the USSR, 1964)
4-months repeated-dose toxicity study, no guideline Reliability: Klimisch 4 as reported by registrant (not assignable because of limited reporting) Guinea pig n = 10 (control) n = 20 (treatment)	TFA EC 200-929-3 / CAS 76-05-1 Inhalation (vapours and aerosols) 4 h/d, 6 d/week 0.025 - 0.05 mg/L	Delayed weight gain (no further details reported) Clinical signs: anxiety of the animals, breath holding, lachrymation, hyperaemia of the conjunctiva, and sanious and sanious-suppurative nasal discharge most likely caused by irritant property of TFA Haematology: increase in the total leukocyte count and blood sugar level Urinanalysis: increased levels of protein in the urines Ophthalmoscopic examination (microscopic): hyperaemia of the conjunctiva and lachrymation; “severe signs of irritation of the respiratory pathway and of the eyes” (from Effect levels table in dossier) Anatomic pathology (microscopic): signs of irritation in the upper respiratory pathways (rhinitis, tracheitis, bronchitis) and lungs (thickening of the alveolar septa, peribronchitis, emphysema, pulmonary collapse). Dystrophy of the liver and kidneys	(Institute of work health and safety of the USSR, 1964)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		LOAEL: < 0.7 mg/L air (severe signs of irritation of the respiratory pathway and of the eyes, effects in the liver and kidney and body weight loss) Testes and epididymes not examined	

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Twelve (ten via the oral and two via the inhalation route) repeated-dose toxicity studies with TFA or its sodium salt are available:

1. OECD TG 452 study (WuXi AppTech, 2019)

   A one-year chronic oral toxicity study performed according to OECD TG 452 with Sprague-Dawley rats using sodium trifluoroacetate (EC no. 220-879-6) in drinking water at doses of 0, 30, 120, and 600 ppm (WuXi AppTech, 2019) resulted in no treatment-related mortality, and no adverse effects in clinical signs, body weight, food consumption, water consumption, ophthalmology and clinical pathology. A dose-proportional increase in plasma concentration of the test substance was observed in males and females. The NOAEL for this study was reported to be 600 ppm (corresponding to 37.8 mg/kg/day in males and 64.0 mg/kg/day in females). Immunotoxicity endpoints were not assessed in the study. Some ophthalmological changes observed in some animals, such as cornea opacity, cataract, ocular discharge, periorbital swelling, iris synechia, retinal vessel tortuosity, fundus hyperreflectivity and incomplete pupil dilation post-mydriatic, were not considered treatment-related, according to the TFA registration dossier (UUID: c0bccced-2a93-4d3e-9cd7-2eda0a7239d1), because there was no dose-response correlation. These data cannot be assessed because they are not reported in the registration dossier. No LOAEL can be determined from this study and the NOAEL was 37.8 mg/kg bw/day for males and 64.0 mg/kg bw/day for females.

2. OECD TG 408 study (Bayer CropScience, 2007)

A 90-day oral repeated dose toxicity study performed according to OECD TG 408 with rats (Wistar Rj:WI (IOPS HAN)) (Bayer CropScience, 2007) using sodium trifluoroacetate (EC no. 220-879-6) in the diet, dosing 9.9, 98, 1043 mg/kg bw/day for males and 12.2, 123, 1216 mg/kg bw/day for females, found statistically significant decreases in body weights and body weight gains in male rats in the high dose group (non-significant decrease in females). The haematological assessment revealed significantly decreased haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haematocrit in high dose females with reductions of ≤ 8 % of each parameter. The urinanalysis showed significantly higher ketone levels in mid- and high dose males and females. The clinical chemistry assessment resulted in significantly reduced glucose and total bilirubin levels in mid and high dose males and females with a maximum bilirubin decrease of −81 %. In females, alkaline phosphatase (ALP) increased up to +95 % in the high dose

group ($p \leq 0.01$) and alkaline amino transferase (ALT) increased up to +38 % ($p \leq 0.05$; +85 % in the mid dose group but non-significant). Absolute and relative liver weights increased significantly in males and females in mid and high dose (e.g., rel. liver weights in males +14 % in mid dose ($p \leq 0.01$) and +33 % in high dose ($p \leq 0.01$)). Liver effects also include increased centrilobular to panlobular hepatocellular hypertrophy in males (high dose only) and females (mid and high dose) and hepatocellular necrosis in females in the high dose group. Corresponding effects indicating disturbed liver cell function are increased ALT values and possibly increased ALP activities (other sources such as kidney or heart could not be excluded). Elevated urine ketone levels may have resulted from insufficient energy supply (supported by reduced glucose level) due to excessive fat metabolism as an alternative source of energy. Ophthalmological findings include corneal opacity in the left eye of one male in the high dose group and anterior synechia in the iris of the left eye of another male at the high dose (no data on severity of effects are reported, no dose relation). The NOAEL is 8.4 mg TFA/kg body weight/day in males and 10.1 mg TFA/kg body weight/day in females, based on increase in liver weight, histopathological changes in the liver, and changes in haematological parameters, clinical biochemistry and urinalysis. The LOAEL is 98 mg/kg bw/day for males and 123 mg/kg bw/day for females or for the corresponding TFA doses: 82.3 mg TFA/kg bw/day for males and 103.3 mg TFA/kg bw/day for females.

3. OECD TG 407 study (BayerCropScience, 2014)

A 28-day oral repeated dose toxicity study according to OECD TG 407 with rats (Wistar) was performed with trifluoroacetate at doses of 50, 149, 436, 1315 mg/kg bw/day for males and 52, 157, 457, 1344 mg/kg bw/day in females via diet (BayerCropScience, 2014). Clinical chemistry showed increased ALT in males and females (m: +37 %; f: +23 %; NOEL: 436/457 mg/kg bw/day), decreased cholesterol in males (m, ≈ -30 %; NOEL: 149/1344 mg/kg bw/day), decreased glucose levels in males and females (m & f, ≈ -30 %; LOEL: 50/52 mg/kg bw/day), increased urinary ketone (m & f; LOEL: 50/52 mg/kg bw/day), and increased urinary volume in males (m, +65 %; NOEL: 436 mg/kg bw/day). Relative liver weights increased in males and females (m: +15 %; f: +13 %; NOEL: 50/157 mg/kg bw/day). Increased ALT activities are indicative of liver cell dysfunction, however without histopathological changes the slight increase in liver weight and enzyme activity cannot be assessed. Decreased glucose levels and increased urinary ketone levels indicate insufficient energy supply. No LOAEL can be determined from this study.

4. OECD TG 422 study (CIT BP, 2012)

A 28-day combined repeated dose toxicity study with reproduction/developmental toxicity screening test according to OECD TG 422 with rats (Sprague-Dawley) was performed with potassium trifluoroacetate/potassium trifluoromethanesulphinate (1:1) at doses of 0, 100, 300, 1000 mg/kg bw/day with male and female animals via oral gavage. In males at hd, haemoglobin content was decreased (-10.2 %, $p < 0.01$), inorganic phosphorus was increased (+13.4 %, $p < 0.05$) and calcium was decreased (-4.9 %, $p < 0.05$). These effects were considered non-adverse because of the amplitude of changes and the absence of relevant effect in other parameters and of minor toxicological significance at hd. The mean absolute and relative liver weights were increased in md and hd males (statistically significant) and in hd females (not statistically significant), which correlated with the minimal centrilobular hypertrophy of hepatocytes in 4/5 hd males, 2/5 hd females and 1/5 md males. However, this was not considered as adverse effect, because associated degenerative microscopic findings or changes in enzyme activities were absent. The incidence and severity of haematopoiesis in the spleen of males were minimally increased compared to control in all doses. However, no dose-relation was observed and the

haematopoiesis may have been compensatory to the changes in red blood cell parameters. The NOEAL was determined at 1000 mg/kg bw/day for both substances, corresponding to 500 mg KTFA/kg bw/day.

5. 14-day oral repeated dose non-guideline toxicity study (BayerCropScience, 2014)

A 14-day oral repeated dose non-guideline toxicity study with rats (Wistar) using trifluoroacetate in the diet at doses for males of 43, 85, 170 mg/kg bw/day and for females of 45, 91, 190 mg/kg bw/day resulted in several liver effects: increased relative liver weights in high dose males (+23 %), increased slight diffuse centrilobular hepatocellular hypertrophy in males (mid dose: 40 % of males, high dose: 20 % of males), increased hepatocellular mitoses in all males and 2/3 of females in the high dose group, increased hepatic cytochrome P-450 content (+19 % in the high dose group) (BayerCropScience, 2014). Dose-dependently increased specific and total palmitoyl-CoA oxidation activities occurred in males from 85 mg/kg bw/day (+84 % and +92 % in high dose, respectively). The haematological assessment revealed decreased white blood cell count in females in the high dose group (−30 %), decreased lymphocyte count (−38 % in high dose) and increased % of neutrophils in the high dose group (+65 %). According to the report summary, TFA is a weak peroxisome proliferator in male rats. A LOAEL of 85 mg/kg bw/day can be determined for males (based on liver findings: ↑ rel. liver weights in combination with ↑ hepatocellular hypertrophy and ↑ palmitoyl-CoA oxidation activities (that yields acetyl-CoA that is used for the formation of ketone bodies in the liver)) and 190 mg/kg bw/day for females (based on ↓ white blood cell count and ↓ lymphocyte cell count).

6. 14-day repeated dose non-guideline toxicity study (Labcorp Laboratories, 2024a)

A 14-day oral repeated dose non-guideline toxicity study with non-mated female rabbits (New Zealand White) administering NaTFA via oral gavage at doses of 0, 750 mg/kg bw/day resulted in several effects on body weight and food consumption as well as clinical chemistry: Treated animals lost body weight (-80 g) whereas control animals gained weight (+90 g). Food consumption in the dose group was reduced throughout the dosing period. ALP and bile acids were reduced by 34 % or 77 %, respectively. Cholesterol and triglycerides were increased by 44 % and 68 %, respectively. D3 hydroxy butyrate was increased but quantification was not possible because the control values were below quantification limits and only dose group values were quantifiable.

7. 5 – 14-day repeated dose non-guideline toxicity study (Just et al., 1989)

A 5 – 14-day repeated dose non-guideline toxicity study with rats (Wistar) using sodium trifluoroacetate in dietary exposure (single dose of 0.5 %) demonstrated a moderate increase in cell size in the liver lobule and increase in the number of 3,3'-diaminobenzidine (DAB)-positive particles, particularly in the centrilobular zone, indicating the occurrence of increased peroxisome proliferation, as well as an increase in the total volume of peroxisomes per cell (Just et al., 1989). No clear LOAEL can be derived from this single-dose study.

8. 5 – 6-day non-guideline repeated dose toxicity study (Stier et al., 1972)

A 5 – 6-day non-guideline repeated dose toxicity study with rats (Wistar) using TFA neutralised with NaOH in drinking water (one dose level corresponding to approximately 148 mg/kg bw/day)

found no treatment-related effects except increase relative liver weight by +43 % (Stier et al., 1972). No clear LOAEL can be derived from this study.

8.–12. Five other repeated-dose toxicity studies (Blake et al., 1970; Institute of work health and safety of the USSR, 1964; Permadi et al., 1993)

Five other repeated-dose toxicity studies with TFA or its sodium salt are rated as not assignable in terms of reliability (Klimisch 4) due to insufficient reporting, and are not further discussed but summarised in Table 17 (Blake et al., 1970; Institute of work health and safety of the USSR, 1964; Permadi et al., 1993).

Table 18: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
(WuXi AppTech, 2019)	No effects; LOAEL > 37.8 mg/kg/day for males and > 64.0 mg/kg/day for females	1 year	Not determined	No classification
(Bayer CropScience, 2007)	LOAEL: 98 mg NaTFA/kg bw/day for males and 123 mg NaTFA/kg bw/day for females corresponding to 82.3 mg TFA/kg bw/day for males and 103.3 mg TFA/kg bw/day for females based on increase in rel. liver weight and histopathological changes in the liver (hepatocellular hypertrophy at LOAEL, necrosis in females at 1216 mg/kg bw/day) The effects observed in eyes of two hd males are not considered sufficient for classification because of low incidence and because effects occur above the guidance values for classification (corneal opacity in the left eye of one male, anterior synechia in the iris of the left eye of another male)	90 days	98 mg NaTFA/kg bw/day or 82.3 mg TFA/mg bw/day	No classification as effects with LOAELs below guidance values (≤ 100 mg/kg bw/day) are not severe enough
(BayerCropScience, 2014)	No adverse effects; LOAEL > 1315 mg/kg bw/day for males and > 1344 mg/kg bw/day for females	28 days	Not determined	No classification
(CIT BP, 2012)	LOAEL > 500 mg/kg bw/day	28 days	Not determined	No classification as effects with LOAELs below guidance values (≤ 300 mg/kg bw/day) are not severe enough

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
(BayerCropScience, 2014)	LOAEL: 85 mg/kg bw/day for males based on increase in rel. liver weight and histopathological changes in the liver (hepatocellular hypertrophy)	14 days	Extrapolation factor: 6.4 (90/14) → 85 mg/kg bw/day / 6.4 = 13.2 mg/kg bw/day	No classification as effects with LOAELs below guidance values (≤ 100 mg/kg bw/day) are not severe enough

10.12.2 Comparison with the CLP criteria

All data provided in the registration dossier as well as publicly available data were considered to conclude on the classification for specific target organ toxicity – repeated exposure. Human data on specific target organ toxicity arising from a repeated exposure to TFA or its salts are not available. Data from sub-acute, sub-chronic and chronic studies with experimental animals are available (Table 17).

TFA-related and dose-dependent liver toxicity was observed in male and female rats after subacute and subchronic exposure but not in the chronic exposure study according to the study summary. Findings such as increased relative liver weights and hepatocyte hypertrophy were observed at doses below the guidance values for classification as STOT RE 2. However, these liver effects were not considered severe enough for STOT RE classification. In the sub-chronic study, adverse effects which are considered relevant for classification, such as hepatocellular necrosis, occurred only at the high dose-level one order of magnitude above the guidance value for STOT RE 2 classification.

Ophthalmological findings (e.g. cornea opacity) were reported for rats after oral administration of the sodium salt (Bayer CropScience, 2007; WuXi AppTech, 2019). In the one-year repeated-dose toxicity study (WuXi AppTech, 2019), the registrant stated that “Cornea opacity, cataract, ocular discharge, periorbital swelling, iris synechia, retinal vessel tortuosity, fundus hyperreflectivity and incomplete pupil dilation post-mydriatic were observed in some animals during the dosing and recovery phase exams.” The study data can be interpreted as the eye being a target organ in adult rats after chronic oral exposure. However, the detailed data of this study are not reported and the effect was not dose-related. In the study by Bayer CropScience (2007), the effects observed in eyes of two hd males are not considered sufficient for classification because of low incidence and because effects occur above the guidance values for classification (corneal opacity in the left eye of one male, anterior synechia in the iris of the left eye of another male). Ophthalmological findings after inhalation of aerosols and vapours of the free acid in rats and guinea pigs include severe hyperaemia of the conjunctiva and lachrymation (red eyes) and may be related to the corrosive properties of TFA (Institute of work health and safety of the USSR, 1964).

Assignment to the STOT RE classification category 2 is therefore not appropriate.

10.12.3 Conclusion on classification and labelling for STOT RE

Classification and labelling for STOT RE is not appropriate for TFA.

10.13 Aspiration hazard

Not assessed in this dossier

10.14 Endocrine disruption for human health

Not assessed in this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Hazardous to the aquatic environment

Not assessed in this dossier

11.2 Endocrine disruption for HH

Not assessed in this dossier

11.3 PERSISTENT, BIOACCUMULATIVE AND TOXIC (PBT) OR VERY PERSISTENT, VERY BIOACCUMULATIVE (VPVB) PROPERTIES UNDER CLP ANNEX I, 4.3

Not assessed in this dossier

11.4 PERSISTENT, MOBILE AND TOXIC (PMT) OR VERY PERSISTENT, VERY MOBILE (VPVM) PROPERTIES UNDER CLP ANNEX I, 4.4

Introductory remark by the dossier submitter

In all test systems on degradability and mobility reported and assessed in this chapter the test substance is always the dissociated anion TFA^-.

Difference may only occur in the practical way which has been used during the preparational phase of each test to bring the test substance, the dissociated anion TFA^-, into solution and consequently into the test system. First option is to use TFA, which has a high water solubility but on the other hand is a strong organic acid with a pKa of 0.23 meaning that it is in its dissociated form in all environmental compartments and under all environmental conditions. If TFA is used in a test setup to bring TFA^- into solution a pH adjustment is required.

Second option is to use one of the several inorganic salts of TFA, e.g. the sodium salt (NaTFA), the potassium salt (KTFA), etc., which has several practical advantages in the test setup like a low vapour pressure, being a crystalline solid and not requiring a pH adjustment. Under this option the degradability or the mobility of the inorganic cation e.g. Na^+, K^+, etc. is not applicable and only the degradability or the mobility of the dissociated anion TFA^- is assessed.

The bioavailability as well as the fate and behaviour of the dissociated anion TFA^- in a test system and in the environment is the same, no matter if TFA itself or one of its inorganic salt has been used to bring the test substance, the dissociated anion TFA^-, into solution. It is indisputable from a scientific point of view that this is not a read-across between TFA and its inorganic salts. Rather, it is simply two distinct practical methods for introducing the relevant test substance, the dissociated anion TFA^-, into solution and consequently into the test system. All test results, assessments and conclusions refer to the dissociated anion TFA^-. Thus, the test results are valid and under CLP apply for both TFA and all its inorganic salts.

11.4.1 Persistence under CLP Annex I, 4.4

Table 19: Summary of relevant information on persistence under CLP Annex I 4.4

Method/ Study type	Test material and purity	Results	Remarks/Reliability	Reference
non-TG scientific research study with laboratory study part and microcosm field study part	TFA^- (analytical purity: no data)	No DT_{50} value in fresh water and fresh water sediment could be derived as no degradation of TFA^- was observed in the 120 days laboratory study part and in the 365 days microcosm field study part	2 (reliable with restrictions) assessed in the registration dossier under REACH dossier submitter: key study for use under CLP	Ellis et al. (2001)
OECD TG 307	^{14}C-TFA^- (analytical purity: no data)	No meaningful DT_{50} value in soil could be derived as no degradation of TFA^- was observed in the 120 days study	study in Draft Renewal Assessment Report for FLUFENACET dossier submitter: key study for use under CLP	Eckermann (2012a)
OECD TG 301D	TFA^- brought into solution by the sodium salt (NaTFA) (analytical purity: 99.9%)	no biodegradation of TFA^- observed after 28 days and prolonged	2 (reliable with restrictions) assessed in the registration dossier under REACH dossier submitter: reliable and suitable for use under CLP	anonymous (1992b)
non-TG experimental study: screening test	^{14}C-TFA^- (analytical purity: no data)	No biodegradation of TFA^- was observed by all nine tested bacterial strains after 13 days	4 (not assignable) assessed in the registration dossier under REACH dossier submitter: supporting evidence under CLP	anonymous (1996)
non-TG scientific research study	TFA^- (analytical purity: 97%)	TFA^- was cometabolically degradable in anaerobic conditions and 35°C in the 630 day study	4 (not assignable) assessed in the registration dossier under REACH dossier submitter: not suitable for use under CLP	Kim et al. (2000)
OECD TG 302A	TFA^- brought into solution by the sodium salt (NaTFA) (analytical purity: no data)	no biodegradation of TFA^- observed after 127 days	2 (reliable with restrictions) assessed in the registration dossier under REACH dossier submitter: reliable and suitable for use under CLP	anonymous (1992c)

11.4.1.1 Water, water-sediment and soil degradation data (including simulation studies)

Study by Ellis D.A. et al. (2001)

The purpose of this non-GLP scientific research study was to investigate the fate of four haloacetic acids (trifluoroacetic acid, monochloroacetic acid, dichloroacetic acid and trichloroacetic acid) in field pond waters (microcosms) and using laboratory sediment water systems from Canada. Studies were conducted over time periods of 2880 hours (laboratory study) up to one year (microcosm field study). The microcosm field studies were conducted over two separate years (1997-98 and 1998-99).

laboratory water sediment system

- Type of culturing flask: 120 ml narrow mouth screw capped Boston round bottles
- Volume of test solution: bottles filled with 5 g dried sediment (from microcosm field study) and 100 mL test solution
- Composition of medium: pond water
- Additional substrate: Wet sediment used in the microcosm field studies was oven dried at 100°C for 3 hours.
- Continuous darkness: No, the exposure to daylight was 12 - 14 h
- Number of culture flasks/concentration: 10 replicates / 20 µg/mL TFA.
- Preparation of test solutions: A stock solution of TFA at a concentration of 2000 µg/mL was prepared in pond water. A 1 mL aliquot from the stock solution was further diluted in 100 mL pond water to give a final concentration of 20 µg/mL. The Experimental solutions (20 µg/mL TFA sample, plus control and blank) were gently poured onto the sediment taking care not to cloud the water, then loosely capped and placed on a north facing windowsill.
- Inoculum blank: Control pond water, with no analyte added, was used as a blank
- Abiotic sterile control: Test solutions of 20 µg/mL TFA were prepared to which 10 µg/mL of HgCl2 (Aldrich, Milwaukee, WI) was added.
- Sampling: Aliquots (5 mL) were taken at regular time intervals from each solution and analysed by IC.

Wet sediment (sand, loam, 20% organic matter) was used in the field studies and obtained from the Guelph Microcosm Facility, and was oven dried at 100°C for 3 h. Dry sediment (5 g) was added to clear 120 ml narrow mouth screw capped Boston round bottles (VWR Canlab Mississauga, Ont.). Experimental solutions (20 µg L^{-1}) sample, plus control and blank) were gently poured onto the sediment taking care not to cloud the water, then loosely capped and placed on a north facing windowsill. The approximate exposure to the sun each day for the solutions during the months of June and July 1999 was 12±14 h. Aliquots (5 ml) were taken at appropriate time intervals from each solution and analysed by IC.

No degradation of TFA^- was observed.

Even when TFA^- was spiked into a solution in which trichloroacetic acid (TCA) had degraded, no degradation was observed; this suggested that even the microbes responsible for TCA degradation were not capable of degrading TFA^-.

The dossier submitter noticed that the laboratory water sediment system does not correspond to common water sediment studies such as OECD TG 308. The test was not conducted in dark and not at a constant temperature. However, most deviation from the OECD TG 308 guidance would support aerobic degradation (e.g. higher test temperature, exposure to sun light, longer test duration of 120 days). Only the low amount of 5g dry sediment used in contrast to 50g dry sediment in OECD TG 308 might reduce the microbiological activity in the test system. The concentration TFA^- in sediment itself was not measure. However, this seems not problematic, since all TFA^- was recovered from the aqueous phase.

Given the absence of any observed degradation of TFA^- at these optimised conditions, it can be deduced with a high degree of certainty that no degradation is to be expected in a standardised OECD TG 309 or 308 test system and at an environmentally relevant temperature of 12°C.

In the laboratory water sediment system no DT50 value in fresh water and fresh water sediment could be derived as no degradation of TFA^- at room temperature and sun light exposure was observed during the extended study duration of 120 days and it can be assumed that the rate constant is not significantly different from zero.

This study may be used as key study under CLP and the test result can be applied directly to the criteria set out in sections 4.4.2.1 and 4.4.2.2.

field aquatic microcosm

- 30 artificial ponds
- Volume of test solution: Microcosms (approx. 1.2 m deep) were used with a water depth of 1 m, a diameter of 3.9 m, a surface area of 11.95 m^2 and a capacity of approx. 12 m^3 of water. Relative to the surrounding landscape, the ponds were located on top of a raised mound in order to avoid potential run-off from precipitation events re-entering the ponds.
- Additional substrate: Each microcosm bottom was filled with trays containing sediment covering approx. 50 % of the total surface area.
- addition of macrophytes, fathead minnows (Pimephales promelas), pumpkinseed sunfish (Lepomis gibbosus) etc.
- Pre-treatment: The source water was circulated through the microcosm for 2 weeks before the start of the test.
- Treatment: TFA solutions were injected into the microcosms using a Proven Pony Pup, high-volume, low-pressure pump system. Each microcosm including controls, were circulated for 15 min. after injection. The compound was weighed out of the day of the actual exposure and suspended in redistilled deionized water. The pH of each solution was adjusted to 8.5 using NaOH (aqueous solution).
- Test concentrations: For the 1997 study with TFA, the concentrations were 10 and 100 µg/L (four microcosms each) and 300 and 1000 µg/L (two microcosms). For the 1998 study, The TFA concentrations were 100, 1000, 3000 and 10,000 µg/L which were applied to three microcosms each.
- Inoculum blank: Thee control ponds (with no analyte added) were used as blanks.
- Sampling: Water samples for TFA analysis and routine water chemistry determinations were taken at day 1, at 1 h, 1, 2, 4 and 7 days and on a weekly or biweekly basis until completion of the study. All sample containers were prepared by soaking overnight in a 10 % (v/v) HCl solution,
cleansing with a hot detergent solution, rinsing with hot tap water, distilled tap water and then drying in a drying oven at 150°C overnight.
- Sample storage before analysis: samples were stored at 4°C until analysis.
- Water chemistry: Maximum and minimum temperatures and dissolved oxygen were taken daily during the course of the study. On selected sampling days, specific water parameters were measured (water hardness, alkalinity, dissolved organic carbon, total nitrogen and phosphates as well as pH).

Microcosm field studies were conducted over two separate years (1997/98 and 1998/99). It was shown that any input of TFA to the ponds from precipitation would be negligible (in the order of ng/l). There was no detectable concentration of TFA present in the ponds prior to treatment.

There was no change in the TFA^- concentration over the period June-October, but following this, a reduction of up to 35 % TFA^- was observed for the months November-January. For the period of February-March, the concentration of TFA^- subsequently returned to initial concentration levels.

No such decrease in TFA^- was observed for the subsequent year.

It is hypothesized that the decrease and subsequent increase in TFA^- over the winter/spring months can be accounted for by an enforced partition of the TFA^- into an as yet not identified phase. The formation of ice within the pond would be expected to lead to the enhancement of TFA^- concentrations in the aqueous phase, due to thermodynamic exclusion from the ice. Indeed, when a solution containing TFA^-, sediment, phytoplankton and pond water was placed in a freezer at -20°C and ice allowed to form, the measured aqueous TFA^- concentration increased. Furthermore, ice samples collected from the pond showed no detectable concentration of TFA^-. It is suggested the partitioning of TFA^- into an as yet undetermined phase can be enhanced at low temperature. It is hypothesized in the period between subsequent years the biotic and/or suspended particulate matter concentrations were different and one of these may be the phase into which TFA^- was partitioning.

No degradation of TFA was observed in the aqueous environment in the field aquatic microcosms over the time scale of up to one year.

In the microcosm field study part no DT_{50} value in fresh water could be derived as no degradation of TFA^- was observed in the extended study duration of 365 days and it can be assumed that the rate constant is not significantly different from zero.

This study may be used as key study under CLP and the test result can be applied directly to the criteria set out in sections 4.4.2.1 and 4.4.2.2.

OECD TG 307 study by Eckermann N. (2012a)

The GLP study was performed to comply with the following Guidelines:

- OECD Guideline for the Testing of Chemicals, No. 307, Aerobic and Anaerobic Transformation in Soil, 2002;
- US. EPA OCSPP Fate, Transport and Transformation Test Guidelines, OPPTS 835.4100 and OPPTS 835.4200, Aerobic and Anaerobic Soil Metabolism, 2008.

Additionally, as the data obtained in the study were kinetically evaluated and the results presented in the study report, it was declared that the following Guideline was used:

- FOCUS (2006): “Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration.” Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference SANCO/10058/2005 version 2.0, 434 pp.;

The aim of the study was to examine the degradation of TFA in aerobic soil. The experiment was performed on four EU-soils (2 x sandy loam, clay loam, silt loam). The test soils were taken from the agriculturally used areas, representing different geographical origin and different soil properties, in line with the requirement of the relevant Guideline. The test soils were sampled shortly before being used (14 days before the experiment began) with shovel from 0-20 cm layer of grassland plot.

The examination of the microbial viability of the test soils showed that they were viable throughout the whole experiment. The monitoring of incubation temperature showed that the samples were kept in the constant temperature T = 20 ± 1°C. The mean incubation temperature was T = 20.00°C and it ranged from T = 19.80°C to T = 20.40°C.

For treatment the pre-incubated biometric flasks were removed from the incubation chamber. After application of the test compound the vessels, except those designated as DAT-0 samples, were fitted with the traps for volatile compounds and returned to incubation chamber to be incubated at constant temperature T = 20.0 ± 0.10C and soil moisture level 55 ± 5% MWHC for up to 120 days. At the designated time points – DAT 3, DAT 7, DAT 14, DAT 28, DAT 34, DAT 59, DAT 92 and DAT 120, duplicate samples of each test soil were removed from the incubation chamber and further processed.

The total recovery was in the range of 97.0 – 103.4 [% AR] for all four soils. The Radioactivity extracted was in the range of 97.0 – 102.1 [% AR] for all four soils. The maximum level of volatiles formed was 0.1 [% AR] for all four soils. The NER level was in the range of 0.6 – 2.0 [% AR] for all four soils.

The kinetic analysis was carried out in line with the recommendations given by FOCUS for the determination of the kinetic endpoints suitable for modelling. All fitted models were visually acceptable and statistically good. Therefore, SFO is proposed as a model giving the acceptable approximate of the kinetic behaviour of TFA^- in soil with a $DegT_{50}$ (20°C) value of 10,000 days (~27.4 years). For the purpose of hazard classification under CLP this $DegT_{50}$ (20°C) would need to be adjusted to 12°C and a $DegT_{50}$ (12°C). However, as no degradation of TFA^- was observed in the experiment a $DegT_{50}$ value is not considered meaningful under CLP.

No meaningful DT_{50} value in soil could be derived as no degradation of TFA^- was observed in the extended study duration of 120 days and it can be assumed that the rate constant is not significantly different from zero.

This study may be used as key study under CLP and the test result can be applied directly to the criteria set out in sections 4.4.2.1 and 4.4.2.2.

Supplementary study by Eckermann N. (2012b)

A supplementary parallel study was performed by Eckermann (2012b), the same author to the above study, with the specific aim to examine a possible influence of concentration of TFA on its rate of degradation in soil. That was done by examining the degradation of TFA in function of concentration and time. The experiment was performed on four EU-soils.

No degradation was observed for none of the tested concentrations of TFA^- and consequently the concentration of TFA^- has no effect on degradation in soil. The results of this supplementary study, although indirectly, conform the appropriateness of the previously summarised study.

This study may be used as supporting evidence under CLP.

11.4.1.2 Ready biodegradability tests

OECD TG 301D study by anonymous (1992b)

A GLP ready biodegradability test was performed with a closed bottle test performed according to slightly modified OECD 301D, EEC 1984 Part C. and ISO Test Guidelines. Microorganisms (Secondary activated sludge at concentration of 2 mg DW/L, obtained from the waste water treatment plant (WWTP) Nieuwgraaf in Duiven: plant treating predominantly domestic wastewater) are inoculated into a chemically defined liquid medium containing the test substance TFA^- brought into solution by NaTFA at 20 mg/L or the reference substance (Acetic acid, sodium salt or Sodium acetate at 6.7 mg/L) under aerobic conditions for a period of 77 days; the test was prolonged because the pass level was not reached at day 28.

Details on inoculum

- Source of inoculum/activated sludge (e.g. location, sampling depth, contamination history, procedure): Secondary activated sludge was obtained from the WWTP Nieuwgraaf in Duiven. The WWTP Nieuwgraaf is an activated sludge plant treating predominantly domestic wastewater. A minor deviation of the test procedures described in the guidelines was introduced: Instead of an effluent/extract/mixture, activated sludge was used as an inoculum.

- Pretreatment: The activated sludge was preconditioned to reduce the endogenous respiration rates: the sludge (approximately 200 mg Dry Weight (DW)/L) was aerated for a period of one week.
- Concentration of sludge: 2 mg DW/L

TEST CONDITIONS

- Composition of medium: 8.5mg/l KH2PO4, 21.75mg/l K2HPO4, 33.3mg/l Na2HPO4.2H2O, 22.5mg/l MgSO4.7H2O, 27.5mg/l CaCl2, 0.25mg/l FeCl3.6H2O. Ammonium chloride was omitted from the medium to prevent nitrification. Due to this omission the pH of the medium decreased slightly.
- Test temperature: The temperature was measured and recorded with a control one (IBT, Rotterdam, The Netherlands). The bottles were incubated at 21+/- 1°C.
- Continuous darkness: yes

TEST SYSTEM

- Culturing apparatus: the test was performed in 250 to 300 ml BOD (biological oxygen demand) bottles with glass stoppers.
- Number of culture flasks/concentration: 2

SAMPLING

- Sampling frequency: 0, 7, 14, 21, 28, 42, 77 days
- Sampling method: two duplicates bottles of all series were withdraw for analyses of the dissolved oxygen concentrations. The close bottle test was prolonged by measuring the course of the oxygen decrease in the bottles of day 28 using a special funnel. This funnel fitted exactly in the BOD bottle. Subsequently, the oxygen electrode was inserted the BOD bottle to measure the oxygen concentration. The medium dissipated by the electrode was collected in the funnel. After withdrawal of the oxygen electrode the medium collected flowed back into the BOD bottle, followed by removal of the funnel and closing of the BOD bottle.

CONTROL AND BLANK SYSTEM

- Inoculum blank: Yes in duplicate
- Abiotic sterile control: No
- Toxicity control: No
- Other: Mineral nutrient solution without test material but with inoculum and mineral nutrient solution with sodium acetate and with inoculum.

Use was made of 10 bottles containing only inoculum, 10 bottles containing test substance and inoculum and 10 bottles containing sodium acetate and inoculum. The temperature and pH were measured, temperatures ranged from 23 to 25°C and the pH of the media was 7.0 and 7.1 at the start and the end of the test, respectively. Two duplicate bottles of all series were withdrawn for analyses of the dissolved oxygen concentration using an oxygen electrode at day 0, 7, 14, 21, 28, 42 and 77 days. Parameter followed for degradation estimation was O_2 consumption.

The percentages biodegradation of TFA^- brought into solution by NaTFA in the closed bottle test were 0% for 0, 7, 21, 28 and 77 days and 8% for 14 and 42 days. However, the results of the prolonged test are invalid because the differences of extremes of replicate values of the removal of the test chemical at 77 days are 95% (> 20%). Moreover, the result of 8% degradation at day 42 is probably an artefact due to the 40% coefficient of variation between duplicate values of the control.

The percentages biodegradation of sodium acetate in the closed bottle test were 0, 83, 92, 85 and 83% for 0, 7, 14, 21 and 28 days respectively. The comparison of the inoculum blank to the test bottles

proves that the test substance caused no reduction in the endogenous respiration. Therefore, NaTFA is considered to be non-inhibitory to the inoculum.

TFA^- brought into solution by NaTFA was not biodegraded in the OECD TG 301D (28 days and prolonged to 77 days) closed bottle test.

This study may be used as reliable and suitable under CLP.

Study by anonymous (1996)

The purpose of this aerobic biodegradation study of trifluoroacetic acid (TFA) and Ammonium perfluoro-octanoate (C8) was to evaluate the ability of aerobic bacteria, previously shown to have a broad range of degradative capabilities, to degrade TFA. Nine different bacterial strains, known to metabolize compounds similar to TFA, were tested in bottle assays for their dehalogenation analysis and ^{14}C-TFA to test for production of $^{14}CO_2$.

^{14}C-TFA degradation or TFA dehalogenation were not detected in incubations with any of the strains tested, even with ^{14}C-TFA incubations of up to thirteen days (JOB5 and ENVOB with propane feed) or two days incubation with TFA for fluorine analysis. Both JOB5 and OB3b were assayed at several different densities (OD = 1, 2 and 3) with negative ^{14}C-TFA results. The levels of ^{14}C carbon dioxide detected in the traps from the incubations with TFA failed to exceed the levels that were observed in the controls which contained no bacteria and/or no labelled TFA. In some experiments, the vials containing live cells produced slightly higher levels of ^{14}C trapped in the NaOH, However, the total was never more than 0.2 % higher than the controls. Soluble and total non-volatile (including cells) ^{14}C was never significantly different in the experimental and killed controls.

The study was not performed according to international standard nor under GLP. The methodology is scientifically acceptable but the report is missing some crucial information to assess its reliability.

No degradation of TFA^- by all nine bacterial strains was observed after 13 days.

This study may be used as supporting evidence under CLP.

Study by Kim B.R. et al. (2000)

A long-term (90-week) study was conducted to assess the anaerobic biodegradability of TFA in an engineered anaerobic reactor. The pH and temperature of the contents of both reactors were maintained at pH 7.2 and 35°C, respectively. The microbial community established in the anaerobic reactor system was able to degrade all of the TFA fed to the system for a period of more than 1 year. TFA and TCA (Trichloroacetic acid) have a similar chemical structure and TCA is able to degrade co-metabolically through anaerobic reductive dehalogenation.

TFA^- was found only under low loading rate to be co-metabolically degradable under laboratory anaerobic conditions at 35°C using ethanol as a source of electrons. Under higher loading rates, the anaerobic degradation virtually stopped.

This study assessed only the co-metabolically degradability in anaerobic conditions in an engineered anaerobic reactor at 35°C and is not suitable for use under CLP.

OECD TG 302A study by anonymous (1992c)

An inherent biodegradability test was performed in compliance with the methods described in OECD TG 302A and EEC Directive 87/302. The semi-continuous activated sludge (SCAS) test was chosen as the most appropriate test due to its potential for promoting biodegradation under aerobic conditions. In this test, TFA- was introduced into the system in solution using NaTFA.

The SCAS test was conducted using secondary activated sludge collected from the WWTP Nieuwgraaf in Duiven, The Netherlands. The sludge was maintained by the daily addition of primary settled sewage from the same location. The test was conducted at an influent concentration of TFA- of 17.6 mg NPOC/L over a period of 127 days. At the start of the experiment, individual settled sludges were mixed, and 50 mL of the resulting composite sludge was added to each unit. The control unit received 94 mL of primary settled sewage, 5 mL of deionized water, and 1 mL of concentrated phosphate buffer. The test unit received 94 mL of primary settled sewage, 1 mL of concentrated phosphate buffer, and 5 mL of the test compound stock solution.

A few minor deviations from the protocol of the SCAS test were introduced:

- the fill and draw procedure was performed only six times per week instead of daily;
- to maintain a constant pH in the SCAS unit, 1 mL of a concentrated phosphate buffer (1.6M, pH = 7) was added six times a week;
- effluent samples were filtered using Schleicher and Schüll membranes (cellulose nitrate) with pores of 8 µm so that the test substance was passed while the sludge was filtered.

To determine the non-purgeable organic carbon (NPOC) in the effluent of the SCAS units, the samples were filtered to remove sludge particles and acidified before injection into a Dohrmann DC-190 NPOC apparatus. The incubation temperature ranged from 20 to 25°C, and the pH of the effluent varied from 6.9 to 7.4.

The closed bottle tests were conducted at various time points (days 0, 28, 62, 99, and 127), and oxygen concentrations and biodegradability percentages of TFA- were determined. Some results suggested slight biodegradation, but these were questionable due to high endogenous respiration, which did not significantly differ from that in the presence of the test substance. No biodegradation was observed in the closed bottle tests conducted on days 62 and 99, aligning with the SCAS test results indicating a lack of biodegradation.

TFA- caused no reduction in the biodegradation of NPOC present in primary settled wastewater, indicating that it is non-inhibitory to activated sludge. Approximately 20% of TFA- was removed from the wastewater in the SCAS test, with the final calculated removal percentage being 14%. However, biodegradation of TFA- should lead to fluoride formation, which was not detected in the effluent of either SCAS unit. This confirms that TFA- was not biodegraded under the test conditions.

TFA^- brought into solution by NaTFA was not biodegraded in the OECD TG 302A (127 days) semi-continuous activated sludge (SCAS) closed bottle test.

This study may be used as reliable and suitable under CLP.

11.4.1.3 Enhanced ready biodegradability tests

To the knowledge of the dossier submitter there is no e*nhanced ready biodegradability tests* available.

11.4.1.4 BOD_5/COD

To the knowledge of the dossier submitter there is no *BOD_5/COD test* available.

11.4.1.5 Hydrolysis

The dossier submitter notes that in the REACH registration dossiers the assessment of the potential to hydrolyse is waived with the argumentation that "*based on the structure of TFA (extremely stable fluorine-carbon bond) there is no potential for hydrolysis and the haloacetates are not identified as chemical structure that hydrolyses by HYDROWIN (version 2.0). This assumption is confirmed by preliminary results presented in Emptage (1993) where no decarboxylation could be detected in water at 100°C and the stability of the substance in aqueous solution observed during the ecotoxicological and biodegradation tests performed"* with the TFA anion.

The dossier submitter supports this scientific argumentation.

11.4.1.6 Photochemical degradation

Not reported as to the knowledge of the dossier submitter there is no test result on *photochemical degradation* in air, water or soil available which would influence or change the conclusion on the hazard classification.

11.4.1.7 Field investigations and monitoring data (if relevant for the hazard class)

Not reported as to the knowledge of the dossier submitter there is no *field investigations and monitoring data (if relevant for the hazard class)* available which would influence or change the conclusion on the hazard classification.

11.4.1.8 Estimated data on persistence, including read-across

Not reported as to the knowledge of the dossier submitter there is no *estimated data on persistence, for example ones derived from computational/QSARs, read-across/grouping, etc.* available which would influence or change the conclusion on the hazard classification.

11.4.1.9 Other convincing scientific evidence

Not reported as to the knowledge of the dossier submitter there is no *other convincing scientific evidence* available which would influence or change the conclusion on the hazard classification.

11.4.2 Mobility under CLP Annex I, 4.4

Table 20: Summary of relevant information on mobility under CLP Annex I 4.4

Method/ Study type	Test material and purity	Results	Remarks/Reliability	Reference
scientific research study acc. to OECD TG 106	TFA^- brought into solution by the sodium salt (NaTFA) (no purity given)	-2.02 ≤ log Koc ≤ 0.19	2 (reliable with restrictions) assessed in the registration dossier under REACH dossier submitter: key study for use under CLP	Richey et al., 1997
OECD TG 106; EU Method C.18	TFA^- brought into solution by the potassium salt (KTFA) (with a purity of 16.7%)	1.27 ≤ log Koc ≤ 2.49	2 (reliable with restrictions) assessed in the registration dossier under REACH dossier submitter: key study for use under CLP	(anonymous, 2013)
OECD TG	TFA^- brought into	no log Koc values	2 (reliable with restrictions)	(anonymous,

Method/ Study type	Test material and purity	Results	Remarks/Reliability	Reference
106	solution by the sodium salt (NaTFA) (no purity given)	could be derived; three soils assessed; adsorption to soil 1: 0.1 – 0.2 % soil 2: 0.5 -.2.6 % soil 3: -1.7%	assessed in the registration dossier under REACH dossier submitter: supporting evidence under CLP	1992a)
OECD TG 106	^{14}C-TFA$^-$ brought into solution by sodium salt (NaTFA)	no log Koc values could be derived; three soils assessed K_{Foc} = 0.0001 mL/g (geometric mean)	study in Draft Renewal Assessment Report for FLUFENACET dossier submitter: supporting evidence under CLP	Moendel and Hein (2011)
OECD TG 312	^{14}C-TFA$^-$ brought into solution by sodium salt (NaTFA), (specific activity of 3.48 MBq/mg and radiochemical purity, determined by HPLC, >98%)	0.65 ≤ log Koc ≤ 1.05 (calculated by dossier submitter)	study in Draft Renewal Assessment Report for FLUFENACET dossier submitter: reliable and suitable for use under CLP	Hein (2014)
QSAR equation from Sabljic and Güsten (1995) class "organic acid"	ambiguous	log Koc = 0.982 (based on a log Kow value of 0.79 (mean of QSAR estimations))	QSAR estimation in the registration dossier under REACH dossier submitter: not suitable for use under CLP	Registrant (2024)
QSAR equation from Sabljic and Güsten (1995) class "organic acid"	TFA$^-$	log Koc = - 0.78 (based on a log Kow value of -4.1 (experimental data))	QSAR estimation by the dossier submitter: supporting evidence under CLP	dossier submitter (2024)

11.4.2.1 Soil adsorption/ desorption studies

OECD TG 106 study by Richey et al. (1997)

Batch equilibrium soil adsorption studies were conducted on 54 soils from 15 terrestrial sites, encompassing a wide range of soil and ecological conditions. Air-dried and sieved (2 mm) soils were characterized for chemical and physical parameters, including soil pH, total soil C and N, % organic matter (LOI), soil texture (% clay, % silt, % sand), exchangeable cations [calcium (Ca^+), magnesium (Mg_2^+), potassium (K^+), sodium (Na^+), iron (Fe_3^+) and aluminium (Al_3^+)], cation exchange capacity (CEC) and water-extractable anions [chloride (Cl-), nitrate (NO3-) and sulphate (SO4 2-), acid-oxalate, pyrophosphate and citrate-dithionite extractable Fe3+ and Al3+ fractions.

For batch equilibrium soil adsorption studies, conducted on each of the soils, 1:5 and 1:20 soil to solution ratio was used for organic and mineral soils, respectively. A range of sorbate concentrations (0, 2, 4, 7, 10, 20, 30, 40 µmol Na-TFA/L) were used for each soil. Suspension, consisting of soil and adsorbate was equilibrated on a reciprocating shaker for 24 h at 25°C. Aqueous fraction was analysed, using ion chromatography.

The difference between the amount of TFA initially in the adsorbate solution and the amount remaining after equilibration was determined to be the amount of TFA adsorbed by the soil. Langmuir and Freundlich adsorption isotherms were plotted and fitted to evaluate the extent of adsorption of TFA on all of the soils. The Langmuir adsorption maximum (Xt) was determined using OPTIMO, an optimization program that allows for the inclusion of uncertainty when fitting data to a Langmuir model. The distribution coefficient for adsorption (Kd) was determined for the special case of the Freundlich model where the Freundlich coefficient (n) = 1. When the adsorption data showed better fit with the Langmuir model, the linear portion of the isotherm was used to determine Kd. Kd was reproducible within a value of 0.09 L/kg for soils with low organic matter content (<17 %) and a value of 0.16 L/kg for soils with high organic matter content (>17 %) in replicate samples.

Table 21: Distribution of **soil samples** based on their **Kd values**

Number of soils	Kd values measured
3	> 10, with maximum of 20
8	2 - 10
15	0.5 - 2
8	0.1 – 0.5
20	no retention

According to OECD TG 106, equation (6), the distribution coefficient for adsorption (Kd) can be normalized with the organic carbon content of the soils, resulting in the calculation of the organic carbon normalized adsorption coefficient Koc. However, log Koc values are not reported by the authors of this study. As the criteria for mobility in CLP, Annex I, part 4.4.2.2.1 and 4.4.2.2.2 are defined as the soil adsorption coefficient (Koc) the dossier submitter calculated log Koc values (presented in Table 22) to enable a direct comparison with the CLP criteria for M and vM classification.

Table 22: Summary of soil properties and Kd values from Richey et al. (1997). The author reported values for 20 of in total 54 soils investigated. Koc and log Koc values were calculated by dossier submitter. The Total Organic Carbon was calculated by the dossier submitter based on the equation Organic matter (%) = Total organic carbon (%) x 1.72.

location; soil class/soil	**pH (0.01M CaCl2)**	**Organic matter (%)**	**Total Organic Carbon (%)**	**clay (%)**	**CEC (cmolc kg^{-1})**	**Xt (µmol kg^{-1})**	**Kd (L kg^{-1})**	**Koc**	**logKoc**
Bonanza Creek, AK; Inceptisol; /organic horizon	5.3	82.5	47.97	3	63.6	245	11	22.93	0.05
Bonanza Creek, AK; Inceptisol; /mineral horizon	4.61	30.3	17.62	5	12.1	10	0.86	4.88	-0.34
Coweeta, NC; Oxisol/A horizon (mesic)	4.16	9.4	5.47	3	2.1	26	1.5	27.45	0.08
Coweeta, NC; Oxisol/AB horizon (mesic)	4.2	6.2	3.60	9	1.4	47	0.68	18.86	0.02
Coweeta, NC; Oxisol/A horizon (frigid)	4.12	24.8	14.42	7	5.4	76	1.2	8.32	-0.16

location; soil class/soil	pH (0.01M CaCl2)	Organic matter (%)	Total Organic Carbon (%)	clay (%)	CEC (cmolc kg^{-1})	Xt (µmol kg^{-1})	Kd (L kg^{-1})	Koc	logKoc
Lysina, Czech Republic; Spodosol/Oa horizon	2.76	38.5	22.38	5	20.6	32	0.71	3.17	-0.58
Lysina, Czech Republic; Spodosol/E horizon	3.11	2.9	1.69	2	4	27	0.3	17.79	0.01
Lysina, Czech Republic; Spodosol/Bs1 horizon	3.34	7.9	4.59	6	11.6	30	0.54	11.76	-0.08
Lysina, Czech Republic; Spodosol/Bs2 horizon	3.81	7.1	4.13	7	7.3	45	0.53	12.84	-0.06
Lysina, Czech Republic; Spodosol/C horizon	4.05	4	2.33	1	3.7	60	0.6	25.80	0.07
Hubbard Brook, NH; Spodosol/Oa horizon	3.04	79.5	46.22	8	17.2	54	3.9	8.44	-0.16
Hubbard Brook, NH; Spodosol/E horizon	3.18	1.4	0.81	2	1	19	0.34	41.77	0.14
Hubbard Brook, NH; Spodosol/Bh horizon	3.58	17	9.88	4	12.5	21	0.68	6.88	-0.22
Hubbard Brook, NH; Spodosol/Bs1 horizon	4.01	8.1	4.71	6	2.3	18	0.42	8.92	-0.15
Hubbard Brook, NH; Spodosol/Bs2 horizon	4.7	0.5	0.29	3	0.05	13	0.17	58.48	0.19
Hubbard Brook, NH; Spodosol/wetland	5.04	75.2	43.72	2	17.7	180	5.8	13.27	-0.05
Lake Agassiz, MN; Histosol/peat core	3.22	93.3	54.24	1	17	260	20	36.87	0.12
Konza Prairie, KS; Mollisol/surface soil	5.85	10.1	5.87	27	23.1	28	1.5	25.54	0.07
Niwot Ridge, CO; Inceptisol/organic horizon	4.33	18.6	10.81	2	5.5	17	0.19	1.76	-2.03
Niwot Ridge, CO; Inceptisol/mineral horizon	4.71	6.1	3.55	11	10.4	4	0.32	9.02	-0.14

The authors of this study reported for only 20 of the 54 soils, from 7 of the 15 terrestrial sites, the soil characteristics and the Kd values (presented in Table 22). The dossier submitter assesses this selection as representative for the whole study as it also includes two of the three highest Kd values (see Table 22) including the maximum. The peat core sample from Lake Agassiz Peatlands with an organic matter of 93.3 %, were found to adsorb the highest concentrations of TFA^-, resulting in the maximum Kd value of 20 and a log Koc value of 0.12 (presented in Table 22).

The maximum log Koc value of 0.19 results from the Hubbard Brook Spodosol with an organic matter of only 0.5 % and a Kd value of only 0.17.

It must also be noted that from the 54 soils investigated no adsorption of TFA^- at all was observed in 20 soils. The authors of this study did not report the soil characteristics for these 20 soils. However, for a Kd value of 0 no Koc value can be calculated anyway. That for these 20 additional soils no adsorption at all was observed clearly supports the overall conclusion that TFA^- has an extremely low adsorption potential.

The study clearly proofs that TFA^- has an extremely low adsorption potential with 20 reliable log Koc values between -2.02 and 0.19.

This study may be used as key study for use under CLP and the test result can be applied directly to the criteria set out in sections 4.4.2.1 and 4.4.2.2.

OECD TG 106 study by anonymous (2013)

A batch equilibrium study according to OECD TG 106 and EU Method C.18 was reported by the lead registrant (anonymous, 2013). The study used a reaction mass of: potassium trifluoroacetate (2923-16-2) potassium trifluoromethanesulfinate (41804-89-1) in water (EC 911-467-3). With that, TFA^- brought into solution by the inorganic salt KTFA constituent.

Three soils and two mixtures of these three soils were used. Soil samples were collected in China. Samples were sieved (2 mm) and air dried (20 – 25 °C). For subsequent calculations the authors used the mass of soil, referring to oven dry mass. Properties of the soils used in the batch equilibrium studies is reflected in Table 23**Fehler! Verweisquelle konnte nicht gefunden werden.**. It is note by the lead registrant, that the clay content was not determined.

Table 23: Properties of soil used for adsorption/desorption batch equilibrium in study (anonymous, 2013) aligned with the distribution coefficient for adsorption (Kd) and the organic carbon normalized adsorption coefficient (Koc). The log Koc values were calculated by dossier submitter.

	Soil Types	pH	organic carbon (%)	texture	Kd (cm^3/g)	Koc	log Koc
A	Black Soil (Hei LongLongjiang)	6.15	2.35	Silt Loam	0.440	18.729	1.27
B	Red Soil (Jiangxi)	4.57	0.71	Clay loam	2.195	309.184	2.49
C	Paddy Soil (Jiangsu)	6.58	3.2	Loam	0.953	29.776	1.47
D	Red Soil/Black Soil (1:1)	5.36	1.21	Clay loam/ Silt Loam	0.992	81.998	1.91
E	Red Soil/Paddy Soil (1:1)	5.58	1.64	Clay loam/ Loam	1.027	62.598	1.80

In a preliminary test (Tier 1 of OECD TG 106), two soil types (A and B) and three soil/solution ratios (1:1; 1;5; 1:25) were adopted over a 48-h period of mixing. The pre-testing revealed that optimal soil/aqueous ratio is 1:1, the appropriate adsorption equilibrium time is 48 h.

Tier 1 tested the adsorption kinetics at one concentration of test substance. Five soils were used. The equilibration time (48h), the soil/solution ratio (1:1), the weight of the soil sample (10g), the volume of the aqueous phase in contact with the soil (10mL) and the concentration of the test substance in the solution (1mg/L) were chosen based on the preliminary study results. Analysis was performed approximately after 2, 4, 8, 24 and 48 h contact time. The experiment was run at least in duplicates, blank and control samples were considered in the study design. The distribution coefficient Kd and Koc at equilibrium was calculated.

The study results are summarised as following by the lead registrant: *The adsorption coefficient (Kd) values of* [TFA^-] *in the fives soils were 0.440, 2.195, 0.953, 0.992 and 1.027 cm^3/g, respectively and*

the Koc values of [TFA^-] *were 18.729, 309.184, 29.776, 81.998 and 62.598 cm^3/g in the above soils respectively. These results show that* […] [TFA^-] *has a low adsorption onto the soils tested.*

The results of desorption kinetics show that the desorption coefficient (Kdes) values of [TFA^-] *in the five soils were 3.733, 3.226, 2.744, 3.682 and 3.056 cm^3/g, respectively and the Kdes values of* [TFA^-] *were 1.358, 1.909, 2.505, 1.874 and 2.410 cm^3/g in above soils respectively; in addition, the max percentage of desorption (Dti) of* [TFA^-] *is 27.3% in above soils respectively and the total desorption is less than 75% of the amount adsorbed. Therefore, the adsorption of* [TFA^-] *on five soils has a low reversibility.*

Based on the organic carbon normalized adsorption coefficients Koc the dossier submitter calculated the log Koc vales as presented in Table 23**Fehler! Verweisquelle konnte nicht gefunden werden.**.

The study clearly proofs that TFA^- has an extremely low adsorption potential with 5 reliable log Koc values between 1.27 and 2.49.

This study may be used as key study under CLP and the test result can be applied directly to the criteria set out in sections 4.4.2.1 and 4.4.2.2.

<u>OECD TG 106 study by anonymous (1992a)</u>

In the GLP OECD TG 106 study by anonymous (1992a) the adsorption of TFA^- brought into solution by NaTFA was investigated in three soils. The study reports the adsorption behaviour after 16 hours adsorption phase as a mass balance (Percentage of TFA^- adsorbed (%)). The mass balance was calculated by the authors considering the TFA^- mass in water layers of the soil-less control samples and in water phase after adsorption phase. In soils itself, TFA^- concentration were not analysed.

The lead registrant considered the study as reliable with restriction (reliability 2) and evaluated the study as the key study. This is in contrast to several deviations to the OECD TG 106 guideline reported:

- the analysis of the aqueous phase for the test substance was done within 5 days, instead of 24 hours.
- the eluent is a 3.5 mM $NaHCO_3$ solution instead of a 35 mM $NaHCO_2$.
- details on the analytical method, including the detection limit is missing in the study summary
- insufficient details on the Tier 1 level e.g. description of selection of optimal soil/solution ratios and estimation of the equilibrium time, instead an adsorption equilibrium duration of 16 hours of was used
- only three different soils instead of the recommended five different soils were tested
- none of the soils used follows the recommendations in table 1 in the OECD TG 106. However, the selected soils originate from a temperate geographical zone, as requested in the OECD TGD 106.

The dossier submitter considers the limitations of the study summary and the deviation from the OECD TG 106 as substantial and is unable to assign a reliability.

The authors reported 0.1 – 0.2 % TFA^- adsorbed to Soil 1, 0.5 – 2,6 % TFA^- adsorbed to Soil 2 and a negative value of -1,7 % TFA^- adsorbed to Soil 3.

Although, calculation of the distribution coefficient for adsorption (Kd) and the organic carbon normalized adsorption coefficient (Koc) is theoretically possible based on the adsorption mass

balance following OECD TG 106, equation 5 and 6, this would require uniquely assigned data descriptions which are not available from the study summary reported in the lead registration dossier.

Also, the study summary confused e.g. wet soil weight with dry weight of soil, which is an essential parameter for the calculation of Kd values.

Although no Koc values could be derived, the study clearly proofs that TFA^- has an extremely low adsorption potential.

This study may be used as supporting evidence under CLP.

OECD TG 106 study by Moendel and Hein (2011)

Moendel and Hein (2011) investigated in an OECD TG 106 study the adsorption/desorption of ^{14}C-TFA^- brought into solution by ^{14}C-NaTFA in five different soils. The study was assessed by Poland in the scope of application for extension of the approval period for the active substance flufenacet according to Regulation (EU) No 11907/2009 (DRAR, 2017) as TFA is a major soil degradation product of flufenacet.

The examination of the adsorption of TFA^- onto soil at equilibrium showed that the test compound did not sorb onto soil to any extent (in most cases adsorption at equilibrium was shown to be negative). Therefore, it was proposed to use for TFA^- the value of Kf = 0 mg/L and consider the adsorption process as fully linear, hence 1/n = 1.0.

Theoretically, log Koc values for the soils investigated in the study could be calculated based on equations (5) and (6) of the OECD TG 106. However, as reflected in the DRAR, the amount of adsorbed TFA^- onto soil was reported to be negative. Consequently, no meaningful calculations are possible for the distribution coefficient for adsorption (Kd), the organic carbon normalized adsorption coefficient (Koc) and subsequently for the log Koc.

Although no Koc values could be derived, the study clearly proofs that TFA^- has an extremely low adsorption potential.

This study may be used as supporting evidence under CLP.

11.4.2.2 Experimental information from soil column leaching studies

OECD TG 312 study by Hein (2014)

In an OECD TG 312 study by Hein (2014), the leaching of TFA as one of the degradation products of Flufenacet through a soil column was examined.

^{14}C-TFA^- brought into solution by ^{14}C-NaTFA was tested for leaching potential on four different soils. The study's design was such to enable the determination of Kd and Koc values for the test item. This was not possible in a previous batch equilibrium adsorption study by Moendel and Hein (2011), which demonstrated that TFA^- basically has no adsorption potential at all. Details on the study design are given in the DRAR (2017). Kd and Koc values are presented in

Table 24.

Table 24: Overview of Kd and Koc values from Hein (2014) and log Koc values calculated by dossier submitter, if log Koc > 1.

Soil geographical descriptor	elution with 392 mL artificial rain (Study design A)			elution with 984 mL artificial rain (Study design B)		
	Kd [mL/g]	Koc [mL/g]	log Koc	Kd [mL/g]	Koc [mL/g]	Log Koc
Laacherhof AXXa	0	0	n.d.	0.1	4.5	0.65
Dollendorf	0	0	n.d.	0	0	n.d.
Höfchen am Hohenseh	0	0	n.d.	0.2	11.3	1.05
Laacherhof Wurmwiese	0	0	n.d.	0.2	7.1	0.85

The study with three reliable log Koc values between 0.65 and 1.05 clearly proofs that TFA^- has an extremely low adsorption potential.

This study is reliable and suitable for use under CLP and the test result could be applied directly to the criteria set out in sections 4.4.2.1 and 4.4.2.2.

11.4.2.3 Estimated data on mobility including read-across

QSAR estimation of Koc value by the lead registrant

The registration dossier by the lead registrant states concerning computational/QSAR data: "*By default, EUSES and CHESAR use QSAR calculation according to equation from Sabljic and Güsten (1995), as reported in the EU TG (2003), using the class of non-hydrophobic chemicals. In the case of TFA, the class "organic acid" is more relevant. Therefore the Koc is calculated as follows:*

*log Koc = 0.6 * log Kow + 0.32, with log Kow = 0.79"* (Registrant, 2024).

Based on a log Kow value of 0.79, the log Koc value is calculated to be 0.982.

It should be noted that the above log Kow value of 0.79 is a calculated average. It includes a total of 10 Kow values estimated by QSAR for the non-ionic TFA form in the range 0.21-1.78. The arithmetic mean of these 10 results is 0.79 +/-0.48.

The reported average log Kow value does not take into account an available experimental value of logPow = -4. Therefore, the calculated log Koc value of 0.982 represents the non-ionic form of TFA. As discussed earlier (section 11.4.), the non-ionic form of TFA is not relevant for the environmental pH range 4.0 to 9.0 where the substance is dissociated and present in its anionic form.

Therefore, the results of this QSAR calculation are considered not reasonable and are not suitable under CLP.

QSAR estimation of Koc value by the dossier submitter

Dossier submitter follows the lead registrant's consideration to use a QSAR calculation based on Sabljic and Güsten (1995) with the equation developed for the class "organic acid".

However, in the view of the dossier submitter, an experimentally based Kow obtained under environmentally relevant pH conditions should be used for the calculation as this represents TFA^-. The lead registration dossier describes an experimentally determined Pow value of -4.1 based on an OECD TG 107 (anonymous, 1991).

Using the equation

log Koc = 0.6 * log Kow + 0.32

Based on a log Kow value of -4.1 the log Koc value is calculated to be -0.78.

This may be used as supporting evidence under CLP and the estimated values could be applied directly to the criteria set out in sections 4.4.2.1 and 4.4.2.2.

11.4.2.4 Water solubility

see section 7.**Fehler! Verweisquelle konnte nicht gefunden werden.**

11.4.3 Toxicity under CLP Annex I. 4.4

Following the assessment in section 10.10 of this report, the substance is toxic for reproduction category Repr. 1B (H360fD; Suspected of damaging fertility; may damage the unborn child).

11.4.4 Short summary and overall relevance of the provided information on the PMT and vPvM properties

11.4.4.1 Short summary and overall relevance of the provided information on persistence

TFA^- shows no degradation in all four reliable and relevant aerobic experimental studies assessed: a OECD TG 301D (anonymous 1992b), a OECD TG 302A (anonymous 1992c), a OECD TG 307 (Eckermann 2012a) and a scientific research study with a laboratory water sediment system and a field aquatic microcosm study part (Ellis et al. 2001). All four studies are utilised as key studies by the lead registrants under REACH or by the reporting member state (RMS) under the Commission Regulation (EU) NO 1107/2009 within e.g. the Draft Renewal Assessment Report for the pesticide flufenacet and consequently are also utilised as reliable and suitable or as key studies for use under CLP.

To the knowledge of the dossier submitter there is no reliable and relevant evidence available e.g. from hydrolysis, photochemical degradation, field investigations, monitoring data, estimated data including read-across and other convincing scientific evidence, which conflicts with the conclusion that TFA^- must be considered ‘very persistent’ in the environment under CLP.

In contrast, the OECD TG 301D screening test and the OECD TG 302A inherent test are reliable and suitable under CLP and give already by themselves a strong scientific indication that TFA^- must be considered ‘very persistent’ in the environment.

Further, TFA^- did not degrade in fresh water, in fresh water sediment and in soil and consequently no or no meaningful $DegT_{50}$ (12°C) could be derived from the experiments and it can be assumed that the rate constant in all three investigated environmental compartments is not significantly different from zero. Consequently, it can be concluded with a high degree of certainty that TFA^- must be considered to fulfil the ‘very persistent’ criterion (vP) under CLP Annex I, 4.4.2.2.1 for all three investigated compartments: (a) fresh water, (b) fresh water sediment and (c) soil.

11.4.4.2 Short summary and overall relevance of the provided information on mobility

TFA^- shows an extremely low adsorption potential in one valid and reliable QSAR estimation and in all valid and reliable experimental studies evaluated: one scientific research study according to OECD TG 106, three OECD TG 106 studies and one OECD TG 312 study.

To the knowledge of the dossier submitter there is no reliable and relevant evidence available e.g. from field investigations, monitoring data, estimated data including read-across and other convincing scientific evidence, which conflicts with the conclusion that TFA^- must be considered as 'very mobile' in the environment under CLP.

Although no Koc values could be derived, already two OECD TG 106 studies (anonymous,1992a & Moendel and Hein, 2011) clearly proof that TFA^- possess an extremely low adsorption potential.

Numerical log Koc values were derived from three experimental studies and one QSAR estimation. The QSAR estimation by the dossier submitter calculated a log Koc value of -0.78 which may be used as supporting evidence under CLP. The OECD TG 312 study by Hein (2014) with three reliable log Koc values between 0.65 and 1.05 provides a clear proof that TFA^- has an extremely low adsorption potential. This study is reliable and suitable for use under CLP and the test result could be applied directly to the criteria set out in sections 4.4.2.1 and 4.4.2.2, however not used as key study under CLP by the dossier submitter.

The two OECD TG 106 studies utilised as key studies for use under CLP result in 20 reliable log Koc values between -2.02 and 0.19 (Richey et al. 1997) and in 5 reliable log Koc values between 1.27 and 2.49 (anonymous 2013).

As the lowest log Koc values in these two independent OECD TG 106 studies are -2.02 and 1.27 it can be concluded with a high degree of certainty that TFA^- must be considered to fulfil the 'very mobile' criterion (vM) under CLP Annex I, 4.4.2.2.2.

11.4.4.3 Short summary and overall relevance of the provided information on toxicity

Following the assessment in section 10.10 of this report, the substance is toxic for reproduction category Repr. 1B (H360fD; Suspected of damaging fertility; may damage the unborn child and consequently fulfil the 'toxicity' criterion (T) under CLP Annex I, 4.4.2.1.3.(b).

11.4.5 Comparison with the CLP criteria and conclusion on classification and labelling for PMT/vPvM hazards

Table 25: Comparison with CLP criteria for persistence, mobility and toxicity

	Criteria fulfilled	TFA^-	Conclusion
persistence criteria CLP Annex I, part 4.4.2.1.1	(b) the degradation half-life in fresh […] water is higher than 40 days; (d) the degradation half-life in fresh […] water sediment is higher than 120 days and (e) the degradation half-life in soil is higher than 120 days.	(b) No DT_{50} value in fresh water could be derived as no degradation of TFA^- was observed in the 120 days laboratory water sediment system and in the 365 days field aquatic microcosm study part (Ellis et al. 2001) and consequently the degradation half-life in fresh water at 12°C is higher than 40 days. (d) No DT_{50} value in fresh water sediment could be	**Persistent (P)**

	Criteria fulfilled	TFA^-	Conclusion
		derived as no degradation of TFA^- was observed in the 120 days laboratory water sediment system (Ellis et al. 2001) and consequently the degradation half-life in fresh water sediment at 12°C is higher than 120 days. (e) No meaningful DT_{50} value in soil could be derived as no degradation of TFA^- was observed in the 120 days study (Eckermann (2012a) and consequently the degradation half-life in soil at 12°C is higher than 120 days.	
mobility criteria, CLP Annex I, part 4.4.2.1.2	For an ionisable substance, the mobility criterion shall be considered fulfilled when the lowest log Koc value for pH between 4 and 9 is less than 3	TFA^- is an ionisable substance and the lowest log Koc values in two independent OECD TG 106 studies are -2.02 (Richey et al. 1997) and 1.27 (anonymous 2013) and both are less than 3.	**Mobile (M)**
toxicity criteria, CLP Annex I, part 4.4.2.1.3	(b) the substance meets the criteria for classification as toxic for reproduction (category 1A, 1B or 2) according to Section 3.7	toxic for reproduction category Repr. 1B (see section 10.10 of this dossier)	**Toxic (T)**

According to the CLP regulation 4.4.2.1. a substance shall be considered a PMT substance when it fulfils the persistence, mobility and toxicity criteria set out in CLP, Annex I, part 4.4.2.1.1, 4.4.2.1.2 and 4.4.2.1.3. and assessed according to CLP, Annex I, part 4.4.2.3.

> The substance fulfils the criteria in CLP, Annex I, part 4.4.2.1.1. Persistence as it meets the P criteria in three situations: (b) the degradation half-life in fresh […] water is higher than 40 days; (d) the degradation half-life in fresh […] water sediment is higher than 120 days and (e) the degradation half-life in soil is higher than 120 days.
>
> The substance fulfils the criteria in CLP, Annex I, part 4.4.2.1.2. Mobility as it meets the M criterion as it is an ionisable substance and the lowest available log Koc value is less than 3.
>
> The substance fulfils the criteria in CLP, Annex I, part 4.4.2.1.3. Toxicity, as it meets the T criteria in the situation (b) the substance meets the criteria for classification as toxic for reproduction (category 1A, 1B or 2) according to Section 3.7 of this dossier.

Consequently, Trifluoroacetic Acid … % (EC Number: 200-929-3, CAS Number: 76-05-1) fulfils the classification criteria for PMT and the hazard Statement "*EUH450: Can cause long-lasting and diffuse contamination of water resources*".

Table 26: Comparison with CLP criteria for very persistent and very mobile

	Criteria fulfilled	TFA^-	Conclusion
persistence criteria, CLP Annex I, part 4.4.2.2.1	(a) the degradation half-life in fresh […] water is also higher than 60 days; (b) the degradation half-life in fresh […] water sediment is also higher than 180 days (c) the degradation half-life in soil is also higher than 180 days.	(a) No DT_{50} value in fresh water could be derived as no degradation of TFA^- was observed in the 120 days laboratory water sediment system and in the 365 days field aquatic microcosm study part (Ellis et al. 2001) and consequently the degradation half-life in fresh water at 12°C is higher than 60 days. (b) No DT_{50} value in fresh water sediment could be derived as no degradation of TFA^- was observed in the 120 days laboratory water sediment system (Ellis et al. 2001) and consequently the degradation half-life in fresh water sediment at 12°C is higher than 180 days. (c) No meaningful DT_{50} value in soil could be derived as no degradation of TFA^- was observed in the 120 days study (Eckermann (2012a) and consequently the degradation half-life in soil 12 °C is higher than 180 days.	**very Persistent (vP)**
mobility criteria, CLP Annex I, Part4.4.2.2.2	For an ionisable substance, the mobility criterion shall be considered fulfilled when the lowest log Koc value for pH between 4 and 9 is less than 2	TFA^- is an ionisable substance and the lowest log Koc values in two independent OECD TG 106 studies are -2.02 (Richey et al. 1997) and 1.27 (anonymous 2013) and both are less than 2.	**very Mobile (vM)**

According to the CLP regulation Section 4.4.2.2. a substance shall be considered a vPvM substance when it fulfils the persistence and mobility criteria set out in CLP, Annex I, part 4.4.2.2.1 and 4.4.2.2.2 and assessed according to CLP, Annex I, part 4.4.2.3.

> The substance fulfils the criteria in CLP, Annex I, part 4.4.2.2.1. Persistence as it meets the vP criteria in three situations: (a) the degradation half-life in fresh […] water is also higher than 60 days; (b) the degradation half-life in fresh […] water sediment is also higher than 180 days and (c) the degradation half-life in soil is also higher than 180 days.
>
> The substance fulfils the criteria in CLP, Annex I, part 4.4.2.2.2. Mobility as it meets the vM criterion as it is an ionisable substance and the lowest available log Koc value is less than 2.

Consequently, Trifluoroacetic Acid … % (EC Number: 200-929-3, CAS Number: 76-05-1) fulfils also the classification criteria for vPvM and the hazard statement "*EUH451: Can cause very long-lasting and diffuse contamination of water resource*".

11.5 Hazardous to the ozone layer

Not assessed in this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier

13 ADDITIONAL LABELLING

Additional labelling is not considered relevant for this substance.

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