SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Carbon Disulphide

EC No 200-843-6

CAS RN 75-15-0

Evaluating Member State(s): France

Dated: March 2022

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2013

Before concluding the substance evaluation a Decision to request further information was issued on: 30 November 2015
Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance, carbon disulphide (CS_2; EC number 200-843-6) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected endocrine disruptor (ED);
- Suspected CMR (Reproductive toxicity);
- High aggregated tonnage;
- High release to the environment;
- High worker exposure.

During substance evaluation, an additional concern was identified:

- Postnatal development related to potential neurodevelopmental and cardiovascular toxicity.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Carbon disulphide is on the list of indicative EU occupational exposure limit values established in the third list of Directive 2009/161/EU (on Protection of health and safety of workers from the risks related to chemical agents at work).

In this directive, a long-term exposure limit (8 hours time-weighted average (8-h TWA)) for carbon disulphide is set at **15 mg/m³** (equivalent to 5 ppm) with a skin notation.
A binding occupational exposure limit (OEL) has also been established in France for the Substance at the same level on the same basis.

No other ongoing processes were identified.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the Substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

Based on the evaluation of the Substance and its conclusion, the eMSCA will prepare a further risk management option analysis (RMOA) in which the appropriate options will be clarified and the most relevant Risk Management Measures (RMMs) identified (see below).

4.1.1. Harmonised Classification and Labelling

Carbon disulphide is an industrial chemical that has a **current Annex VI entry** in the CLP regulation (EC 1272/2008). Three main hazards were identified following exposure to carbon disulphide: neurotoxicity, cardiotoxicity and reproductive toxicity. These findings are already covered by the classification of carbon disulphide as STOT RE 1 including specific concentration limits. Nevertheless, nervous system, cardiac system would need to be indicated in the classification as specific target organs.

For reproductive toxicity since effects on both fertility and pre-/post-natal development are either confirmed or new, classification as Repr. 2 (H361df) is still warranted. Reproductive toxicity in Category 1B may need to be considered and will be discussed as a first step for potential further RMM in the RMOA.

In addition, classification as Acute Tox. 4, H332 would need to be added in the harmonised classification.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Carbon disulphide is a known neurotoxicant that can induce damages in the central nervous system (CNS) and peripheral nervous system (PNS) in experimental animals and in humans. In addition, carbon disulphide induce cardiovascular toxicity in animals and humans. The substance is classified as STOT RE 1, in high potency class, under the CLP Regulation.

Serious health concern has been identified for carbon disulphide which is a highly potent neurotoxicant and cardio-vascular toxicant. Identification under Article 57(f) could be considered as a first step of further RMMs.

It may be noted that two out of the four exposure scenarios (intermediate uses and manufacture) of the substance would not be covered by authorisation. Nevertheless, authorisation would favour substitution and a reduction of worker exposure.

At its 20th meeting (October 2021), the Endocrine Disruptor Expert Group (ED EG) supported the outcome of this evaluation that, based on the data presented in this document including the EOGRTS requested under SEv, the substance does not meet the criteria to be identified as an endocrine disruptor.

4.1.3. Restriction

There is a current indicative OEL of 15 mg/m^3 (5 ppm) and a biological limit value of 1.5 mg thiazolidine-2-thione-4-carboxylic acid (TTCA)/g creatinine for carbon disulphide. The OEL is based on the neurotoxicity and cardiotoxicity potential of carbon disulphide (SCOEL, 2008). The existing indicative OEL may not be protective enough for these effects and also not for potential effects on the reproductive system and the identified effect on thyroxine (T4) level disturbance.
As summarised in section 7.9.9, using a revised long-term DNEL of 2 ppm (3 mg/m^3) for inhalation, the Risk characterisation ratios (RCRs) were above the trigger value of 1.0 for the use of the substance in the manufacture of regenerated cellulose the use of the substance as an intermediate.

Moreover, biomonitoring studies (Table 33 in section 7.12 of the report) indicate that the total exposure may exceed the BLV (TTCA measurements) for the use of the substance in regenerative cellulose. This suggests that the risk is not adequately controlled compared to the current indicative OEL.

Therefore, a restriction for these specific uses may be considered as a first step for further RMM in the RMOA.

4.1.4. Other EU-wide regulatory risk management measures

A revised, lower binding EU-wide occupational exposure limit (BOEL) appears to be also an option to further protect workers. The current indicative OEL is insufficiently protective of potential reproductive toxicity effects in human and may also be insufficiently protective of subtle neurotoxicity and cardiovascular effects that may already be indicative of adverse effect.

It is noted that a BOEL would cover all the exposure scenario of the substance. The revision of the BOEL instead of a restriction will be considered as a first step for further RMM in the RMOA.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
RMOA	2022 at the earliest	France

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance, carbon disulphide (CS2; EC number 200-843-6) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected endocrine disruptor (ED);
- Suspected CMR (Reproductive toxicity);
- High aggregated tonnage;
- High release to the environment;
- High worker exposure.

During substance evaluation, an additional concern was identified:

- Postnatal development related to potential neurodevelopmental and cardiovascular toxicity.

Table 3 below summarises the evaluated endpoints and conclusions from the eMSCA.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Potential ED	Concern confirmed Findings, possibly ED related, were observed in experimental animals and in humans. Nevertheless, neurotoxicity and excessive oxidative stress induced by the Substance may also be a plausible non-endocrine MoA for the observed effects. In case additional/future data become available showing a plausible link between endocrine activity and adverse effects, this conclusion should be reconsidered. No further action for possible ED properties as agreed at the 20th ED EG meeting (October 2021).
Suspected CMR (Reproductive toxicity) Neurodevelopmental toxicity	Concern partly confirmed The effects observed in the newly generated EOGRTS and in the updated data on developmental toxicity confirm the need to classify the substance as at least Repr. 2, H361fd. Stronger classification cat. 1B will be considered as an option in the RMOA. Moreover, neurotoxicity was observed at similar dose levels in pups and in parental animals. A higher sensitivity of pups to CS2 toxicity was not observed in the EOGRTS. Therefore, no further action is proposed.
High aggregated tonnage High release to environment High worker exposure	Concern confirmed Widespread uses of the substance and high worker exposure have been identified. Further action at EU level would be needed to further reduced worker exposure and adequately manage the risks identified for some scenarios. The updated environmental exposure assessment does not lead to unacceptable risk provided that the proposed risk mitigation measures are applied.

7.2. Procedure

Carbon disulphide was included in the Community rolling action plan (CoRAP) for evaluation in 2013.

All the physico-chemical, human health and environmental hazards that were part of the registration dossier were evaluated.

Based on the evaluation of the available data, the evaluating MSCA concluded that there was a need to request further information to clarify the concerns related to substance identity, worker and environmental exposure, ED, reproductive and neurodevelopmental toxicity potential. Therefore, eMSCA prepared a draft decision to request further information. The decision was submitted to ECHA and was agreed by the member state Committee in October 2015.

The substance evaluation conclusion was prepared based on the updated registration dossier from December 2019. In addition, a literature review from 2013 to 2019 was performed on pubmed.

7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Carbon disulphide
EC number:	200-843-6
CAS number:	75-15-0
Index number in Annex VI of the CLP Regulation:	006-003-00-3
Molecular formula:	CS_2
Molecular weight range:	76.141
Synonyms:	Methanedithione

Type of substance: Mono-constituent

Structural formula:

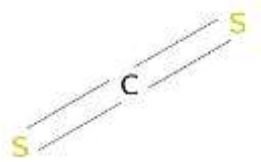

Table 5b

Constituents	Typical concentration	Concentration range	Remarks
Carbon disulphide *EC n°* 200-843-6 *CAS RN* 75-15-0	99.9% (w/w)	99.5 - 100.0% (w/w)	-

The composition submitted by the registrant is considered as monoconstituent according to REACH guidance for identification and naming of substances.

Analytical information is provided (Utraviolet/Visible, Infrared, Nuclear magnetic resonance, Mass spectra and Gas chromatography) to confirm the composition and the structure of the substance.

7.4. Physico-chemical properties

Table 6

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	**Value used for SEV:** Colorless and odourless liquid
Melting / freezing point	**Value used for SEV:** Below -76.0 °C *Melting point was determined according to the test procedure OECD TG 102.*
Boiling point	**Value used for SEV:** 42.2°C at 101.3 kPa *Boiling point was determined according to the test procedure OECD TG 103.*
Relative density	**Value used for SEV:** 1.2635 at 20.0 °C *Relative density was determined according to the test procedure OECD TG 109.*
Granulometry	Not applicable. Substance is marketed or used in a non solid or granular form.
Vapour pressure	**Value used for SEV:** 274 hPa at 25.0 °C (extrapolated) and 412 hPa at 31.0 °C (measured) *Vapour pressure (extrapolated and measured) was determined according to the test procedure OECD TG 104.*
Partition coefficient n-octanol/water (Log Kow)	**Value used for SEV:** Log Kow (Pow): 2.7 at 25.0 °C and pH 6.6 *Partition coefficient was determined according to the test procedure OECD TG 117.*
Water solubility	**Value used for SEV:** 2.9 g/L at 20.0 °C and at pH 5.9 *Water solubility was determined according to the test procedure OECD TG 105.*
Solubility in organic solvents / fat solubility	*Handbook Merck Index 14th specifies that CS_2 is miscible in chloroform, ether, anhydrous methanol, ethanol, benzene, CCl4, oils. This data is consistent with the data provided in the peer reviewed CRC Handbook 86th ed.*
Surface tension	**Value used for SEV:** 71.9 mN/m at 20.0 °C and 1000 mg/L *Surface tension was determined according to the test procedure OECD TG 115.* CS_2 is not surface active.
Flash point	**Value used for SEV:** < -20.0 °C at 990 hPa *Flash point of CS_2 was determined according to the test procedure EC A.9 method.* CS_2 is classified as highly flammable liquid according to CLP regulation (See Flammability point above).
Autoflammability/self-ignition temperature	**Value used for SEV:** 102.0 °C at 1013.0 hPa *Auto-ignition temperature of CS_2 was determined according to the test procedure EC A.15 method.*

	CS_2 is not auto-flammable at ambient temperature.
Flammability	**Value used for SEV:** Highly flammable *According to CLP regulation, CS_2 is classified as highly flammable as its flash-point is < -20.0 °C ant its boiling point is 42.2 °C.* **Classification: Flam. Liquid 2 (Hazard statement: H225: Highly flammable liquid and vapour.)**
Explosive properties	**Value used for SEV:** Non explosive *Explosivity of CS_2 was evaluated according to the test procedure EC A.14 method.*
Oxidising properties	**Value used for SEV:** Non oxidizing properties *In accordance with column 2 of REACH Annex VII, the oxidising properties do not need to be conducted as the substance is highly flammable.*
Stability in organic solvents and identity of relevant degradation products	*In accordance with column 1 of REACH Annex IX, the stability in organic solvents does not need to be tested as the stability of the substance is not considered as critical.*
Dissociation constant	*In accordance with column 2 of REACH Annex VII, the dissociation constant does not need to be performed as the substance does not contain any ionic structure.* *Moreover, based on the structure of the substance no functional groups with relevant acidic or basic character can be found. No significant dissociation is expected.*
Viscosity	**Value used for SEV:** Kinematic viscosity at 20.0 °C: 0.363 mm²/s (static) *Data on viscosity of CS_2 come from Handbook Merck Index 13th.*
Conversion factor (SCOEL, 2008)	25°C, 1 bar 1 mg/m^3 = 0.317ppm 1 ppm = 3.16mg/m^3

7.5. Manufacture and uses

7.5.1. Quantities

Table 7

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☒ 100,000 t – 1 000 000 t	☐ Confidential

7.5.2. Overview of uses

Table 8

USES	
	Use(s)
Manufacture	
Use at industrial sites	
Manufacturing of regenerated cellulose	Sector of use : manufacture of textile, scientific research
Uses as intermediate	Product category: biocidal products, plant protection products, polymer preparations and compounds, products such as ph-regulators, flocculants, precipitants, neutralisation agents
Formulation as a solvent in industrial processes	Product category: polymer preparation and compounds

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 9

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)						
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**	
006-003-00-3	Carbon disulphide	200-843-6	75-15-0	Flam. Liq. 2 Repr. 2 STOT RE 1** Skin Irrit. 2 Eye Irrit. 2	H225 H361fd H315 H319 H372	Repr. 2; H361fd: C ≥ 1 % STOT RE 1; H372: C ≥ 1 % STOT RE 2; H373: 0,2 % ≤C < 1 %

** asterisks were inserted in the Annex VI entry following the translation from DSD to CLP (translation from R48/23): they do refer to "other route of exposure cannot be excluded".

7.6.2. Self-classification

In the registration(s) the following self-classifications are proposed in addition to the current harmonised classification:

Acute Tox. 4, H332
STOT RE 1, H372 (Cardiovascular system, eye, nervous system)

7.7. Environmental fate properties

7.7.1. Degradation

Carbon disulphide is considered as hydrolytically stable. Carbon disulfide will mainly partition to the air compartment. The main atmospheric degradation products identified are COS and SO_2. However, the contribution of carbon disulphide degradation in the production of atmospheric SO_2 and COS is considered negligible compared to the other anthropogenic and natural sources of COS and SO_2 (BUA report 83, 1991; Klimont *et al.*,

2013). The phototransformation half-life is between 5 to 15 days. The biodegradation of carbon disulphide in water was >80 % after 28 days of exposure in closed bottle screening test (OECD TG 301D), therefore carbon disulphide is readily biodegradable.

7.7.2. Environmental distribution

The adsorption coefficient log Koc of carbon disulphide was estimated to be 1.5, i.e. Koc = 34 L/Kg according to a test conducted to the OECD TG 121. This estimation indicates a low adsorption potential of carbon disulphide on organic particles.

The Henry's law constant, calculated from SimpleTreat V4 is 384 Pa. m3/mol at 20°C, indicating high volatility of carbon disulphide from water. Then, once released into the environment, carbon disulphide would distribute over time predominantly into air.

7.7.3. Bioaccumulation

The bioaccumulation potential of carbon disulphide is expected to be low. Carbon disulphide has a log Kow value lower than 3 (it was measured to be 2.7, see section 6.4). The expected low bioconcentration potential is in line with the observed and estimated bioconcentration factors. Estimated BCF, based on Kow is of 28. This was confirmed by literature values that range between 6 and 60.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

Two studies are available for the short-term acute toxicity of carbon disulphide to fish. Both studies were carried out in closed systems with *Poecilia reticulata*. The lowest reliable LC50 for freshwater fish is 3 mg/L (unpublished report, 1991).

A short term fish study on early life stage of *Brachydanio rerio* was conducted (similar to OECD guideline 212). According to guidance, in spite less sensitive than the FELS OECD 210 chronic test since shorter exposure duration, OECD 212 can be regarded as a long-term fish test, in which sensitive life-stages are exposed. The study was carried out with special precautions to avoid evaporation losses of carbon disulphide. Given the high volatilisation or high fugacity of carbon disulphide from water to air, a 21 days study was not feasible. Then, fish were exposed to carbon disulphide during 8 days, from the newly fertilised egg to the end of the sac-fry stage at concentration ranging from 0.024 to 6.25 mg/L. A $NOEC_{hatching}$ of 1 mg/L was determined. No effect concentrations were determined at 2.5 mg/L for both survival and specific malformation of the notochord (corkscrew spine) (unpublished report, 1991).

7.8.1.2. Aquatic invertebrates

A static test in a sealed system was carried out to determine the toxicity of carbon disulphide to *Daphnia magna*. The 48-h EC_{50} of carbon disulphide for *Daphnia magna* was 2.1 mg/L (Leuwen *et al*., 1985).

7.8.1.3. Algae and aquatic plants

A test in sealed flasks was carried out to determine the toxicity of carbon disulphide to the algae *Chlorella pyrenoidosa*. The 96-h E_rC_{50} for *Chlorella pyrenoidosa* was 21 mg/L (Leuwen *et al*., 1985).

7.8.1.4. Sediment organisms

According to Annex X of Regulation (EC) No 1907/2006, long-term toxicity tests for sediment organisms data are not needed, as the results of the chemical safety assessment does not indicate the need to investigate further the effects of the substance and/or relevant degradation products on sediment organisms. Then, the equilibrium partitioning method is used for assessing the hazard to sediment organisms.

7.8.1.5. Other aquatic organisms

No relevant information available

7.8.2. Terrestrial compartment

One reliable long term study is available on the toxicity of carbon disulphide for soil micro-organisms (Bremner and Bundy, 1974). Carbon disulphide was incubated with several soil types in closed flasks and the effect on the rate of nitrification (formation of nitrate from ammonium) was measured. Carbon disulphide appeared to be a strong inhibitor of nitrification. The 5-d EC_{50} in a clay loam soil was found to be 0.21 mg/kg dw.

7.8.3. Microbiological activity in sewage treatment systems

Relevant studies on the toxicity of carbon disulphide to STP microorganisms are not available. Consequently, a NOEC of 2.6 mg/L (nominal concentration) based on results from the biodegradation study (unpublished report, 1992c) is used to derive the PNECSTP. In this study performed in closed vessel, the authors showed that inhibition of the endogenous respiration of the inoculum by 2.6 mg/L of carbon disulphide was not detected. Therefore, this concentration could be used as NOEC.

7.8.4. PNEC derivation and other hazard conclusions

Table 10

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	PNEC $_{freshwater}$ = 1.0E-02 mg/L	NOEC of 1 mg/L for fish (nominal concentration) with the assessment factor: 100
Marine water	PNEC $_{marinewater}$ = 1.0E-03 mg/L	NOEC of 1 mg/L for fish (nominal concentration) with the assessment factor: 1000
Sediments (freshwater)	PNEC $_{sediment}$ = 1.5E-02 mg/kg ww	No data on sediment organisms is available. According to the Guidance on information requirements and chemical safety assessment – Chapter R.10: Characterisation of dose [concentration]-response for environment, in the absence of any ecotoxicological data for sediment-dwelling organisms, the PNECsed may be provisionally calculated using the equilibrium partitioning method. This method uses the PNECwater for aquatic organisms and the suspended matter/water partitioning coefficient as inputs. The following formula can therefore be applied: PNECsed = (Ksusp-water / RHOsusp) * PNECaqua * 1000 with:

		RHOsusp: bulk density of wet suspended matter = 1150 kg/m^3 and Ksusp-water = partition coefficient suspended matter water = 1.75 m^3/m^3 (estimated from the equation R16.7 of the guidance document of ECHA (2016) and an estimated logKoc value of 1.53 using the equation for non hydrophobic substances in the Guidance on Biocidal Products Regulation: Volume IV Environment - Assessment and Evaluation (Parts B+C) (2017).
Sediments (marine water)	PNEC $_{sediment}$ = 1.5E-03 mg/kg ww	No data on sediment organisms is available. According to the Guidance on information requirements and chemical safety assessment – Chapter R.10: Characterisation of dose [concentration]-response for environment, in the absence of any ecotoxicological data for sediment-dwelling organisms, the PNECmarine sediment may provisionally be calculated using the equilibrium partitioning method. This method uses the PNECsaltwater for aquatic organisms and the marine suspended matter/water partitioning coefficient. This method results in a PNEC value of 10 times lower than the PNEC value for freshwater sediment.
Sewage treatment plant	$PNEC_{stp}$ = 0.26 mg/L	No reliable study assessing the toxicity of the registered substance to microorganisms is available. Based on the biodegradation study where no adverse effect on STP micro-organisms is expected up to 2.6 mg/L. To this value the assessment factor of 10 was applied to derive the PNECstp.
Soil	PNEC $_{soil}$ = 1.86E-04 mg/kg ww	NOEC of 0,21 mg/kg dw (nominale concentration) with the assessment factor: 1000 PNEC has been recalculated to wet soil: PNEC soil wet = PNEC soil dry /1.13.
Air	-	-
Secondary poisoning	No potential for bioaccumulation	Low BCF value of < 60

7.8.5. Conclusions for classification and labelling

Based on the lowest aquatic acute toxicity value higher than 1 mg/L (96h LC_{50} = 2.1 mg/L) and the chronic toxicity value higher than 0.1 mg/L (NOEC = 1 mg/L), carbon disulphide does not warrant a classification for aquatic toxicity according to the Regulation (EC) No 1272/2008.

7.9. Human Health hazard assessment

The available data were retrieved from the registration dossier of carbon disulphide. Moreover, a literature search was performed covering the period between 2013 and 2020. The literature search was done using PubMed database using the search term "75-15-0 [CAS]" in abstract-title-key words. Only full-text publication in English were retained.

In addition, this evaluation also takes previous international evaluations or reviews into account, in particular: EU (1997), ACGIH (2006), AEGL (2009), ATSDR (1996), BUA (1991,

1993), DFG (2005), Gelbke *et al*. (2009), HCNL (2011), IPCS (2002), SCOEL (2008), Silva *et al*. (2013), Wrc-NSF (2002).
Animal studies were scored according to Klimisch score using toxRTool criteria and a risk of bias analysis was performed for human data on key studies.

Data summarised in form of tables are presented from the most recent studies to the oldest ones.
Exposure expressed in ppm in the inhalation studies were converted to mg/m^3 using the conversion factor 1ppm = 3.16 mg/m^3.

7.9.1. Toxicokinetics

The Substance is a very volatile liquid at ambient temperature.

Absorption

The substance is readily absorbed via oral, dermal and inhalation.

Human and animal studies reported that carbon disulphide is extensive absorbed following inhalation exposure (70-80%).

Carbon disulphide is absorbed through the skin and vapour can also be absorbed dermally.

For the dose selection in the requested EOGRTS study, a comparison of route of exposure was performed by the registrant as a non-guideline study (GLP-compliant) in male rats only using both oral and inhalation route of exposure. The aim of the study was to determine the whole blood toxicokinetics of carbon disulfide and the plasma toxicokinetics of the carbon disulphide metabolite 2-thiothiazolidine-4-carboxylic acid (TTCA) in male rats (Wistar Han) following a single oral (gavage) or inhalation administration of carbon disulphide and to determine the routes of elimination and excretion of carbon disulphide and TTCA (Unpublished report, 2018).

In this study, carbon disulphide was administered by gavage in corn oil with 5% acetone to 75, 150 or 300 mg/kg or by a single whole-body 3h inhalation exposure of 150 ppm. Blood samples were collected from 3 animals/administration group/time point at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h post dose. For excretion, urine and feces were collected from 3 animals/group at 0-6, 6-12, 12-24, 24-4 and 48 to 72 hours postdose. TTCA was the only metabolite measured.

The following summary of the results was provided in the endpoint study summary of the REACH dossier: "A*fter a single oral (gavage) dose of carbon disulfide administered to rats at 75 mg/kg, the whole blood Cmax was 15,500 ng/mL, and AUClast was 188,000 h*ng/mL. The terminal elimination phase T1/2 was 11.1 hours. After a single oral (gavage) dose of carbon disulfide administered to rats at 150 mg/kg, the whole blood Cmax was 29,500 ng/mL, and AUClast was 459,000 h*ng/mL. The terminal elimination phase T1/2 was 16.1 hours. After a single oral (gavage) dose of carbon disulfide administered to rats at 300 mg/kg, the whole blood Cmax was 53,400 ng/mL, and AUClast was 766,000 h*ng/mL. The terminal elimination phase T1/2 was 14.7 hours.*

*After a single inhalation exposure to carbon disulfide administered to rats at 150 ppm over 3 hours, the whole blood Cmax was 11,300 ng/mL, and AUClast was 110,000 h*ng/mL. The terminal elimination phase T1/2 was 22.6 hours.*

*Dose-normalised AUClast ranged from 2510 (h*ng/mL)/(mg/kg) to 3060 (h*ng/mL)/(mg/kg) for the oral dose groups. The narrow range of values and the absence of any trend relative to dose level indicates that the whole blood exposure to carbon disulfide was dose-proportional. Summing the whole blood exposure of 110,000 h*ng/mL measured after the completion of a 3-hour inhalation exposure plus the calculated exposure during the Inhalation Phase (Cmax 11300 ng/mL * 3 h) results in overall carbon disulfide exposure (AUC) of 143,900 h*ng/mL following a 150 ppm inhalation dose. Extrapolating the inhalation exposure on the plot of oral dose versus exposure indicated that a 110,000 h*ng/mL inhalation dose is roughly equivalent to an oral dose of 30 mg/kg and an inhalation dose of 143,900 h*ng/mL is rougly equivalent to an oral dose of 44 mg/kg.*

*The 2-thiothiazolidine-4-carboxylic acid concentrations in plasma were much lower than carbon disulfide concentrations in whole blood, with Cmax ranging from 242 ng/mL to 421 ng/mL across all groups and dose-normalized AUClast in the range of 20.6 to 27.8 (h*ng/mL)/(mg/kg) following an oral dose. Like carbon disulfide, 2-thiothiazolidine-4-carboxylic acid exposure was also dose-proportional among oral doses. The terminal elimination phase T1/2 ranged from 3.86 to 6.73 hours in the oral dose groups and was 1.58 hours in the inhalation group. Based on the excretion data, approximately 11% to 19% of the dose was recovered as carbon disulfide in the urine and 17% to 32% of the dose was recovered as carbon disulfide in the expired air following an oral dose. The amount of carbon disulfide recovered in the air and urine increased with increasing oral dose level, suggesting saturation of binding sites and/or metabolic clearance processes at higher doses. However, there was no notable change in the fraction eliminated in either matrix (urine or expired air) at the 2 lowest doses, and a minor shift to greater pulmonary excretion at the highest dose. Carbon disulfide in expired air was primarily excreted in the first 12 hours, while carbon disulfide in urine continued to be excreted across the time course. Using a calculated inhalation dose of 30 mg/kg extrapolated as above, but only using the AUC after the completion of the inhalation exposure, approximately 44% of the dose was recovered as carbon disulfide in urine and expired air, and the distribution between the 2 matrices reflected a similar pattern as observed after the oral dose.*

Approximately 1% of the dose was recovered as 2-thiothiazolidine-4-carboxylic acid in the urine following an oral dose, reflecting the lower circulating concentration of 2-thiothiazolidine-4-carboxylic acid versus carbon disulfide in the whole blood and plasma. Similar recovery was observed after inhalation dosing.

With the exception of 1 time point, all feces samples were below the quantitation limit for carbon disulfide. In addition, the 2-thiothiazolidine-4-carboxylic acid concentrations measured accounted for 0.01% of the dose or less across the entire 72-hour collection period, indicating that biliary excretion is not a route of carbon disulfide or 2-thiothiazolidine-4-carboxylic acid excretion in rats.

Overall recovery of carbon disulfide and 2-thiothiazolidine-4-carboxylic acid represented approximately 31% to 52% of the administered dose among all dose levels and routes of administration. Because notable concentrations of carbon disulfide were still being eliminated in the urine at 72 hours postdose, it is likely that carbon disulfide and metabolites remained in the rat at the termination of the study. In addition, while quantitative recovery is desired, carbon disulfide is known to undergo multiple metabolic transformations in vivo, and these metabolites were not measured as part of this study. Potential changes in biliary excretion of CYP-related metabolites would not be detected in this experiment, but even with apparent variations in the degree of total excretion in urine and expired air between dose groups, the relative amount excreted in each matrix was consistent and there was no indication that biliary excretion was a significant contributor to clearance of carbon disulfide or 2-thiothiazolidine-4-carboxylic acid.

The study demonstrates that systemic exposure to carbon disulfide, as evaluated by whole blood AUClast, is dose-proportional and that the distribution of carbon disulfide and 2-thiothiazolidine-4-carboxylic acid in the urine and expired air does not differ by dose or by dose route. In addition, single and, based on the data collected for 2 -thiothiazolidine-4-carboxylic acid and carbon disulfide, repeated oral doses can be scaled to match the desired equivalent inhalation exposure."

Based on these data the estimated equivalent oral dose for 3 h exposure to 150 ppm was calculated to be 44 mg/kg bw/day. For 6h inhalation exposure, the registrant assumed an equilibrium after 3h inhalation and estimated an equivalent dose of 57 mg/kg.

Overall, there are uncertainties on these results for extrapolation of oral exposure vs inhalation exposure in the EOGRTS study is that the TK results were only available for acute exposure (single gavage or 3h inhalation exposure) and not following repeated administration.

In addition,only one sex (males) was used in the study. There is gender differences in metabolism between males and females, the latter metabolising carbon disulfide more quickly than males. The toxicokinetics of carbone idsulfide in rats was studied as part of the collaborative NIEHS study (Moorman et al. 1998): "*Male and female F344 rats were*

exposed nose-only 50, 500, and 800 ppm CS_2 for 180 minutes and blood samples were taken 4, 8, 15, 30, 60, and 180 minutes after the start of exposure. The concentration in blood at 180 minutes increased proportionally with dose and was significantly (about 40 %) lower in females than in males. No true steady-state during the exposure was reached".

Approximately 1% of the dose was recovered as TTCA in the urine following an oral dose (75 to 300 mg/kg) and after inhalation dosing (150 ppm). This is in line with the previous oral study in rat of Kivisto et al., 1995. Saturation of TTCA production was observed in rats treated with a single gavage dose of 1, 10, 30, or 100 mg/kg, 4.6%, 2.4%, 1.7%, and 0.8%, respectively, of the dose was excreted in the urine as TTCA. Neverthless, the value of 1% TTCA seems low by inhalation compared to the study of Kivisto et al., 1995. Rats were exposed to carbon disulfide at 50 ppm for 6 hours. After 7 days the pretreatment regimens were repeated in the same rats, and the rats were again exposed to carbon disulfide at 500 ppm for 6 hours. About 7.6% and 2.3% of the dose was excreted as TTCA at 50 and 500 ppm, respectively, suggesting saturation. However, the investigators speculated that saturation may not have occurred because the physical activity level of the rats was reduced at 500 ppm suggesting that carbon disulfide uptake at 500 ppm may also have been reduced because of the lowered respiratory rate (ATSDR, 1996).

Metabolism

Carbon disulphide is extensively metabolised. There are two pathways that are described in the literature.

Carbon disulphide can be metabolised by cytochrome P450 (CYP450) to an unstable oxygen intermediate. Oxidative metabolism of carbon disulphide produces intermediary oxides and reactive elemental sulphur. The latter binds to cysteine residues (R-SH) in microsomal fraction and in apocytochrome P450, generating hydrodisulfides (R-S-SH) . Carbon disulphide was shown to behave as a suicide substrate of CYP450 (De Matteis and Seawright, 1973; Catignani and Neal, 1975).

The second metabolic pathway is the formation of dithiocarbamate and gluthatione conjugates. Indeed, carbon disulphide can reacts directly with sulfhydryls of glutathione and cysteine to generate TTCA. Carbon disulphide can also form dithiocarbamates following direct combinaison with the amine groups of amino acids. Dithiocarbamates are seen in both animals and human. Dithiocarbamates have been shown to be able to react with amino acids and to chelate essential metals (e.g. Zn++ and Cu++) and affecting important enzymes (e.g dopamine-beta-hydroxylase) (Caroldi *et al.,* 1984; ATSDR, 1996).

The contribution of each pathway in the toxicity of carbon disulphide is not fully elucidated. Carbon disulphide can also direcly react with free amino groups and interact with biological macromolecules such as protein and nucleic acids.

The following metabolic scheme is proposed in animals and human for carbon disulphide (ATSDR, 1996):

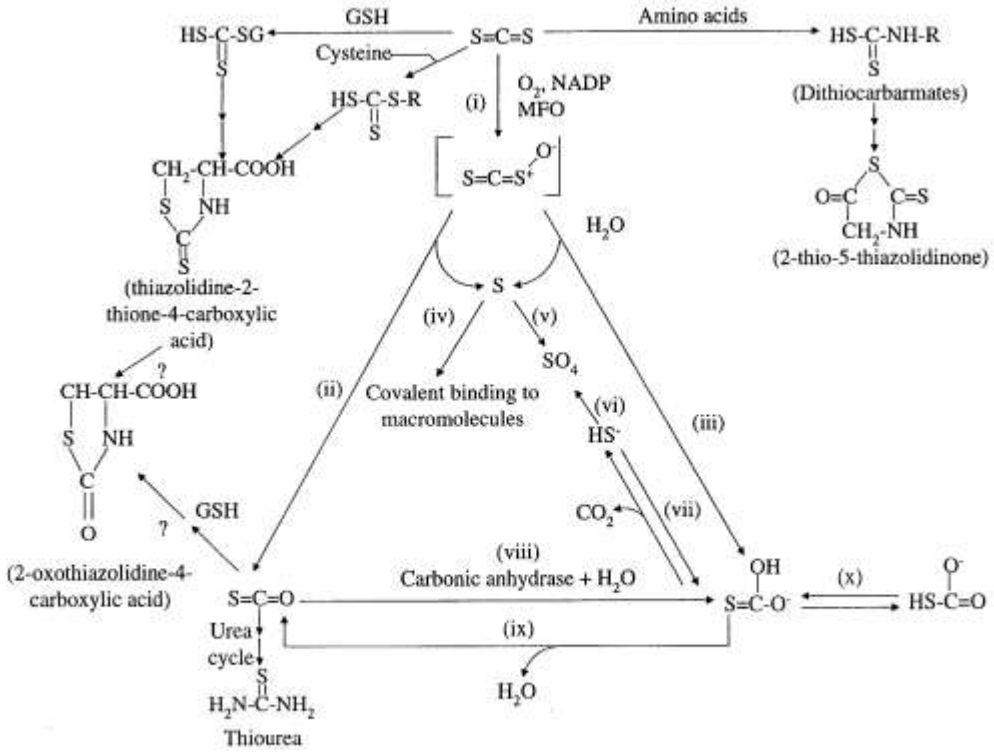

Figure 1: Carbone disulphide proposed metabolic scheme (Beaucamp *et al.*, 1983)

Elimination

Carbon disulphide is eliminated in the expired air or the urine. In human, about 10-30% of the inhaled dose and 3% of the dermal absorbed dose is exhaled unchanged. In urine, TTCA represents less than 6% of absorbed carbon disulphide and unchanged carbon disulphide in urine is less than 1%. The majority of urinary exctretion is in form of inorganic sulfates (ATSDR, 1996).

7.9.2. Acute toxicity and Corrosion/Irritation

Acute toxicity

The registrants concluded the substance is acutely toxic by inhalation (LD_{50} of 10.35 mg/l, vapour) and should be classified as Acute Tox. 4, H332 "Harmful if inhaled" (unpublished report, 2010a) and the evaluating MSCA supports this conclusion.

Skin irritation

An *in vitro* study was performed to assess the corrosive potential of carbon disulfide by means of the Human Skin Model Test (OECD 431). Although borderline, carbon disulfide was not corrosive in the condition of the study (Unpublished report, 2010b).
An *in vitro* study was performed to assess the irritation potential of carbon disulfide by means of the Reconstructed Human Epidermis (RhE) Test Method (draft guideline of December 2009, similar to OECD TG 439). The study does not fully comply with the new test guideline of 2010 but was considered acceptable. Nevertheless, carbon disulfide was not a skin irritant in this study (Unpublished report, 2010c).

Two non-standard *in vivo* studies investigating the corrosive/irritating potential of carbon disulfide were identified in the public domain:

- In the old study of Hueper *et al.* (1936) small cotton plugs soaked with 2 ml of carbon disulfide were placed into the tip portions of the ears of five rabbits, for 3 -5 consecutive days. The study was performed in order to examine effects on rabbits that have been previously described in viscose rayon workers. The workers were reported to develop serious blisters that evolved to hemorrhagic blisters. Nonetheless, they were not exposed exclusively to carbon disulfide, but to a mixture, that consisted of carbon disulfide, sulfuric acid, sodium sulfate, and hydrogen sulfide. The analytical composition

was not tested. In the rabbits, early epidermal and sub-epidermal vesicles were formed in the skin that progressed to ulcers, accompanied by an inflammatory response. The skin manifestations resulting had the clinical and anatomical characteristics of second and third degree chemical burns. However, the documentation was insufficient for assessment. As with the workers, no information on the analytical purity of the test substance is provided and thus, the causative agent of such effects could be an impurity and not carbon disulfide itself. Moreover, exposure time, observation period, strain and sex of the animals are not given. Therefore, the study is deemed not suitable for the evaluation of the irritating properties of carbon disulfide.

- In the investigation of the second *in vivo* study from Chou *et al.* (2005) topical carbon disulfide exposure exerted a dose-dependent increase in transepidermal water loss (TWEL) and lipid extraction. Cell death in viable epidermis at the highest exposure level, suggests epidermal cell damage. Although the determination of the TWEL is an important endpoint to investigate skin irritation, it does not meet the current classification criteria and hence, carbon disulfide cannot be classified as a skin irritant based exclusively on these results.

No *in vivo* study performed with current guideline is available.

According to the current classification, carbon disulfide is classified as a skin irritant. Carbon disulfide demonstrates non-irritating properties and non corrosive properties in two *in vitro* studies. Some positive results were obtained in 2 *in vivo* studies and in workers; however, the adequacy and the reliability of these studies were low. Taking all existing data into account, and the negative results in the in vitro assay, the current classification Skin irrit 2- H315 did not seem warranted.

Eye irritation

A Bovine Corneal Opacity and Permeability (BCOP) assay was conducted to assess the eye irritating properties of carbon disulfide (Unpublished report, 2010d). No *in vivo* study performed with current guideline is available. No relevant information on this endpoint was available in the human data.

According to the current classification, carbon disulfide is classified as an eye irritant. Carbon disulfide was negative on a well-conducted *in vitro* BCOP assay. Nevertheless, according to the OECD TG 437, a chemical that is not predicted as causing serious eye damage or as not classified for eye irritation/serious eye damage with the BCOP test method would require additional testing (*in vitro* and/or *in vivo*) to establish a definitive classification. No relevant information on this endpoint was available in the human data. Overall, based on the negative results on the *in vitro* BCOP study, current classification of carbon disulfide did not seemwarranted.

7.9.3. Sensitisation

Skin sensitisation

A Local Lymph Node Assay (LLNA) was performed, according to the OECD TG 429 (Unpublished report, 2010d). Female mice were tested at concentrations of 25, 50, and 100% (w/v) in acetone:olive oil (4:1); the concentrations were chosen based on a pre-test. Simulation indices relative to the mean control values were as follow: 1.22, 0.99, and 3.46. The authors of the study noted that as the lymph nodes were pooled, there is no information on the presence of potential outliers at 100%. Although no dose-response was observed, a stimulation index ≥ 3 is observed in the LLNA test at 100% and the substance may be regarded as a weak skin sensitiser. There is no specific data available in the dossier to conclude that the result of the LLNA could be a false-positive. At 50% and 100%, the animals did not show any sign of systemic toxicity or local irritation. No other information was found, regarding the skin sensitising properties of carbon disulphide in experimental animals.

According to the registration dossier, no case of skin sensitisation has been reported in humans although there are a huge amount of human data investigating potential health effects of the substance.

Based on the results of the LLNA study, a classification as Skin Sens. 1B, H317 would be warranted. Nevertheless, evaluating MSCA notes the uncertainties in the results as no dose-response was observed. In addition, a huge amount of human data are available with the substance and no human case were reported. Human data does not support that the substance is a skin sensitiser. Overall, taken all together, carbon disulphide may not warrant to be classified for skin sensitisation.

Respiratory sensitisation

In the registration dossier, from experience from human occupational exposure no reports on respiratory sensitisation were available.

7.9.4. Repeated dose toxicity

7.9.4.1. Human data

Neurological effects and cardiovascular toxicity are the critical health effects in humans following repeated exposure to carbon disulphide. In addition, effects on kidneys, reproductive toxicity and endocrine systems have been reported as target systems in humans. Effects on reproductive and hormone disturbance are assessed in details in sections 6.9.7 and 6.10.

Neurological effects

Carbon disulphide is able to produce neurological damages to both the central and the peripheral nervous systems. Exposure to carbon disulphide induces neuropathy for myelinated axons (axonal swelling with accumulation of neurofilament proteins in distal motor and sensory nerve tracts). Such effects were reported in workers and consisted of subtle neurobehavioral changes (irreversible motor and sensory nerve conduction velocity reduction), polyneuropathy and impaired performance in psychomotor testing. Autonomic nervous system effects were also reported in humans. Effects on nervous system were clearly observed at concentrations ≥ 30 mg/m^3 (10 ppm) and started to be reported at exposure > 10 mg/m3 (3 ppm) (HCNL, 2011; SCOEL, 2008; ACGIH, 2006). Johnson *et al*. (1983) identified a dose-related reduction in motor nerve conduction velocities of workers exposed to median 7.6 ppm. The study establishes this value as a chronic LOAEL for neurological effects.

In 2017, Yoshioka *et al.* published the results of a 6-year prospective Japan cohort study at baseline (1992-93) and follow-up (1998-99). In this study, 382 male workers exposed to carbon disulphide were compared to 368 unexposed male workers. Workers were enrolled from 11 Japanese viscose rayon factories and were followed-up during 6-years. During the study, 4 factories ceased rayon production and among the workers, 145 ceased to be exposed (ex-exposed workers). The mean cessation period was 4.1 years. The authors reported that at a mean concentration of 6 ppm (19 mg/m3) (measured urinary TTCA: 1.74 mg/g Creatinine) for workers and 4 ppm (13 mg/m3) (measured urinary TTCA: 1.38 mg/g Creatinine) for ex-exposed workers, motor nerve conduction velocity (MCV) of the exposed and ex-exposed workers were slower than the unexposed workers at baseline and follow-up, but the mean reduction in MCV at the 6-year timepoint was not different from unexposed workers. At baseline, sensory nerve conduction velocity (SCV) were not different between the groups. Nevertheless, SCV reduction was significantly reduced in exposed workers compare to unexposed workers or ex-exposed workers at follow-up. The SCV reduction effect was still observed after adjustement for potential confounding variables (e.g. aging) in exposed workers compare to non-exposed workers (
The authors also suggested that the effects in peripheral neurological system may be reversible at 4 ppm since SCV was almost the same at follow up and since regression coefficient workers/ex-exposed was positive when SCV was used as an outcome variable.

Table 11
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Table 11: **Sensory nerve condution velocity (SVC) of the median nerve (m/s) (Yoshioka *et al.*, 2017)**

	n	Baseline study Mean ± SD	Follow up study Mean ± SD	Reduction in SCV during the 6-year follow-up (mean ± SD)
Unexposed workers	337	53.81 ± 4.32	50.43 ± 4.97	–3.38 ± 3.97
Ex-exposed workers	121	52.82 ± 5.07	49.56 ± 5.29	–3.26 ± 3.79 #1
Exposed workers	226	53.29 ± 4.70	48.82 ± 5.49 ***	–4.47 ± 3.94 ***

***: p< 0.001 compared to the unexposed workers. #1 p<0.05 compared to the exposed workers.

Overall, this cohort study showed that 6-year carbon disulphide exposure around a mean level of 6 ppm did not affect MCV reduction but induced significant SCV reduction beyond the influence of aging (Yoshioka *et al*., 2017).

Cardiovascular toxicity
As regards cardiovascular effects, serious effects have been observed in human exposed to carbon disulphide in viscose rayon factories. The effects consisted mainly of arteriosclerosis and excess mortality from coronary heart disease.

Mean cholesterol was reported to be increased in exposed subjects in several studies. An effect on lipid metabolism enhancing the development of atherosclerosis has been hypothesised. Higher LDL and lower HDL cholesterol were observed in exposed workers in several studies. Effects on cardiovascular system were clearly observed at doses ≥ 10 ppm. Ischaemic findings (minor ECG abnormalities) were already noted at a mean concentration of 5 ppm (Takebayashi *et al*., 2004). SCOEL did not considered this effect of toxicological relevance, whereas HCNL considered that this could predict an increased risk for cardiovascular toxicity (HCNL, 2011). Schramm *et al*. (2016) showed an increase in intima-media thickness especially in workers with high (maximum exposure > 10 ppm in the last 3 years of the published study) and long-term exposure (> 20 years, > 2170 ppm x years and > 33 mg/g creatinine x years). In contrast, Domergue *et al.* (2016) observed no relation between cardiovascular risk markers and carbon disulphide exposure at a concentration below 5 ppm, with a TTCA < 2.2 mg/g creatinine. In may be noted that in the studyn 'recent exposure index' was calculated for each worker and was much lower than 2.2 TTCA mg/g creatinine [median (min–max), mg/g of creat (n = 101) 0.05 (0.02–3.80)].

Overall, based on subtle neurological changes in workers observed in Yoshioka *et al.* (2017) at 6 ppm and the ischaemic ECG findings in Takebayahi *et al.* (1994) at 5 ppm, a LOAEC of 5 ppm can be considered for both neurotoxicity and cardiovascular toxicity.

Cardiovascular effects

According to the summary in a SCOEL report (2008) from BUA (1993) "functional and morphological changes of the heart, including necrosis of the myocardium have been observed at relatively high concentration either due to a direct effect of carbon disulfide on the heart or due to the increased incorporation of cholesterol and lipoproteins into heart vessels leading to arteriosclerosis."

7.9.4.2. Animal data

Oral

No standard oral repeated dose toxicity studies were available in the dossier or in the litterature. Some non-standard studies were identified in international report. Decreased body weight has been observed in rats following oral exposure to carbon disulfide for a short term (4 weeks) exposure period to 253 mg/kg bw (Hoffman *et al*., 1990; Klapperstück *et al*., 1991). Additionally, in the same studies, carbon disulfide had a cardiodepressive effect (electrophysiological and mechanical parameters), increased the blood pressure and caused electrocardiograph alterations indicative of myocardial

ischemia, following administration of epinephrine or norepinephrine (Hoffman *et al.*, 1990; Klapperstück *et al*., 1991). Body weight depression, and neurological deficits, i.e. abnormal gait, were observed in the two studies of Song *et al.* (2006a & b), after oral administration of 300 mg/kg bw for 12 weeks. These effects were accompanied by significant alterations in the cytoskeletal proteins of the cerebral cortex and spinal cord homogenates, suggesting that they might be implicated with the manifested neuropathy.

Inhalation

Inhalation is the most relevant route of exposure to carbon disulphide . Two key guideline 90-day inhalation studies in rats are available (Unpublished reports, 1983a and 1983c). A third 90-day study performed in mice is used as supporting data (Unpublished report, 1983b). Each study was performed under almost identical conditions and were similar to OECD TG 413; they differed as regards the test animals: 2 strains of rats (Fischer and Sprague-Dawley) and one strain of mice (B6C3F1). Animals were exposed to carbon disulphide vapours at 0, 50, 300, and 800 ppm (equivalent to 153, 948, 2528 mg/m^3), for 6 h/d, 5 d/w for at least 89 consecutive days:

i- Fischer F344 rats (Unpublished report, 1983a)
At the top dose of 800 ppm (2528 mg/m^3), the following findings were statistically significant or showed increase incidences:

- Lower body weights compare to control after 1st week for both sexes. Food consumption was mainly decrease in male,
- Clinical signs: crusty muscle and ataxia,
- Haematology: decreased erythrocyte count in males with concommitant increased MCH and MCHC. Increased lymphocytes in males and females, decreased platelet in males,
- Biochemistry: significant increased transaminases, calcium depression, increased urea nitrogen, decreased total protein, decreased potassium levels in male only,
- Increased urine specific gravity in both sexes, trace of ketones and blood in urine samples
- Organ weight: Brain, heart, liver, kidney and testiscule relative weight were statistically significantly increased in males. Statistically significant decrease in brain, spleen and testicle absolute weight. Decreased brain and ovary absolute weight. Increased heart, kidney and liver relative weight;
- Necropsy: axonal swelling of nerve fibers of the ventral and lateral funiculi of the spinal cord for both sexes, segmental degeneration of fibers in the sciatic nerve in few animals of the high dose group. Slight increase in iron positive pigmentaion of the spleen in high dose males and females.

At 300 ppm (948 mg/m^3), the following findings were significant:

- Decreased male body weights after the 5th week
- Decreased absolute brain weight in males and females

At 50 ppm (153 mg/m^3)

- Decreased male brain absolute weight (1.87 g vs 1.96g in controls)

Cholesterol was not examined in the study. No change in thyroid or adrenals (weight or at necropsy) was noted in the study. The study yielded a LOAEC of 50 ppm (153 mg/m3), based on statistically significant dose-related brain absolute weight decrease observed in males. No NOAEC was identified in the study.

ii- Sprague-Dawley rats (Unpublished report, 1983b)
At the top dose of 800 ppm (2528 mg/m^3), the following findings were statistically significant or showed increase incidences:

- One male was found dead.
- Ataxia observed in animals.
- Statistically significant lower body weights after 1st week of exposure in both sexes.
- Haematology: changes in leukocyte counts measured in males. In females, haemoglobin and hematocrit were decreased; mean corpuscular volume, as well as mean corpuscular haemoglobin levels were also slightly increased (still significant) in females.
- Biochemistry: Decreased potassium and calcium levels;

- Organ weight: absolute brain weights decreased significantly in both sexes. Kidney absolute weights depressed in males. All relative weight were increased significantly in males (liver, brain, spleen, testicles, heart, kidney), while for females it was only heart and liver ratios. Similarly, heart and liver/brain weight ratios were also increased in females;
- Necropsy: axonal swelling of nerve fibers of the ventral and lateral funiculi of the spinal cord was seen for both sexes, with most commonly affected the thoracic cord, followed by lumbar and cervical cords. Segmental degeneration of fibers in the sciatic or tibial nerve in some animals (6/10 per sex) were also noted. A slight increase in iron positive pigmentation was observed in macrophages of the spleen in males and females

At 300 ppm (948 mg/m^3), the following findings were significant:
- Decreased body weight at weeks 4 and 6 in males
- Haematology: increased MCH in females; Changes in leukocyte counts in males
- Decreased absolute brain weight in females. Decreased liver relative weight in females

Cholesterol level was not analysed. No change in thyroid or adrenals (weight or at necropsy) was noted in the study. The NOAEC was identified at 50 ppm (158 mg/m^3) in the study.

iii- B6C3F1 mice (Unpublished report, 1983b)
At the top dose of 800 ppm (2528 mg/m^3), the following findings were statistically significant or showed increase incidences:
- Survival: 2 males and 2 females died before schedule necropsy;
- Clinical signs: 4 animals from the highest concentration group did not respond to artificial light stimulus
- Significant decrease in body weight gain in both sexes
- Haematology: red blood cells, haemoglobin and haematocrit were significantly reduced at the highest concentration in both sexes
- Biochemistry: total serum protein was diminished in both sexes; urea nitrogen was reduced.
- Organ weight: Brain absolute weight depression for both males and females, as well as in kidneys and testicular of males and ovaries of females. Relative heart, kidney weight were increased in both sexes, as well as ovary weight in females.
- Necropsy: segmental degeneration in peripheral nerves at the high dose group, axonal swelling in ventral and lateral funiculi of the thoracic and lumbar spinal cord, renal lesions diagnosed as nephropathy, iron positive pigmentation (haemosiderin) observed in macrophages of the red pulp of the spleen in high dose animals.

Cholesterol was not investigated. No change in thyroid or adrenals (weight or at necropsy) was noted in the study. The study yielded a NOAEC of 300 ppm (948 mg/m^3).

In addition to these guideline unpublished studies, a large number of non-standard published studies was identified based on the most recent and elaborate literature reviews, which address the toxicity of carbon disulfide after subchronic or/and chronic exposure and most particularly on neurological effects.

Neurological effects:

Nerve conduction velocity alterations: Subacute, subchronic and chronic inhalation exposure of rats to carbon disulfide at 285 and 507 ppm (equivalent to 901 and 1602 mg/m^3) produced reduced conduction velocity in the sciatic and tibial nerve, which was fully or partly reversible, depending on the concentration and exposure time (Knobloch *et al.,* 1979). Exposure of rats to 500 or 800 ppm carbon disulfide (1580 and 2528 mg/m^3) for 13 weeks decreased the ventral caudal tail nerve conduction velocity (Herr *et al.,* 1988). Nerve conduction dysfunction in the auditory brainstem responses was detected by Hirata *et al*. (1992), who exposed female rats to 800 ppm carbon disulfide (2528 mg/m3) by inhalation 6 h a day, 5 days a week, for 15 weeks. Gagnaire *et al.* (1986) demonstrated that exposure of rats to 500 ppm of carbon disulfide via inhalation, 5 days a week for 25 weeks, results in reduced peripheral nerve conduction velocity (sensory & motor tail nerve).

Change in neurobehaviour: In the study by Moser *et al*. (1998), neurobehaviour was investigated according to the result of the functional observational battery analysis over 2, 4, 8 and 13 weeks (0, 50, 500, 800 ppm, inhalation). The major effect was exerted on neuromotor function, typically affecting the hindlimb. A dose-related response was observed regarding the neuromuscular changes. Hindlimb and forelimb grip strength values were decreased. Gait changes were recorded evolving by the 13th week to disturbed hindlimb control. Mild tremors, as well as decreased responsiveness to visual stimulus, were seen in the 13-week inhalation study. 50 ppm (158 mg/m^3) was considered as a NOAEC in this study.

Other studies provided supporting information on neuromuscular dysfunction and changes in behaviour:

- Symptoms of motor impairment (Frantik *et al*., 1970; 770 ppm for 8 weeks, by inhalation), manifested in the hind-limbs (Colombi *et al.,* 1981 - rats, 700 ppm, 5 h/d, 5d/w for 12 weeks); Moser et al., 1998 - rats, 500 ppm, for 13 weeks) or decreased neuromuscular integrity (Clerici and Fecher, 1991 - rats, 500 ppm, 5 -12 weeks).
- Two weeks of inhalation exposure to carbon disulfide to 250 ppm onward, exhibited effects on behaviour, with an inhibition of avoidance response to shocks (Goldberg *et al.* 1964). Marked gait abnormalities were seen in rats exposed to 396 ppm (1251 mg/m^3) of carbon disulfide for 12 weeks by inhalation (Sun *et al.,* 2009), or treated orally with 300 mg/kg bw for 12 weeks (Song *et a*l., 2006a & 2006b).

Other studies also provided supporting information on hearing and visual damage:

- Irreversible severe reductions in visual acuity with degeneration of retinal ganglion cells (Merigan *et al.* 1988), axonal swelling of the optical nerve (Eskin *et al.* 1988), after inhalation exposure of monkeys to 256 ppm for 6 h/d, 5 d/w, 5-13 weeks as well as loss of hearing (Rebert & Becker, 1986) at 400 ppm in rat.

Neuropathology: In a 90-day inhalation study by Gottfried *et al*. (1985), a NOAEC for peripheral nervous system and central nervous system at 50 ppm was observed in rats. Degeneration and axonal swelling were observed in spinal cord and peripheral neurons at 300 ppm (948 mg/m^3) and above. Neuron axonal swelling in the peripheral as well as in the central nervous system (Sills *et al.,* 1998; Valentine *et al.,* 1998; Valentine *et al.,* 1997, Towes *et al.,* 1998, Sills *et al.,* 2000), usually accompanied by neurofilamentous accumulation, due to covalent cross-linking (Valentine *et al.,* 1998; Valentine *et al.,* 1997; Song *et al*., 2006a & 2006b) and myelin thinning (Sills *et al.,* 1998). Axonal swelling was usually detected at concentrations of 500 ppm (1580 mg/m^3) and above, while the neurofilamentous cross-linking was seen already at 50 ppm (158 mg/m^3) after inhalation of carbon disulfide (Valentine *et al.,* 1997).

With regards to cardiovascular effects, the following summary is available in ATSDR, 1996. "*Rats administered carbon disulfide at 16 ppm and greater for up to 6 months exhibited concentration-related structural and functional changes (distention of the lumen, attenuation of myocardial vessels, irregular thickening of the aorta wall, as well as microscopic histological changes). Although an increase in the enzyme activity (fructose-1,6-phosphatase, glutamate dehydrogenase, and glucose-6-phosphate dehydrogenase) was reported at the lowest concentration (3.2 ppm), the statistical significance of this finding was not reported. Also, no structural changes were seen at 3.2 ppm. However, when rats exposed to the same concentration of carbon disulfide were administered an atherogenic diet, there was an increase in mortality, a decrease in albumin and increase in globulin fractions in the serum, and serious metabolic and structural changes in the myocardium and the aorta (Antov et al. 1985).*

Rats chronically administered 321.1 ppm carbon disulfide (5 hours a day, 6 days a week, for 15 months) did not develop any gross or histological lesions in the aorta; however, lipid droplets were occasionally noted on histological examination of the coronary arteries (Wronska-Nofer et al. 1980). In this same study, rats simultaneously fed an atherogenic diet had more advanced lipid infiltrates of the coronary arteries, which suggests that carbon disulfide may have an accelerating effect on atherosclerotic changes induced by dietary hypercholesterolemia. Thus, carbon disulfide may have promoted the development of

atherosclerosis and coronary heart disease via altered cholesterol metabolism within the arterial wall'".

7.9.5. Mutagenicity

The genotoxicity of carbon disulphide was investigated in a battery of both *in vitro* and *in vivo* studies. They are presented in Table 12 and table 12, respectively.

Table 12: Summary of *in vitro* genotoxicity studies performed with carbon disulfide

Test	Cell type	Concentration	Metabolic activity	Observation and remarks	Ref.
Ames test OECD 471, GLP Purity: > 99.8% No limitation compare to TG	*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537	0.005% to 0.1% v/v	+/- S9 mix	Negative	Unpublished report, 1991
In vitro mammalian cell gene mutation test GLP, OECD 476 Purity > 99% No limitation compare to TG	Mouse Lymphoma L5178Y Cells	23.8 to 761 µg/mL	+/- S9 mix	Negative	Unpublished report, 2010e
In vitro chromosome aberration and sister chromatid exchange (SCE) Limitations: no positive controls	Cultured human lymphocytes	3-60 µg/mL	+/- S9 mix	Chromosome aberration: negative SCE: - S9: negative + S9: equivocal	Garry *et al*., 1990
Effect of CS_2 on human sperm chromosomal aberration N= 9 healthy man Sperm was incubated with female golden hamster oocytes Limitations: invalidated positive control, low number of cells investigated, no information on cytotoxicity, no metabolic investigation	Human sperm cells	0, 1, 5, 10 µM Positive control: Pingyanmicin	-S9 only	Increased in aberrant rate (%) and average breaks at 10 µmol/l at the highest concentration only	Le *et al*., 1996

Table 13: Summary of *in vivo* genotoxicity studies performed with carbon disulfide

Test	Cell type	Concentration	Observation and remarks	Ref.
In vivo micronucleus assay (by inhalation) OECD 474, GLP Purity > 99.8% 6h exposure 10 animals/sex/dose (5 for positive control) Limitations: insufficient proof of bone marrow exposure	CD-1 mouse Male/female (bone marrow erythrocytes)	150, 500 and 1500 ppm	Negative	Unpublished report, 1992d

Test	Cell type	Concentration	Observation and remarks	Ref.
In vivo chromosome aberration test (Meeting extract) 3-week exposure Mated with unexposed male mice	Oocyte of mice	10-100 mg/m^3 (3.2 to 32 ppm)	Incidence of defective zygotes and chromosomal aberrations in female pronuclei of zygotes, were significantly higher than that of the control group. The incidence of oocyte chromosomal aberrations in the CS_2 exposure group without mating was also significantly higher than that of control and the dose-response relationships were demonstrated. This finding was associated with a lower fertilization rate in another study	Bao *et al*., 1996
Sperm head shape abnormality test Intraperitoneal exposure Limitations: studies investigating germ cell toxicity, does not provide direct evidence of genotoxicity	rats	25, 50, 100, 200 mg/kg bw	↑ sperm head shape abnormalities, significant at 200 mg/kg only decrease in sperm count (≥ 100 mg/kg)	Kumar *et al*., 1999
Single cell gel electrophoresis assay: DNA damage detection Published (abstract only, article in Chinese) 3 dose groups (unknown route of exposure)	Mice sperm	Not available	Dose related increase in: - DNA damage - Index of DNA damage intensity - Tail moment Decreased of the percentage of the head of the comet	Tang et al., 2003
In vivo chromosome aberration test Published (abstract only, article in Russian) Treatment during gestation (by inhalation)	Female rats, foetuses	Not available	Chromosomal aberrations	Vasil'eva, 1982

Genetic toxicity *in vitro*

Two unpublished guideline studies (GLP-compliant) provided negative results: Ames assay using carbon disulphide as gas and an *in vitro* gene mutation assay in mammalian cells using carbon disulphide solubilised in ethanol.

With regards to chromosome aberration *in vitro*, no GLP guideline study is available. Two published studies are rated Klimisch score 3 (unreliable) by evaluating MSCA due to lack of detailed information on study methods and results and due to study deficiencies. In the study from Garry *et al.,* 1990, performed on primary human lymphocytes, a significant and dose-dependent increase of sister chromatid exchange was found, while in the same cells no positive effect was observed on chromosomal aberrations (excluding gaps). In this study, no positive controls were used. In the *in vitro* study from Le and Fu (1996) using human sperm cells, an increase in chromosomal aberrations was observed at 10µmol/L.

Nevertheless, the technique employed in this test is not validated and few details were available, moreover no information on cytotoxicity was provided.

Genetic toxicity *in vivo*

Carbon disulphide was negative in a well-conducted GLP *in vivo* micronucleus study in mouse (unpublished report, 1992d). Mice exposed at the highest dose (1500 ppm ; 4740 mg/m^3) showed a small increase in the ratio of polychromatic/mature cells, 24 h post-exposure, which may indicate disturbance of erythropoiesis. However, the biological relevance of this result is unclear and therefore there is no convincing proof (decrease in the ratio polychromatic erythrocytes / normochromatic erythrocytes) that the bone marrow was reached.

There are four published *in vivo* positive studies. A meeting abstract from Bao *et al*. (1996), reported an increase incidence of chromosomal aberrations in oocytes and prenuclei zygotes of exposed adult female mice (dose level at which the effects occurred are unknown). In another study (Vasil'eva, 1982), oral exposure to carbon disulphide gave a genotoxic response, manifested as chromosomal aberrations and polyploid cells in the bone marrow of female rats and in rat embryos exposed on days 10–13 of gestation. The sperm head abnormality findings in Kumar *et al.* (1999) at 100 and 200 mg/kg of (intraperitoneal route) support that carbon disulphide may have the potential to induce adverse effect on male reproductive system of rats but do not give direct evidence of germ cell genotoxicity of carbon disulphide. Finally, Tang, 2003 (abstract only), shows that carbon disulphide induced DNA damage in mice sperm with single cell gel electrophoresis assay. However, no detail on the test method and results was available. More recent studies (Zhang *et al*., 2014; Yang *et al*., 2014) observed DNA damage (comet assay) in endometrial cells in mice after carbon disulphide exposure at peri-implantation at ≥ 157 mg/kg. Yang *et al*. (2014) showed that oxidative stress was also observed (Malondialdehyde analysis) and 8-OH-G was increased at 631 mg/kg.

Conclusion

Although carbon disulphide has been tested for genotoxicity *in vitro* and *in vivo*, it is difficult to draw firm conclusions as regards to this endpoint in the context of this evaluation. The compound was found to be negative in fully adequate standard *in vitro* tests for gene mutations with bacteria and mammalian tissue culture cells and in an *in vivo* micronucleus assay. Nevertheless, no clear proof of bone marrow exposure was available. The studies that yielded the clearest positive results *in vitro* and *in vivo*, notably on germ cells, cannot be fully judged as regards their validity, due to a lack of information on essential methodological details in one hand or because they were not published in English.

Currently, carbon disulphide is not classified for its genotoxic potential and based on both *in vitro* and *in vivo* results of the available database, no classification concerning genetic toxicity is warranted according to CLP criteria. Furthermore, although DNA damage on germ cell has been observed from comet assay in several studies, they are insufficient data to conclude on direct germ cell mutagenicity. It is also plausible that the DNA damage may be secondary to the oxidative stress produced by the substance.

7.9.6. Carcinogenicity

No data on animals were available in the registration dossier. No evidence of carcinogenicity of carbon disulphide has been found in epidemiological studies provided in the dossier.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Table 14: Summary of key toxicity studies on male fertility and hormonal effects in humans

Reference, study design & population	Exposure	Statistical analysis	Results
(Guo *et al.*, 2016) Study type: case-control study Male workers exposed to CS_2 **N:** 76 exposed + 97 controls **Location**: not stated (most probably china factory), unknown number of location, unknown job type **Age:** mean 32 years for unexposed and 33 years for exposed. Working age: 10 years for exposed and 9.8 years for unexposed **Health outcome and analysis: male sexual function.** Blood samples: LH, FSH, Testosterone, SHBG measured by immunoassays. Semen samples: semen routine analysis, sperm chromatin structure assay, semen plasma total antioxidant capacity, flow cytometric analysis, sperm mitochondrial function assay, mitochondrial membrane potential assay, respiratory chain measurements **Exclusions**: know medical or surgical condition that could affect fertility **Author conflict of interest:** not reported **Risk of bias assessment:** **-** Selection bias: only married males included in the study. - Selection of exposed and unexposed male workers not specified - Air static measurements over 2 years may underestimate individual cumulative exposure and skin absorption not taken into account. No information on RPE used by workers - No dose-response investigated - Confounding factor such as co-exposure with hydrogen sulphide noted but not	Concentration of CS_2 measured by gas chromatography. 486 monitoring spots during past 2 years Average value: the concentration range of CS_2 in the workshop air was 0.1 to 44mg/m3 between 2010 and 2014. TWA : 9.73 ± 2.76 mg/m^3 (10h/d, 5d/w for over 2 years). Unexposed: no or negligible exposure	t-test, anova, **Adjustment factor**: age, working age, BMI, education level, smoking and alcohol use were control for semen quality parameters **Statistical power**: not discussed	No statistical differences between age, working age, BMI, education levels, alcohol consumption and smoking and alcohol use between groups. Statistically significant decreased mean serum SHBG in exposed group within the normal range. Statistically significant decreased in testosterone level in exposed group below range of normal value. Statistically significant increase in LH and FSH above normal range values in human. Semen analysis: increased liquefaction time, decreased semen viability compare to unexposed group. Sperm morphological abnormalities were more severe in exposed group. Semen antioxidant capacity was lower in exposed group. The number of apoptotic cells was statistically increased compared to unexposed group. The mitochondrial membrane potential was decreased in exposed group compared to unexposed group. Mitochondrial dysfunction was observed in exposed group compare to referent. More abnormal structural chromatins were observed in spermatozoa from the exposed workers.

adjusted - Limited number of individual - Temporal causal association cannot be studied			
(Ma *et al*., 2010) Study design : cross-sectional study Male workers exposed to CS_2 from the filature and cotton pulp departments of a fabric factory **N:** 80 exposed + 49 controls **Location**: China **Age:** 34 yr. for exposed and 30.5 yr. for non-exposed. Duration of exposure: 15.3 year for exposed and 12.5 years for the controls. **Health outcome: male sexual function.** Semen samples in 43 exposed and 35 controls, self-administered questionnaire **Exclusions**: not specified. The disturbance of other diseases was controlled through interview and medical examination. **Author conflict of interest:** fundings from the national science foundation of china and the department of occupational health disease prevention and cure, china **Risk of bias assessment:** **-** High number of non-participants - No measurements for unexposed workers - Average exposure for 2 years may not reflect individual cumulative exposure (exposure may be underestimated), air measurements include an inadequate assessment of individual exposure. - Low number of cofounding taken into account (ex: co-exposure, smoking not taken into account). No information if groups were comparable for body mass index, smoking, education levels) - No dose-response investigated	Job type. Workers in the filature and cotton pulp departments: exposure concentration > 10 mg/m3 (based on three different workshops of 728 monitoring spots during past 3 years) Average concentration in cotton pulp department: 10.9 mg/m3; No statistical significance with filature department (9.4 mg/m3) Unexposed: power department where no exposure to CS_2 arise.	t-test **Adjustment factor**: age, alcohol drinking **Statistical power**: not discussed	Sexual dysfunction was higher in the exposed group compare to control. Alteration of libido was reported in the wives exposed to CS2 compare to controls. Spermatogenesis: statistically significant differences for: semen volume, liquefaction time, viability, acrosomal membrane integrity rate, semen density, total count, sperm morphological abnormalities ($p<0.01$). Semen quality was more damaged with the longer exposure to CS2, but there was not statistical differences between the two groups
(Takebayashi *et al*., 2003)	Biomonitoring of TTCA in urine	ANOVA, Tukey's	Levels of serum or plasma hormones used to assess pituitary and

<table>
<tr>
<td>Study design: 6-year follow-up investigation of the baseline study of Omae et al., 1998.
Male Japanese rayon workers

N: 259 exposed + 133 former exposed + 352 referent workers
Location: 8 viscose rayon factories (stable fiber or filament fiber) in Japan (3 factories closed since initial cohort study)
Age: means of 35.5 to 36.8. Mean duration of CS2 exposure: 19.3 years at the end of the study.
Analysis: hypophysic function, gonad function and thyroid function. Fasting blood samples: blood glucose, glycosylated hemoglobin, serum insulin, FSH, LH, testosterone, ACTH, TSH, T4, T3, TBG (thyroxine binding protein). Baseline study was performed on non-fasting blood collection.
Exclusions: Workers with age > 50 years
Author conflict of interest: the study was supported by the Japan Chemical Fiber association
Limitation:
- No data on former exposure
- 84 non participant (but similar number in referent and exposed group)
- 10% of baseline subject were not follow up (resigned, died or transfer of job)
- missing data from questionnaire or absence during the day of research team visit. No relation with health status or exposure status according to the authors.
-healthy worker effect cannot be excluded</td>
<td>at the end of shift twice a year from 1992. Personal air sampling for each worker the same day as the TTCA measurement from 1993
Exposure status was categorized into 3 groups: exposed, ex-exposed (some factory have been closed) and referent workers. Further categorization into quartiles of TTCA levels for 6 years if sufficient measurements were available (cut-off values: 2.69, 1.79, 1.14 mg TTCA/g Creatinine)
Geometric TTCA means for all exposed workers was 1.61 mg/g Creatinine and the geometric mean for the air concentration was (5.02 ppm).
Geometric mean of maximum exposure over the 6-years were 5.69 mg TTCA/g cr. and 10.68 ppm for the spinning/refining workers.
The TTCA levels dropped from 1993 onward but not CS_2 air concentration. According to the authors, may be explain by effectiveness of a respiratory protection program developed since 1993.
Potential co-exposure: 16/133 and 15/152 were exposed to acetone or isopropanol.</td>
<td>method for comparison with referent group
Adjustment factor: age, BMI, education levels, alcohol drinking, smoking status
Referent and exposed were roughly match for age.</td>
<td>gonad function were comparable in all three groups.
Blood glucose was unchanged. In the baseline study, increased level of glycosylated heamoglobin and decreased blood insulin (non-fasting blood samples) was seen in exposed group compare to control. This was not seen in the follow up study (fasting blood samples). Glucose tolerance test was not performed in the study.
T4 concentration was statistically significantly lower in exposed groups ($p \leq 0.05$) but no exposure-relation reduction was seen. No effect was seen in the baseline study.
<table>
<tr><th></th><th>B/F</th><th>CS_2</th><th>Ex CS_2</th><th>Referent</th></tr>
<tr><td rowspan="2">Fasting blood glucose (mg/dl)</td><td>B</td><td>100 (1.2)</td><td>100 (1.2)</td><td>102 (1.2)</td></tr>
<tr><td>F</td><td>100 (1.1)</td><td>100 (1.1)</td><td>99 (1.1)</td></tr>
<tr><td rowspan="2">Insulin (µU/ml)</td><td>B</td><td>7.7(2.6)</td><td>6.7 (2.5)*</td><td>8.8 (2.5)</td></tr>
<tr><td>F</td><td>4.9 (2.0)</td><td>5.4 (1.9)</td><td>5.2 (2.1)</td></tr>
<tr><td rowspan="2">HbA_{1c}</td><td>B</td><td>5.1 (1.1)*</td><td>5.1 (1.1)</td><td>5.0 (1.1)</td></tr>
<tr><td>F</td><td>5.0 (1.1)</td><td>5.0 (1.1)</td><td>5.0 (1.1)</td></tr>
<tr><td rowspan="2">T4 (µg/dl)</td><td>B</td><td>8.6 (1.7)</td><td>8.8 (1.8)</td><td>8.7 (1.6)</td></tr>
<tr><td>F</td><td>8.3 (1.5)*</td><td>8.7 (1.4)</td><td>8.6 (1.5)</td></tr>
</table>
B: baseline survey
F: Follow up survey
ExCS_2: workers from factorties that have closed
The use of multivariable analysis to controls the confounders did not change the results.</td>
</tr>
<tr>
<td>Takebayashi et al., 1998
Study design: cross-sectional study. Baseline observation in a cohort study of Omae et al., 1998.
N: 432 male workers exposed to CS_2 and</td>
<td>Job types (1992): n=309 at spinning and refining processes (higher exposure than other jobs) and n=123 workers at other jobs.</td>
<td>X^2 or fisher’s exact test for frequencies of symptoms without</td>
<td>Effect on the endocrine system and other biochemical indices:
- Decreased serum insulin concentration, unchanged blood glucose. Slight increase in glycosylated haemoglobin concentration. Difference higher in other job type than in the spinning or refining workers.
- Effects on other hormones were not observed.</td>
</tr>
</table>

402 reference workers **Location**: 11 viscose rayon factories (stable fiber or filament fiber) in Japan **Age:** means of 34.8 and 36.9 years in the refining and other type jobs. **Mean duration** of CS_2 exposure: 13.8 years fir spinning and refining workers and 12.6 years for other exposed workers **Analysis:** peripheral nerve conduction velocity, neurobehavioral test, subjective symptoms and psychological tests, effects on the endocrine system and biochemical variables: one blood sample without fasting : blood glucose, glycosylated haemoglobin, serum insulin concentration, serum or plasma FSH, LH, testosterone, ACTH; TSH, T3, T4, TBG, aspartate aminotransferase, Alanine aminotransferase, GTP, total protein, LDH, total bilirubin, uric acid **Exclusions**: none reported **Author conflict of interest:** the study was supported by the Japan Chemical Fiber association **Limitation:** - Missing data from questionnaire or absence during the day of research team visit. No relation with health status or exposure status according to the authors. - Healthy worker effect cannot be excluded - No air measurements data to support biomonitoring results. Difficulties to estimate past exposure due to the improvement in the use of PPE and exposure has decreased over time. - Temporal causal association cannot be studied - Potential co-exposure not discussed and not taken into account	5.5% of the spinning and refining workers had work experience at the other exposed processes and 15.4% of the other exposed workers were engaged in the spinning and refining processes. Reference workers were chosen from non-rayon factories or jobs not exposed to CS_2. Biomonitoring: two TTCA measurements in 1992. 3.13 ±2.27 mg/g Creatinine for the spinning and refining workers and 1.28 ±2.01 mg/g creatinine for other exposed workers. 104 workers of the spinning and refining workers had > 5 mg/g creatinine of TTCA Reference workers had TTCA < LOD (0.5 mg/l). 2% had worked at exposed processes for more than 5 years in the past. None were transferred due to deterioration of health.	adjustments. For continuous variable, t test or Welch's method or Anova followed by Tukey's. Wilcoxon and Kruskal Wallis **Adjustment factor**: age, BMI, education levels, smoking habit, alcohol intake Comparable BMI and drinking habit between groups. Differences in education levels and smoking.	The authors tested the hypothesis that the disturbance of glucose metabolism may cause disorders of the peripheral nerves that may confound the association between exposure to CS_2 and reduction in the nerve conduction velocities seen in the study. Multiple regression models adding concentration of glycosylated haemoglobin as an explanatory variable did not change the regression coefficient of exposure to CS_2 variable suggesting that disturbance of glucose metabolism was not confounding factor.
***(*Vanhoorne *et al*., 1994)** No full-text available, summary as described in the registration dossier	See Vanhoorne *et al*., 1993 Cumulative exposure in exposed at time of investigation: 224	Fisher's exact method and Wilcoxon rank	No statistically significant differences between the exposed and unexposed with respect to age, alcohol intake, BMI, smoking, or stress Sperm sample analysis: 43 exposed and 35 non exposed males only.

Study type: cross-sectional **Location**: A viscose rayon plant in Belgium **N:** 116 male viscose rayon manufacturing workers exposed to CS_2 and 75 male workers not exposed (metalworks, plastic processing and amylum processing factory) **Exposure duration** > 1 year**.** Median: 4.5 years of exposure **Age:** 33.2/33.3 in exposed, non-exposed groups (median) **Analysis: male reproductive toxicity :** sperm samples analysis Self-administered questionnaire used to gather demographic data and work history information **Exclusion:** medication that may impact libido, welders and person exposed to lead were excluded from semen sample analysis, no Dutch speakers, not Caucasian. **Author conflict of interest:** unknown ***Limitations:*** **-**46% of eligible controls participated vs 100% of eligible workers (selection bias) **-** Semen samples only in 43 exposed and 35 control - recall bias is possible as sexual effect of CS_2 is well known to viscose workers - cross sectional study do not allow to determine temporal relationships - Questionable assessment of former exposure - potential co-exposure not taken into account - missing good epidemiology practices information about informed consent - study period not given	mg/m3 x year	Multiple linear regression: effects of continuous exposure on outcomes of interest, adjusting for confounders. Logistic regression: effect of CS_2 on the prevalence of sexual complaints. Regression model goodness of fit was checked using Pearson chi-squared statistic. **Adjustments**: age, alcohol, smoking, body mass index, and stress levels	No exposure effects on semen quality (sperm mobility, concentration, morphology, vitality, pH, fructose, GMGT, ATP concentration. Statistically significant decrease in seminal round cells in exposed workers. The average number of children did not differ between the exposed and unexposed groups. The interval between children did not differ between the exposed and unexposed groups Significant increase in the prevalence of complaints of impotence and decreased libido with high or low present exposure. Relationship to exposure observed after adjustment for impotence and decreased libido. Only two workers knowing that impotence was a potential health effect of CS_2 exposure.

(Vanhoorne *et al*., 1993) No full-text available, summarised as described in the registration dossier Study type: cross-sectional **N:** 117 male viscose rayon workers (manufacturing) exposed to CS_2 and 66 male workers not exposed (metalworks, plastic processing and amylum processing factory) **Exposure duration** > 1 year. **Locatio**n: A viscose rayon plant in Belgium **Age**: median age: 32 year in CS_2 exposed and 34.8 in non-exposed **Analysis:** self-administered questionnaire were used to gather demographic data and work history information. Fasting blood sample were taken for hormone analysis: thyroxine, LH, FSH, prolactin, testosterone **Exclusions**: workers no Dutch speakers, not Caucasian. **Author conflict of interest:** unknown **Reported limitations:** - Only 46% of eligible controls participated compare to 100% of eligible exposed workers (selection bias) - cross sectional study do not allow to determine temporal relationships - low number of samples for exposure measurements, no biomonitoring - previous report have shown fluctuating exposure at workplace - potential co-exposure not taken into account - low number of referent workers compare to exposed workers	Randomly personal monitoring of current CS_2 exposure for 17 jobs (mean for each job title) using charcoal tubes. Exposure range: 1.3-37.3 ppm (often > 10 ppm) Large range of CS_2 exposure in different function, even within the same department 6-14 samples were collected over 5 years per job (Vanhoorne *et al*., 1991) Cumulative exposure index: < 300 mg/m3x years (n=70) and > 300 mg/m^3 x years (n=47). Average concentration: 383 mg/m^3 x year; median: 180 mg/m3*year	Wilcoxon and chi-squared tests : baseline characteristic distributions Wilcoxon and Kruskal-Wallis tests: distributions of endocrinological factors **Adjustments:** age, alcohol, smoking, body mass index, stress levels (Multiple linear regression analysis)	An age-related decrease in thyroxine and increase in LH and FSH was observed. No effect on shiftwork on hormone analysis. Statistically significant decrease in prolactin levels in exposed group (high and moderate). No association following adjustments for potential confounders. No association with cumulative exposure index. No effects on thyroxine, LH, FSH, testosterone.

- missing information on informed consent			
(Selevan (NIOSH), 1983) Summary based on abstract and Silva *et al*., 2013 review Study type: retrospective cohort study Tennesy viscose rayon filament facility (US) N=326 exposed and 304 unexposed Caucasian workers. Analysis: patterns of fetal loss, births, time between live births Limitations: - 91% of wives in exposed group were interviewed compare to 86% in control group - potential recall bias	Exposure of father prior to pregnancy estimated using company employment and industrial hygiene records (4-month before the estimated date of conception 73% exposed below 10ppm, 94% exposed < 20 ppm	Exposed were younger than unexposed. No differences in number of year employed	Results as described in abstract "There was a deficit of fetal loss with increasing exposure immediately preceding conception, but an increase with increasing time since first employment in the exposed areas. Only minimal differences were found between exposed workers and comparisons for numbers of live births. There were no significant differences for standardized fertility ratios. The authors conclude that CS. exposure at the concentrations found in the facility do not have an effect on pregnancy experience."
(Vanhoorne *et al*., 1981) Based on Wrc-NSF, 2002 brief summary > 10 years exposure N=20 exposed viscose workers, N=10 controls (slightly exposed viscose workers)	50-100 mg/m^3		No effects on serum FSH, thyroxine and testosterone levels
(Meyer *et al.* (NIOSH), 1981) Based on review from Silva et al., 2013 Tennesy viscose rayon filament facility (US) N=175 workers Duration of exposure: 1 to 15 years Analysis: semen quality	High: > 10 ppm Moderate: 2-10 ppm Low: < 2ppm		No statistically significant difference between the control and CS_2 exposed groups was observed. According to the authors, the lack of effects on semen parameters may be due to regulations calling for lower CS_2 exposure levels in factory conditions than were previously allowed. Additionally, CS_2 exposures (i.e. length of employment) may have been too brief to induce measurable effects.
(Wagär *et al*., 1983) Based on abstract and review from Silva et al., 2013 and Gelbke, 2009 Male viscose rayon factory workers (Hernberg et al.,1970 cohort) **N:** 69 exposed to CS_2 and 22 non exposed **Age:** mean age of 40 years for exposed and 39 years for non-exposed. Mean **duration of exposure**: 1 to 36 years (mean 12.5)	Stationary sampling sites 1960s : 4 to 20 ppm 1970s: 3 to 13 ppm 1980s: 1 to 10 ppm		Overall, significant increase in FSH (13.6 vs 10 IU/l but significant decreased in SHBG (44 versus 53 nmol/l). < 39 years of age (1-9 years CS_2 duration of exposure): ↑LH, FSH (after 10 years exposure only), free testosterone index, ↓ SHBG ≥ 40 years of age: ↑FSH, LH only after ≥ 10 years exposure duration No effects in serum prolactin, cortisol, thyroxine and TSH and testosterone levels

Exposure period: 1947-1983 Limitations: small sample size			
(Cirla and Graziano, 1981) **Study type**: cross-sectional **Location:** Viscose rayon factory in Italy **N**: 50 workers exposed to CS_2 (yarn washing department) and 50 non exposed workers (same factory) **Analysis**: interview with nurse, questionnaire, physical exam and blood sample: glycemia, thyroxine, FSH, LH, testosterone, serum lipid (cholesterol, triglicerid) **Limitations:** - missing good epidemiology practices information - Lack of stratification of the workers by exposure period. The workers have between 3 and 12 years of exposure, but none of the results reflect that time difference. The group size of 50 workers and 50 controls is relatively small, and there is no information on participation rates of either exposed or unexposed workers. - The small number of abnormalities detected in this population limits the statistical power. The cross-sectional study design is inherently weak due to an inability to determine temporal relationships between exposure and outcome, and difficulty in avoiding bias in the selected study sample.	Stationary air sampling 3.2-8 ppm for 3-12 years exposure Always below 10 ppm	For quantitative variables, paired Student's t-test was used. For abnormal percentages in matched pairs, McNemar test (marginal chi-squared) was used Exposed and unexposed workers were matched on sex, age, physical features, work shift, smoking history, alcohol history	No evidence of adverse effects
(Cirla *et al*., 1978) Summary based on abstract, reviews from Silva *et al*., 2013 and Gelbke *et al.,* 2009 Viscose rayon factory in Italy	Air stationary and personal samplings after 1970 Grouping of exposure by level and duration: "Very light": < 19 ppm for < 4		For the very light/light, heavy, and heavy in the past exposed, bound T4 was significantly lower as compared to controls LH and FSH significantly decreased in heavy exposed as compared to controls. No effect on testosterone levels. Decrease in prolactin in relation to exposure.

N: 254 exposed and 54 control workers Analysis: bound T4, U UTBG, free thyroxine index, LH, FSH, testosterone, prolactin Sexual behaviour studied by questionnaire Limitations reported: insufficient exposure data, prolactin was not analysed in controls	years; "light" : ≤ 19ppm; "moderate": 20-40 ppm "heavy": 38.4-76.8 ppm; higher peak values possible in the past; "Heavy in the past": 57.6-76.8 ppm; higher peak values possible in the past; last 12 years < 19 ppm. "heavy but suspended in the past": 40-80 ppm, exposure was terminated		By questionnaire: complaints about reduced sexual potency already after a few years of exposure. No definite correlation seen with hormonal deficit. The authors suggested that the data suggest a direct effect of CS_2 on pituitary activity.
(Lancranjan *et al*., 1972) 140 young exposed viscose workers with intoxication symptoms and 50 controls Location: spinning section of an artificial fibre factory Average age: 30 years 3.5 years exposure Analysis: questionnaire for libido, sexual dynamic troubles. Semen analysis (n=103). Follow-up in 18 patients after removal from exposure	No data		75% of the subject showed sensitive-motive polyneuritis. Decreased libido, ejaculation or orgasm troubles in patients compare to controls. Hypospermia, asthenospermia and teratospermia in high number of patients compare to controls. Decreased excretion of urinary 17 ketosteroid in these patients. High frequency of spermatogenesis alteration (78% in patients). 66% (12) had favorable evolution 3-30 month following cessation of exposure. Three had progressive disorders (17%). No correlation with length of exposure was proved.
(Wink, 1970) Based on Wrc-NSF, 2002 brief summary Exposure period: 12 years N: 15 exposed and 15 control workers	30 mg/m^3		No effects on 17-keto and 17-hydroxy steroid levels in urine.
(Lancranjan *et al*., 1969) Based on Wrc-NSF, 2002 brief summary and abstract 33 exposed young workmen with intoxication symptoms (neurological, vascular or other effects of chronic poisoning) and 31 control subjects Localisation: Bucharest, spinning section of an artificial fibers factory	40-80 mg/m^3	Control and poisoned workers were matched for age	Disorders of spermatogenesis: terotospermia (25/33 exposed, 4/31 controls), asthenospermia (18 exposed, 3 controls), hypospermia (11 exposed, 3 controls) Decreased excretion of 17-keto-steroids (32 patients grouped according to length of exposure, 9 controls), no significant differences between exposure groups. 25/32 patients with toxic polyneuritis suffered from various disabilities of sexual function.

Exposure duration: 7- 42 months			Urinary excretion of total gonadotropins (immature mouse test): 10-25 mice units/ 24h in 10/10 controls and 20/23 < control values. The 3 patients with normal values however had low values for 17-ketosteroid excretion and also malformations of spermatozoa.
(Cavalleri *et al*., 1966) Based on Wrc-NSF, 2002 brief summary	185-525 mg/m^3		Decreased excretion of 17-keto and 17-hydroxy steroids dependent on exposure period

Table 15: Summary of key toxicity studies on female fertility and hormonal effects in humans

(Zhou *et al*., 1988) **Study type:** retrospective cohort study **N**: 265 women with no menstrual disorder at the beginning of exposure. Women exposed since 1964 or later for at least 1 year by the end of 1985 in viscose rayon plants; 291 age-matched non-exposed female workers from a thread factory. **Location:** 5 viscose rayon plants in Shanghai **Analysis:** survey performed by an interviewer: occupational history (job types, type of chemical exposure, exposure duration), menstrual history (age at menarche, days of menstrual cycle, duration and quantity of menses and symptoms during menstruation) and factors that might affect menstruation (change of living conditions, previous diseases, drugs..), term and outcome of pregnancy for married women and factors that may influence **Author conflict of interest:** no information **Reported limitations:**	Environmental monitoring of viscose rayon plants in Shanghai since 1970. Air samples taken from various worksites of the plants analysed once a week. Calculation of monthly median concentration of CS_2 for each plant and average yearly concentration. Parallel sampling study in one plant: air samples from every worker sites were taken and analysed twice per 8h shift for two shifts. Data were obtained from 153 air samples Average CS_2 concentration in the early years of the 1970s were 10 mg/m3. The levels decreased gradually since 1975, and the average level has been below 10 mg/m3 throughout 1980s. Based on CS_2 concentration in the work environment, the five plants were grouped into "low contamination" with an average C_{S2} concentration of 3.1 mg/m3, intermediated contamination at 6.5 mg/m3 and high contamination at 14.8 mg/m3 (eq to 0.98, 2.1 and 4.7 ppm according to default conversion factor).	t-test, chi-square test, Mantel-Haenszel, Cox Adjustments: age, observation duration, reproductive and birth control status	Increased menstrual disturbance was significantly higher: RR=2 ($p< 0.01$) Irregularity of menstruation was the most common disturbance followed by abnormal quantity of bleeding. Association was observed between these incidences and exposure levels. Effects on menstrual disturbance still observed after adjustments. No effects on the outcome of pregnancy was observed.

- Interviewer were trained but not stated if there were blind to exposed/unexposed - information bias possible (recall bias, prevarication bias) - Uncertainties on exposure: only air data, no individual monitoring exposure or biomonitoring, variability between job types observed. - unclear how the 5 plants out of 12 were chosen			
(Hemminki and Niemi, 1982) Based on Wrc-NSF, 2002 brief summary Type of study: population study 9000 chemical female workers in Finland	No exposure data		Increased spontaneous abortion in female of the viscose rayon industry
(Cai and Bao, 1981) Female workers and breast-fed babies in spinning in a viscose rayon factory. Control: breast fed babies and female workers in finishing in the same factory **N:** 183 spinning women and 197 finishing women for the survey on menstruation 100 spinning and 104 finishing for the survey on pregnancy toxemia and abnormal delivery Milk content in 38 spinning women and 4 controls. Umbilical content in 3 exposed newborn, no controls Age: 18 to 32 years in both groups **Work duration** > 1 year (6 years maximum) Analysis: questionnaire, milk collection, umbilical blood content	Co-exposure H_2S and CS_2. Both substances are not present in finishing. 30 representative spot in summer and winter in the spinning room Concentration of CS_2 in the air. CS_2 content in mother milk, umbilical cord blood and urine Concentration of H2S in the air was measured and was low: 8 mg/m3 in summer and 5 mg/m3 in winter Concentration of CS_2: 56 mg/m3 in summer (average 22 to 135 mg/m3) and 37 (11-92) mg/m3 in winter Measure 8hTWA (men and women): 50.5 mg/m3 for spinners, 40.5 mg/m3 for throwster and 37.3 mg/m3 for Doffer groups Average CS_2 content I urine (men and women after work): 6.9 µg/ml.	Not clearly described	Absenteeism higher in the spinning workers than in finishing workers Statistically significant increase incidence of disturbances in menstruation in exposed females. Still significantly higher considering only workers with no disturbance at the entrance of the factory. Most frequent effect reported: length of menstrual cycle. Statistically significant increase in pregnancy toxemia in exposed group. CS_2 present in milk and in urine of babies Related to air and exposure time. Not detected in finishing workers. Detected in the umbilical cord of the newborns (delivery room from the workplace).

Table 16: Summary of key toxicity studies on fertility and sexual organ toxicity in animals (rated Klimisch 1 or 2)

Method	Type of effect

Unpublished report, 2019 Extended one-generation reproductive toxicity study including DNT and F2 generation (OECD 443) 2 (reliable with limitations) Test material: carbon disulphide Vehicle: corn oil Purity: confidential Crl:WI(Han) rats Oral: gavage Doses / Concentrations: 0, 1.2, 12, 120 mg/kg bw/day TK parallel comparative oral vs inhalation route of exposure: dose levels corresponding to around 10, 100, 950 mg/m^3. F0 male exposure: 10 weeks before mating, throughout mating until their termination; F0 females: 10 weeks before mating, throughout mating, gestation and at least 21 days after delivery up to the day before scheduled necropsy. F1 animals cohort 1A, 1B, 1C and 2A: dosed up to and including the day before scheduled necropsy. F2B, cohort surplus and spares, F2 animals were not dosed. Observation: body weight, food consumption, oestrous cyclicity, sperm parameters, Limitations: - Although some neurotoxicity was observed in parental animals, higher dose level may have been considered. Mid dose selection is questionable in view of the large interval between the low and the high dose.	**<u>General toxicity, clinical chemistry and organ toxicity</u>** **Body weight (120 mg/kg)** P0: ↓ slight decrease in bw and bw gain in males and females during premating (top dose, mostly statistically significant). At termination, no differences in bw in treated and control females. F1-generation: ↓ bw gain in males and females (97% of controls in females and 89% of controls in males at the end of the treatment period, statistical significance in males at some time point). **Food consumption/survival** P0/F1: no effects on food efficiency, no effect on survival **Clinical chemistry (at 120 mg/kg)** P0: ↓* T4 in males (0.51x) and lactating females (0.69x); No change in TSH. F1-generation:↓* glucose levels (mean 0.73x control) outside historical control range ↓* T4 levels in males, outside historical range (0.74 x controls). No effects in females. No change in TSH. **Organ specific toxicity** <u>Thymus (120 mg/kg)</u> P0: - ↓** absolute and relative weight (-26% and -17% of control, respectively, p<0,01), - ↑ incidence of lymphoid depletion: 16/25 males (graded minimal to slight) *vs* 1/25 in control males (graded minimal). - No degenerative changes observed. F1-generation: - Dose-related ↓** absolute and relative thymus weight in males (-33 and -20% of control). - ↑ incidence of lymphoid depletion in males (9/20) and females (8/20) graded minimal to slight. Not observed in controls. - No degenerative changes observed. <u>Spleen (</u>≥ 12 mg/kg) P0: dose-related ↑** relative spleen weight at ≥ 12 mg/kg in males (+10% at 12 mg/kg and + 19% at 120 mg/kg compare to controls, p<0.01). P0: dose-related ↑ incidence and severity of hematopoiesis at ≥ 12 mg/kg in males (11/25 in controls, graded minimal, 19/25 at 12 mg/kg and 23/25 at 120 mg/kg, graded minimal to slight) P0: ↑ incidence and severity of hemosiderine pigment at 120 mg/kg in males (11/20 in controls and 22/25 at 120 mg/kg) and females (20/25 in controls and 25/25 at 120 mg/kg), graded slight to moderate. <u>Adrenals (120 mg/kg)</u> P0: ↑** relative weight (males) <u>Liver</u>: (120 mg/kg) P0: ↑** relative liver weight (males)

- Methodological deficiencies in the DNT: • Appropriate statistics that would test for interactions between the major dependent variables is needed. Statistical re-analysis is however necessary for any conclusion: data analysis should include an overall ANOVA that includes treatment, sex and time blocks. • No positive control was used in the DNT study • No historical control data were provided in the DNT study - No histopathology of organs/tissues performed in cohort 1B although histopathological findings were observed in cohort F1A.	Eyes (120 mg/kg) P0: ↑ incidence and severity of retinal atrophy: 3/25 females graded severe and in males (incidence not provided), graded minimal-moderate. F1-generation: ↑ incidence of loss of cell layers in the retina, slight severity in males and females Brain *P0 (120 mg/kg):* - ↓** absolute brain weight (-6% and -5% relative to controls in males and females respectively, p< 0.01) *F1-generation (120 mg/kg):* - ↓** absolute brain weight (-10% and -8% of controls in males and females respectively, p< 0.01). - ↑* relative brain weight (+9% of control) in males. **Litter observations: F1** No effects on survival, food consumption, clinical signs. Slight effect on body weight (around 5%). No effects on T4 levels at PND4. No effects in mean serum level T4 and TSH in the cohort surplus and Cohort 2B, PND 21-22. Decreased anogenital distance (AGD) in male pups, statistically significant when normalised for body weight. Within historical control range. No tabulated data provided. No information whether cube root of body weight was used for normalisation. Brain ↓* absolute brain weight in both sexes. No effect in relative brain weight. **Litter observations: F2** No effect on body weight. No effects on TSH and T4 levels. No effect on eyes. Brain ↓* absolute and relative brain weight in male pups at 120 mg/kg (0.95x and 0.92x control, respectively), outside historical control range **DNT (Cohorts 2A and 2B)** Motor activity and auditory startle were not affected by treatment. *Functional observed battery, 120 mg/kg:* - ↓ mean footsplay value in males and females, individual value partly outside historical control data range: 98, 93, 65% of control in males from low to high dose and 93, 88 and 75% in female from low to high dose. Not statistically significant but dose-related in both male and females and < 20%.

	F1 Cohort 2B (120 mg/kg, terminated PND 21-22): morphometry - ↓* brain width in females at ≥ 12 mg/kg (-3% vs -2% in controls) - ↑* mean caudate putamen width in females in all treated groups *F1 Cohort 2A, PND76-90: morphometry* - no changes in test item-related brain dimension (length and width) - ↓* mean corpus callosum thickness in males. Almost all (8/10) of the individual values for corpus callosum thickness in males were lower than the control group. - ↑* higher mean caudate putamen width in females. 7 out of 10 have values inside historical control range at 120 mg/kg. **Developmental toxicity** P0: ↓ Post-implantation survival index: 94, 94, 88 and 87% for the control, 1.2, 12 and 120 mg/kg/day groups, respectively. ↓ mean litter sizes were 10.6, 11.4, 11.8 and 9.8 living pups/litter for the control, 1.2, 12 and 120 mg/kg/day groups, respectively. Individual values remained within the concurrent control range and mean values remained within the available historical control range. Cohort 1B (=F1-generation) Post-implantation survival index was 98% for the control and 1.2 mg/kg/day groups and 93% for the 12 and 120 mg/kg/day groups. ↓ mean litter sizes: 12.2, 11.1, 11.7 and 10.8 living pups/litter for the control, 1.2, 12 and 120 mg/kg/day groups, respectively. Decreased number of implantation sites (> 10%) at the top dose: 12.6, 11.5, 11.9 and 11.1 at 0, 1.2, 12 and 120 mg/kg, respectively **Reproductive function/performance** Estrous cycle P0: A slight increase in irregular cycle was noted at 12 and 120 mg/kg (3/25 at 12 mg/kg and 4/25 at 120 mg/kg, vs 1 in controls and low dose). Ovaries F1 cohort 1A: ↓** primary and primordial follicle count at 120 mg/kg (34 vs 49 in controls). Decreased number of corporea lutea at the top dose (22.5% decrease compare to control, not statistically significant, not investigated at the mid and low dose levels).

	<u>Sperm measures (120 mg/kg)</u> P0, within historical control range (according to IUCLID study summary): ↓ percentage of motile sperm (0.86x control) ↓ Percentage for progressive sperm (0.74x control). Normal progression of the spermatogenic cycle. ↓ number of cells with coiled tail (0.64x control) F1-generation (cohort 1A): ↑* cells with detached head (4x controls). Mean values outside historical control range (mean: 3.7, Percentile 95: 8). ↑* Mean total sperm count in the epididymis, and percentage motile and progressive sperm in males of the 12 mg/kg/day group only. The mean values for percentage motile and progressive sperm remained within the historical control range. General toxicity: LOAEL= 120 mg/kg, NOAEL = 12 mg/kg (brain, retinal atrophy, thymus, spleen) DNT: LOAEL = 120 mg/kg, NOAEL = 12 mg/kg (footsplay, brain and morphometric changes) Developmental toxicity: LOAEL = 120 mg/kg, NOAEL = 12 mg/kg (post-implantation losses, number of total implantation, mean litter size) Reproductive function toxicity: LOAEL= 120 mg/kg, NOAEL = 12 mg/kg (sperm changes, effects on primordial follicles)
(Unpublished report, 1992b; Nemec *et al.*, 1993) Non-guideline female one-generation reproductive and developmental toxicity study (GLP-compliant) Inhalation; 6h/d. Exposure: 14 days prior to mating, during mating and until gestation day 19. 0, 395, 790, 1580 mg/m3 (125, 250, 500 ppm) Potential adverse effects on gonadal function, estrous cycles, conception rates, parturition and lactation of the F0 maternal generation were examined. Viability, growth and development of the F1 litters were also assessed Klimisch score: 2 (reliable with limitations)	Maternal toxicity at 500 ppm: Body weight losses, decreased food consumption Reproductive toxicity in females: No effects on estrous cycle, mating index, fertility index. Dystocia observed in exposed females at 500 ppm. Developmental toxicity at 500 ppm: mortality of the pups, decreased viability, decreased litter size

Limitations: Non-guideline study Few parameter investigated.	

Table 17: summary of toxicity studies on fertility and sexual organ toxicity in animals rated Klimisch 1 or 2 or from secondary litteraure (Klimisch 4) and provided mechanistic insights

Method	Type of effect
(Gao *et al*., 2014) Male Sragues Dawley rats N=8/group 0,equivalent to 158, 790, 3950 mg/m^3 (0, 50, 250, 1250 ppm), 0 +Cyclosporine A, 1250+cyclosporine A ppm in groups I, II, III, IV, V and VI, respectively 2h-exposure, 5d/w during 10 weeks Analysis: testis Klimisch score 2 (mechanistic data) Limitations: - low number of animal per groups - only 2h exposure - information on general health status of animals not provided	No bw effects in male rats Marked statistically significant decreased in absolute testis weight at ≥ 250 ppm (3.24±0.35g in control vs 2.56±0.26g at 250 ppm and 2.56±0.26g at 1250 ppm) Cellular damage at ≥ 250 ppm Ultrastructural observation: deformed chromatin in Sertoli cells, disrupted membrane junction, vacuoles in spermatocytes suggesting cellular damage of testes. Increased apoptosis in germ cells in CS_2 exposed groups. Dysregulation of mitochondrial function.
(Guo *et al*., 2015) Male Sprague Dawley rats **N=10/groups** 0, 158, 790, 3950 mg/m^3 (eq. to 0, 50, 250, 1250 ppm) 2h-exposure, 5d/w during 4 weeks Analysis: testis Klimisch score 2 (mechanistic data) Limitations:	Morphological changes in testis induced by CS_2. Microscopy: loose structures of seminiferous tubules and disordered cell arrangements Ultrastructural lesions: effects on chromatins, vacuoles formed from Swollen endoplasmic reticulum Increase apoptosis in Sertoli cells primary culture.

- information on general health status of animals not provided - only 2h exposure	
(Huang *et al*., 2012) Male Sprague-Dawley rats Inhalation, whole body, 2h/d, 5d/w, 10 weeks Controls, 50, 250, 1250 mg/m^3 (eq. to 16, 79, 396 ppm), 1250 mg/m^3 + sodium prusside and 1250mg/m^3 and NG-monomethyl-l-arginine N= 6/groups Klimisch score 2 (mechanistic data) Limitation: - Low number of animals per groups - No information on source and purity of CS_2 - Only 2h/d exposure - No information on general health status of treated animals	<u>Activity of NOS, iNOS and NO concentration</u> ↓NOS, iNOS and NO concentration in serum, hypothalamus, pituitary and testis at 1250 mg/m^3 CS_2 groups. Some changes also at mid and low dose groups in pituitary <u>Concentration of sex hormones in serum</u> ↓ GnRH, LH and T in serum, ↑FSH (statistically significant) at 1250 mg/m^3 No effects when rats were all treated with NG-monomethyl-l-arginine. Sodium prusside reverse the effect of CS_2 <u>Sperm quantity and quality</u> Statistically significant decrease in sperm concentration at the mid and high dose <u>Sperm morphology and motility</u> Dose related statistically significant increase in abnormal sperm in all treated groups (7.17% in high dose compare to 1.5% in controls). No difference following treatment with sodium prusside or NG-monomethyl-l-arginine.
(Kumar *et al*., 1999) Non guideline reproductive organ toxicity study in males Charles-Foester rats Intraperitoneal route of exposure Klimisch score: 4 (summarized as reported in wrc-NSF, 2002)	↓ sperm count at ≥ 100 mg/kg ↑ sperm-head shape abnormalities at 200 mg/kg
(Patel *et al*., 1999) Non guideline reproductive organ toxicity study in males Charles-Foester rats Intraperitoneal route of exposure 10 males/groups	- ↓ body weight Thickening and rupturing of seminiferous basement membrane Degeneration/disorganisation of spermatogonial cells Fewer/absent sperm in the lumen at ≥ 100 mg/kg No histopathological findings in epididymal tissue ↓ Serum testosterone (ng/dl) at all doses: Serum testosterone levels (ng/dl)

<table>
<tr>
<td>Vehicle: cotton seed oil
0, 25, 50, 100, 200 mg/kg
30-day exposure

Klimisch score: 4 (summarized as reported in Silva et al., 2013)

<u>Limitations</u>
- Full text article not available
- i.p. route is not the representative of exposure in human</td>
<td>
<table>
<tr><td>Doses (mg/kg)</td><td>0</td><td>25</td><td>50</td><td>100</td><td>200</td></tr>
<tr><td>Mean</td><td>505</td><td>224*</td><td>208*</td><td>84*</td><td>32*</td></tr>
<tr><td>SD</td><td>162</td><td>54</td><td>73</td><td>32</td><td>10</td></tr>
</table>
Silva et al., 2013 performed Benchmark dose analysis on testosterone levels but no models fit.
</td>
</tr>
<tr>
<td>(Zenick et al., 1984)

Male Long Evans Hooded rats
Inhalation, 6h/d, 5d/w, 10 weeks
0, 1896 mg/m^3 (eq. to 600 ppm, actual concentration)
N= 14/group + 10/group unmated, unbled and unmanipulated animals

Mating once a week throughout the study.
Treated and controls were match for mean sperm count.
Analysis: mating behaviour and semen parameters (preexposure, 1, 4, 7 and 10 weeks of exposure) 1h post-exposure. After 10 week exposure, analysis of testosterone, LH and FSH. Hormone response to human chorionic gonadotropin (HCG) and GnRH challenges at week 10 in 5 rats per groups.

Weight of organs: testis, epididymis, vas deferens, seminal vesicle, prostate
Necropsy: epididymis, testis

Klimisch score: 2 (mechanistic data)
Limitations:
- Non guideline study
- No GLP status</td>
<td><u>General toxicity</u>
According to the authors, animals were in good health. No signs of neurotoxicity was observed.
↓* body weight at the end of the 10-week exposure period in CS_2-treated groups 10%

<u>Copulatory behavior.</u>
High variability was observed;
A decrease in ejaculation latency was seen from Week 4. No effects on the number of mounts or intromissions or in the analysis of these behaviors combined. Treated animals performed the same number of events within an abbreviated time frame.

<u>Semen evaluation</u>
<table>
<tr><td>Mean</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Weeks</td><td>Baseline</td><td>Week 4</td><td>Week 7</td><td>Week 10</td></tr>
<tr><td>Sample size</td><td>12</td><td>12</td><td>12</td><td>12</td></tr>
<tr><td>Epididymal sperm count (x10^6)
Control
CS_2</td><td>
52
58</td><td>
54
53 (97%)</td><td>
57
39
(72%)**</td><td>
54
35
(64%)**</td></tr>
</table>
In parentheses: % compare to baseline
</td>
</tr>
</table>

<table>
<tr>
<td>- Only one dose level
- Source of test material not provided
- According to the authors, there were occasional missing values on animals
Because of problems of refrigeration, baseline values for hormonal levels were obtained for 6/13 control and 8/13 exposed animals</td>
<td>Decreased sperm counts at weeks 7 and 10 of exposure. No effects on sperm mobility.

<u>Hormone analysis</u>
No effects on testosterone, FSH and LH. No effects seen following HCG or GnRH challenges.

<u>Termination</u>
The only difference was the decrease in prostate weight in the mated CS_2 group relative to their mated controls. Similar trend was seen in the relative prostate weight.
Decrease in seminal plug weights also observed.
No effects on sperm counts from the cauda epididymis.
No alteration at necropsy of epididymis or testis. Values observed in the CS_2 exposed group fell within the normal range obtained in the laboratory whereas elevated values were observed in controls.</td>
</tr>
<tr>
<td>(Tepe and Zenick, 1984)

Long-Evans hooded male rats
Inhalation, 5h/d, 5d/w for 10 weeks

Experiment I: unmated rats
0, 1106, 1896 mg/m3 (eq. to 0, 350, 600 ppm)
LH, FSH, testosterone levels in blood.
Microscopic examination in testis, cauda epididymis, epidermal sperm count in 5 animals/group

Experiment II : sexual active rats
0, 1896 mg/m3 (eq to 600ppm)
Males mated with females. Evaluation of copulatory behaviour, semen and plasma hormone levels 1 week prior to exposure and after 1, 4, 7 and 10 weeks of exposure (8 to 10 hr post exposure).
After 10-week exposure: same analysis as in experiment I

Klimisch score: 2 (mechanistic information)

<u>Limitations:</u>
- Non guideline study</td>
<td><u>Experiment I</u>
↓* body weight at the end of the 10-week exposure period in CS_2-treated groups. No changes in reproductive organ weight compare to control (data not shown).
Epididymal sperm count: similar in all treated groups. Slightly decreased at 600ppm but not statistically significant.
No histopathological findings were observed.
No effects on plasma gonadotropin values.
↓* plasma testosterone levels

<table>
<tr><td>Mean ± SD</td><td></td><td></td><td></td></tr>
<tr><td>Doses (mg/m3)</td><td>0</td><td>350</td><td>600</td></tr>
<tr><td>Sample size</td><td>29</td><td>15</td><td>15</td></tr>
<tr><td>Body weight (g)</td><td>548±10</td><td>518±9</td><td>503±15*</td></tr>
<tr><td>Plasma testosterone levels (ng/ml)</td><td>3.45±0.60</td><td>5.36 ±0.80</td><td>1.75±0.26*</td></tr>
<tr><td>Epididymal sperm count (x10^8/g cauda)</td><td>8.6±0.7</td><td>8.6±0.4</td><td>7.5±0.8</td></tr>
<tr><td>% normal sperm morphology</td><td>98.8±0.3</td><td>98.8±0.2</td><td>98.3±0.5</td></tr>
</table>
*: p<0.05

<u>Experiment II</u>
Copulatory behavior:
- ↓* mount latencies after 7-week exposure
- ↓* ejaculation latencies after 4-week exposure
- The number of mounts and intromissions remained similar in both CS_2-exposed and control groups (data not shown).
- When an ejaculated sperm count was obtained from the same animal on several occasions, there was a significant decrease in count from the CS_2- exposed animals ($P< 0.01$). A marked decrease in sperm count occurred after 7 weeks of CS_2 exposure and remained depressed throughout the 10 weeks of exposure.</td>
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</table>

<table>
<tr>
<td>- No information on GLP status
- Temperature and humidity the cages and inhalation chamber not specified
- Source of test material not provided
- Number of animals at the start of each experiment in each group not specified.
- Low number of animals per groups for the necropsy of male reproductive organs
- Only two dose levels in experiment 1 and only one dose level in experiment 2.
- Actual data not provided for CS2 concentration.
Unknown if experimenters were blind</td>
<td>- No effects in plasma testosterone, FSH or LH values. Lower plasma testosterone in treated group mostly due to increase in testosterone during week 1 in control group.
After 10-week exposure (7 control and 11 treated animals to 600ppm):
- No statistically significant effects on body weight and on reproductive organ weight.
- ↓ epididymal sperm count and ejaculated serum counts
- No effects in plasma testosterone, FSH or LH values
<table>
<tr><td colspan="3">Mean ± SD</td></tr>
<tr><td>Doses (mg/m3)</td><td>0</td><td>600</td></tr>
<tr><td>Sample size</td><td>7</td><td>11</td></tr>
<tr><td>Body weight (g)</td><td>507±12</td><td>476±12</td></tr>
<tr><td>Plasma testosterone levels (ng/ml)</td><td>5.38±0.85</td><td>4.85±0.64</td></tr>
<tr><td>Epididymal sperm count (x10^8/g cauda)</td><td>11.5±1.3*</td><td>8±0.9*</td></tr>
</table>
*, p<0.05</td>
</tr>
<tr>
<td>(Agadzhanova et al., 1978)

Adult female rats

Inhalation
0, 1, 10, 100 mg/m^3
4-month exposure

Klimisch score: 4 (summarized as reported in wrc-NSF, 2002)</td>
<td>Significant prolongation of oestrous cycle (relative to controls) at 10 and 100 mg/m^3</td>
</tr>
</table>

Table 18: Summary of key toxicity studies on development in animals

Method	Type of effect
RATS	

(Saillanfait *et al*., 1989) Prenatal developmental toxicity study (similar to OECD 414) Pregnant rat (Sprague-Dawley) Inhalation: vapour 0, 100, 200, 400 or 800 ppm (nominal conc. equivalent to 316, 632, 1264, 2528 mg/m^3), 100 ppm H2S, 100 ppm H_2S + 400 or 800 ppm CS_2 Exposure: 6 h/d during gestation days 6-20 (daily) 20-23 rats/group Sacrifice: GD 21 Klimisch score : 2 reliable with limitations Limitations - non GLP study	**Effects of CS_2 alone** <u>Maternal toxicity</u> No mortality Stat. significant decrease in body weight gain and corrected bw gain at ≥ 400 ppm NOAEL = 200 ppm <u>Developmental toxicity</u> - No effects on number of implantations, number of resorptions and number live foetuses and fetal sex ratio - Decreased fetal body weight at ≥ 400 ppm (7% and 14% of the male controls and 6% and 20% of the female controls at 400 and 800 ppm, respectively) - Increase unossified sternebrae at 800 ppm - Increased club foots at ≥ 400 ppm (not statistically significant), 1 in one litter at 400 ppm and 7 in 5 litters at 800 ppm, not seen in other groups - No visceral or skeletal anomalies NOAEL = 200 ppm **Effects of CS_2 and H_2S combination** Slight enhancement of maternal toxicity. Lower fetal body weight was the only parameter indicating fetal toxicity to be significantly potentiated by co-exposition. Club foot: 2 in 2 litters at 400 ppm CS_2 + 100 ppm H2S and 1 in one litter at 800 ppm CS_2 + 100 ppm H2S
Lehotsky *et al*. (1985) Lati: CFY Rat, inhalation 3, 230, 640 ppm (eq. to 0, 10, 700, 2000 mg/m^3) GD 7-15, 6h/d, vertical inhalation chamber 8 dams in treated groups, 10 controls Analysis: development of gait, motor coordination (rotarod) and activity (open field), avoidance learning and swimming Klimisch score : 3 (unreliable) Limitations - Non guideline, non GLP study - Actual concentration not provided	<u>Maternal toxicity</u> 33% mortality among dams at 640 ppm (tremor, muscle weakness) <u>Developmental toxicity</u> Mortality among pups: 50% at 600 ppm, at 35% 225 ppm. Survivor were hyperirritable Decreased mean pup weight from 230 ppm Eye opening and auditory startle were retarded. The righting response was not mature There were immature gait, motor incoordination, diminished open field activity and altered behavioural patterns on day 21 and 36 but there were nearly age-appropriate on day 90. A signs of disturbed learning ability, there were diminished performed and lengthened latency of the conditioned avoidance response, related to the concentrations administered (statistically significant). Effects more severe at 640 ppm than 230 ppm (effects similar at 3 ppm and 230 ppm)

- Very low number of animals per groups used at the start of the study, only 2 to 4 animals per groups during experiments due to mortality - No information on source and purity of CS_2 - No information on the age of dam at the beginning of the study - Statistical significance basis not reported - Unknown number of animals used on the behavioral studies - Data on a new mixture aromatol, also used in the study not shown (no effects)	

(Jones-Price *et al.* (NTP), 1984a) Prenatal developmental toxicity study (similar to OECD 414) CD rats Oral : gavage 0, 100, 200, 400, 600 mg/kg GD 6-15 Sacrifice: GD20 Vehicle : corn oil Klimisch score 4 (IRIS chemical assessment summary) Limitations - No detailed information	<u>Maternal toxicity</u> Decreased body weight gain at all dose levels <u>Developmental toxicity</u> Decreased average fetal body weight at ≥ 200 mg/kg No effects on resorptions or malformations at any dose levels NOAEL dev: ≥ 100 mg/kg
(Tabacova *et al.*, 1983) Neurobehavioral and developmental toxicity study Rats Inhalation, 8h/d F1 exposed during whole gestation to 10,100, 1184, 1991 mg/m^3 (eq. 3.2, 32, 315, 630 ppm) and mated to produce F2 Half of pregnant F1 females were exposure throughout gestation to the same concentration 30-32 females/groups Klimisch score 3 (unreliable) Limitations - Non guideline study, not similar to OECD TG - Non GLP study - No information on CS_2 source and purity - Uncertainties on exposure concentration - Unknown strain of rats - Low number of litters - Behavioral tests - Behavioral tests were only performed at the lower exposure levels - Only weight of dams was investigated, no information on survival, clinical signs, neurotoxicity of dams	<u>Maternal toxicity</u> F0 : decreased maternal weight gain at ≥ 100 mg/m^{3,} oxygen consumption of the placenta and free fatty acids in maternal liver at ≥ 200 mg/m^3, during gestation in F0 and in F1 with continued CS_2 exposure <u>Developmental toxicity</u> F1: Increased malformed fetuses at ≥ 100 mg/m^3 (hydrocephalus, generalized oedema, club foot, tail deformation, hypognathia) F2 (continued exposure): increased malformed fetuses from 10 mg/m3 (same type of malformation as in F1) Hexobarbital sleeping time (suggestive of retarded development of liver metabolising system): effects at 10 mg/m^3 in F1 and F2 <u>Behavioral and functional disturbance</u> Motor activity in open field: effects at 10 mg/m^3 and 100 mg/m^3 in F1 and F2 (continued CS_2 exposure) Crossing narrow path (PND 12):effects at 10 and 100 mg/m^3 in F2 Disappearance at the end of the first month of life, new challenges produce more pronounce effects.

(Hardin *et al.*, 1981) Female rats and rabbits inhalation at 0, 62.3 and 124.6 mg/m^3 (eq. to 20 ppm or 40 ppm) from 34 weeks before breeding and during pregnancy Klimisch score 4 (As summarised in WRc-NSF report) Limitations: -exposure period not given	No significant teratogenic effects and no effects on course of pregnancy (relative to the controls) at any test dose for either species NOAEL dev > 40 ppm
(Tabacova and Balabaeva, 1980) Rats 0.03, 10 mg/m^3 Klimisch score 4 (As summarised in abstract and WRc-NSF report)	No increase in malformation or functional biochemical changes. At 10 mg/m3: effects on viability, retardation of morphological and sensory development At ≥ 0.03 mg/m3 : behavioral deviations
(Beliles *et al.*, 1980) Female rat Inhalation, 7h/d, 5d/w 0, 60-120 mg/m3 (eq. to 190 – 379 ppm) Exposure: from 3 weeks before mating to GD18 Klimisch score 4 (As summarised in WRc-NSF report)	No significant teratogenic effects and no effects on course of pregnancy
(Tabacova *et al.*, 1978) Pre-guideline reproductive/developmental toxicity study Wistar rats 8h/d, gestation period 0, 50, 100, 200 mg/m^3 (eq. to 158, 315, 631 ppm) 32 animals/groups Sacrifice: at term (n=18), after delivery (n=14) F2 generation produced (no further CS_2 exposure) Analysis: number of *corpora lutea*, implantation sites, resorption. Lipid metabolism in liver extracts. Fetal data in F1 and F2: survival, weight, anomalies, hall's open field test (PND 21, 30, 90).	Maternal toxicity: no information No effects on reproductive capacity of females. Increased pre-implantation losses F1 developmental effects: Reduced fetal body weight at ≥100 mg/m^3

Klimisch score: 3 (unreliable) Limitations: - Not similar to OECD TG study on developmental toxicity, no GLP status - No control of exposure and no detailed description of inhalation chamber and measurements. - Number of animals for second mating not provided - Lack of information on maternal toxicity (body weight, food consumption, clinical signs) - Exact exposure duration not clear (gestation period) - Source of test animals and of test material not provided. - No information on environmental condition (temperature, cage size, humidity) - Only few detailed results	Dose-related external malformation in all treated groups (hydrocephaly, club foots, tail malformations). Delayed ossification and deformation of skull bones in all treated groups. Mild parenchymatous dystrophy and reduced glycogen content of the hepatic cells of the fetuses treated with 200 mg/m^3 CS_2. Decreased weight still observed at PND45. Behavioral alteraions (reduced exploratory activity, increased emotional activity) observed in all test groups. F2 developmental effects: No effects on weight and pre or post-implantation losses Same congenital malformations and behavioral changes as in F1
RABBITS	

(Unpublished report, 1991) Prenatal developmental toxicity study (similar to OECD 414), GLP-study New Zealand rabbits Inhalation, whole body, 6h/d, GD 6-18 N=24/group Sacrifice: GD29 0, 60, 100, 300, 600, 1200 ppm (0, 190, 316, 948, 1896, 3792 mg/m^3) (nominal conc.) Klimisch score 2 Limitations: - GD6-18 exposure period instead of from implantation to the day prior to scheduled caesarean section, as currently recommended, may decrease the senisitvity of the study	<u>Maternal toxicity (</u>at≥ ppm) At 1200 ppm: mortality, ↓ food/water consumption, ataxia, wheezing, tremor, laboured respiration Statistically significant decrease in body weight compare to control at 1200 ppm ↓hemoglobin, hematocrit, MCHV, MCH concentration, neutrophils, lymphocytes altered at 1200 ppm; NOAEC = 600 ppm <u>Developmental toxicity (</u>at≥ 600 ppm) At both 600 and 1200 ppm, increased in post-implantation losses (early and late resorption) and reduced number of live foetuses were observed. Reduced fetal body weights were also noted at 600 and 1200 ppm. In the 1200 ppm (3720 mg/m^3) group, increase incidence of visceral (hydrocephalus) and skeletal malformations was observed. One incidence of internal hydrocephalus was also observed at 300 and 600 ppm. LOAEC = 300 ppm

(Jones-Price *et al.* (NTP), 1984b) Prenatal developmental toxicity study (similar to OECD 414) New Zealand rabbits oral: gavage 0, 25, 75, and 150 mg/kg bw Exposure: from gestation day 6 to 19 (daily) Sacrifice: GD30 23-28 dams/group Data collection: gravid uterus weight, number of implantation sites, live, dead or resorpted fetuses; Assessment of weight and malformations of live fetuses. Klimisch score 4 Limitations - Unknown GLP status - No detailed data available - GD6-18 exposure period instead of from implantation to the day prior to scheduled caesarean section, as currently recommended, may decrease the senisitvity of the study	<u>Maternal toxicity</u> At ≥ 75 mg/kg Statistically significant decrease in maternal weight gain compare to control Decreased gravid uterine weight Significant increased liver relative and absolute weight NOAEL maternal = 25 mg/kg <u>Developmental toxicity</u> Stat. significant ↑ in fetal resorption at ≥ 25 mg/kg: percentage per litter resorbed, nonlive (dead + resorbed) or affected (nonlive plus malformed) fetuses. Incidence of resorption: 12.3, 32.47, 41.6, 61.16% in vehicle, 25, 75 and 150 mg/kg, respectively ↑ incidence of malformed fetuses per litter at 150 mg/kg (19.51% vs 5.72% in controls) LOAEL developmental = 25 mg/kg
(Beliles *et al.*, 1980) Rabbits Inhalation at 0, 60-120 mg/m^3 (equivalent to 19-38 ppm) for 7 hours a day, 5 days a week from 3 weeks before mating to gestation day 21 Klimisch score 4 (As summarised in WRc-NSF report)	No significant teratogenic effects and no effects on course of pregnancy, embryos, number and weight of foetuses (relative to the controls) at any test dose

Fertility and sexual function

Experimental animals

In the animal experimental studies, most of the studies were performed via whole body inhalation as it is the most likely route of exposure to the substance. Nevertheless, some studies were also performed using an intraperitoneal route.

With regard to published data, two studies investigated the effects of carbon disulphide on male spermatogenesis and mating behaviours. In a first study (Zenick *et al.,* 1984), rats were exposed to carbon disulphide at 600 ppm (1896 mg/m^3), 6h/d, 5d/w. After 10 weeks, the copulatory behaviour (ejaculation latency, sperm count and mount latency) was affected. Males have decreased body weight compared to control. In a second study, Tepe & Zenick (1984) exposed rats to 350 or 600 ppm of carbon disulphide at for 10 weeks. At 600 ppm (1896 mg/m^3), body weight and epididymis sperm count were decreased without changes to reproductive organ weights or in gonadotropin levels. A NOAEC of 350 ppm can be derived from this study. The authors hypothesised that the effects on copulatory behaviour may be due to alteration of brain monoamine levels. Indeed, it has been described elsewhere that increased dopamine stimulation stimulates mating behavior.

In the study by Kumar *et al.* (1999), adult male albino Charles-foster rats (5/dose) were treated i.p. with carbon disulphide at 0, 25, 50, 100 and 200 mg/kg bw for 60 days. Effects on epididymitis, adrenal weight, sperm count, and sperm head shape abnormality were studied. Sperm count was decreased at doses ≥ 100 mg/kg bw day. Sperm head shape abnormality was increased at 200 mg/kg bw. In the study by Patel *et al.* (1999) rats were exposed by means of intraperitoneal injection for 30 days. The observations indicated effects of carbon disulphide on the male reproduction system (histologic examination, serum testosterone levels) even at the lowest concentration of 25 mg/kg bw (intraperitoneal administration).

An oral EOGRTS (extended one generation reproductive toxicity study including DNT and F2 generation), requested during the SEV process, performed in rats by oral route was provided in 2019 (Unpublished report, 2019). Dose levels used were 0, 1.2, 12 and 120 mg/kg. These dose levels are approximately equivalent to 3, 30 and 300 ppm according to the extrapolation from the requested comparative TK study performed by the registrant (unpublished report, 2018). In view of the low general toxicity observed in the study, evaluating MSCA questions the selection of the top dose and the dose-spacing between the groups that is not in line with OECD TG 443. The top dose was lower than the dose with effect levels in the repeated-dose toxicity studies. Moreover, despite the comparative TK study which is useful to have a direct comparison between the internal dose (e.g. AUC) by oral route and by inhalation, limitations were identified (See section 7.9.1). Differences in toxicokinetics such as differences in brain exposure is expected at the same dose levels as the inhalation route can bypass the blood brain barrier in contrast to oral route (unpublished report, 2018).

With regard to effects on sexual function and fertility, effects on sperm (increase cells with detached head) was noted above historical control values in the F1-generation (cohort 1A) at the top dose of 120 mg/kg (corresponding to around 300 ppm by inhalation according to the comparative TK study provided in the REACH dossier). In P0 other findings were noted but were of equivocal toxicological significance as not observed in F1 : decrease percentage of motile sperm, progressive sperm and number of cells with coiled tail. No other findings in male reproductive organs were found in the F1-generation. **Histopathology investigation of the extended cohort 1B was not conducted, although the substance is a suspected reproductive toxicant.** Evaluating MSCA notes that the effect seen in the study is in line with the effects seen on the male reproduction system in the study by Kumar *et al*. (1999). Nevertheless, as different route of exposure were used, a direct comparison cannot be performed. Moreover, higher dose levels would have been needed to be able to compare with the results obtained by Zenick *et al*. (1984) and Tepe and Zenick (1984) at 600 ppm (1896 mg/m^3) and to investigate potential effects on fertility.

In females, an effect on the ovaries was noted consisting of a decrease in primary and primordial follicles in the F1 generation (cohort 1A) at the top dose of 120 mg/kg (eq. to 950 mg/m3). Although not statistically significant, a decrease in corpoa lutea was also noted at 120 mg/kg. In P0 females, a slight increase in irregular cycle was noted at 12 and 120 mg/kg (3/25 at 12 mg/kg and 4/25 at 120 mg/kg, *vs* 1 in controls and low dose). This finding was not considered treatment related by the study authors as it was not reproduced in F1.

Histopathology investigation of the extended cohort 1B was not conducted, although effects were observed in cohort 1A. No other findings were noted on fertility or on reproductive function in females.

Only slight general toxicity was observed on the body weight at the top dose in F0 and F1-generation. Organ weight changes and/or histopathological findings at the top dose were noted in thymus, spleen, adrenals, brain, eyes, liver in the F0 and F1-generations. A treatment-related decrease of T4 levels was noted at the top dose in F0 males and females and in F1 cohort 1A males but no effect on TSH was reported.

Human data

Human data in men report various effects including effects in sperm quality and decreased libido. Already in the early 70's, Lancranjan *et al*. (1969, 1972) reported in patients working in a viscose rayon plants and intoxicated with carbon disulphide (toxic polyneuritis) anomalies in spermatogenesis, libido complaint and decrease excretion of 17-ketosteroids. In these studies, most of the patients showed polyneuritis.

Effects on testosterone, LH, FSH were not consistently seen in the available human data.

The table below summarised effects seen in males in selected well reported studies (See table above for detailed assessment of the studies).

Table 19: Effects reported on male fertility and sexual function in the available epidemiological studies

Study type	Exposure levels (mean)	T	LH	FSH	Semen quality	Other	Reference
Cohort (n=432)	13-yr, 1.28, 3.13 mg/g Creat.	no	no	no		No effect on ACTH	Takebayashi *et al.,* 1998
Cross sectional (n=392)	19-yr, 0.98, 1.61, 1.94 mg/g Creat. (mean 3, 5 and 6 ppm)	no	no	no			Takebayashi *et al*., 2003
Cohort (n=326)	> 1 yr 0, <5ppm, > 5ppm					OR fetal loss: 1.14-1.18	Selevan *et al.*, 1983
Cohort (n=175)	1-15 yr, <2 to >10 ppm				No		Meyer *et al*., 1981
Cross-sectional (n=43)	12.5yr, 3.2 ppm				↓* (within normal WHO range)	↓* libido	Ma *et al*., 2010;

Case-control (n= 76)	10 yr, 0.03-14 ppm (3.1 ppm, 8hTWA)	↓* (<N)	↑* (>N)	↑* (>N)	↓* (motility, other p. within normal range)	↓SHGB, semen apoptotic cells, ↑* semen antioxidant capacity and abnormal structure chromatin ↓*	*Guo et al.*, 2016
Cross-sectional (n=117)	>1yr, 0.3-95ppm*yr, >95ppm*yr	no	no	no			Vanhoorne, 1993
Cross-sectional (n=43)					no	↓* libido, impotence complaints ↓* seminal round cells	Vanhoorne, 1994

Grey: not investigated; no: no effect observed; SHGB: Sex-hormone binding globulin

In women, disturbances in the menstrual cycle is the main reported effect but fewer data are available. In the retrospective cohort study by Zhou *et al*. (1988) a higher incidence of menstrual disturbance (irregular cycle) was found compared to non-exposed women workers (RR = 2, $p < 0.01$) at doses ≥ 3.1 mg/m^3. An exposure-response was seen in the study as shown in the table below. Nevertheless, evaluating MSCA noted uncertainties on exposure levels that may have been underestimated.

Table 20: Menstrual cycle disturbance in women, reported in Zhou *et al.* (1988)

Symptoms	CS_2 concentration			
Dose (mg/m3)	0	3.1	6.5	14.8
No. subjects	291	134	66	65
Abnormal cases (%)	15.2	32.1	34.9	44.6
Irregular cycle (%)**	7.9	16.4	18.2	32.3
Unusual bleeding (%)**	6.5	12.7	22.7	16.9

** p< 0.01

Decrease in the age of menopause and increase in spontaneous abortion was also reported in some studies but the studies were either of low quality or not available to evaluating MSCA (paper not in English).

Overall, the EOGRTS performed in rats confirmed that there is a concern for both male and female reproductive toxicity as seen in humans. Nevertheless, probably due to insufficient dose levels in the EOGRTS, no fertility effects were seen and the effects are judged insufficient to warrant a modification of the existing harmonised classification of carbon disulphide as Repr. 2, H361f "Suspected of damaging fertility".

Due to effects observed in both men and women workers exposed to carbon disulphide at/or around 5 ppm, this dose can be considered as a LOAEC for this endpoint in humans and 120 mg/kg (eq. to around 300 ppm) was identified as a LOAEC in experimental animals for this endpoint. As this findings in animals were seen in presence of slight neurological changes, this highlights the need to protect workers from these subtle neurological changes in order also to protect workers from a potential reproductive toxicity.

Developmental toxicity

Inhalation

There is clear evidence that the development of pups is affected by carbon disulphide after *in utero* exposure. Toxicokinetics showed that carbon disulphide and its metabolites can pass through the placenta at all stages of gestation and distribute throughout the fetal body, mainly to the brain, blood, liver and eyes (Danielson *et al*., 1984).

Two studies were identified as key studies for this endpoint by inhalation (rated Klimisch 2), Saillenfait *et al*. (1989) in rats and the prenatal developmental study (Unpublished report, 1991) in rabbits.

Saillenfait *et al.* (1989) exposed rats via inhalation to 0, 100, 200, 400 or 800 ppm carbon disulphide for 6h/d during days 6-20 of gestation. Lower exposures (100 or 200 ppm eq. to 310 or 620 mg/m^3) were not associated with maternal toxicity or adverse effects on the developing embryo or fetus. Higher concentrations (400 or 800 ppm; 1240 or 2480 mg/m^3) yielded a significant reduction of maternal weight gain as well as reductions of fetal body weight. A dose-related increase in clubfoot was observed at doses ≥ 400 ppm. Significant increase in unossified sternebrae were reported following 800 ppm (2480 mg/m^3) exposures. A NOAEL of 200 ppm was identified for developmental and maternal toxicity in this study in rats. This NOAEL is supported by other studies such as Hardin *et al*. (1981) and Beliles *et al*. (1980) where no developmental effects or maternal toxicity were seen at doses up to 190-379 ppm (600-1200 mg/m^3). However, the latter studies were not included in the REACH registration dossier. The registrant concluded that the study by Saillenfait *et al*. (1989) is supportive (Klimish 4: not assignable) and was not considered further.

In the GLP guideline study provided in the registration dossier (Unpublished report, 1991), New Zealand white rabbits (24 per group) inhaled 0, 60, 100, 300, 600 or 1200 ppm (equivalent to 0, 190, 316, 948, 1896, 3792 mg/m^3) carbon disulphide for 6 h/d on gestation days 6 to 18. At the top dose, ataxia, labored respiration, wheezing, and tremors were observed, as well as scant feces and low food consumption, that were clearly associated with carbon disulfide treatment. Three animal deaths at 1200 ppm were considered treatment- related. At the top dose, group mean body weight was statistically significantly reduced compare to control. Haematological findings were also noted mainly at the top dose (haemoglobin and haematocrit levels, MCV, MCHC, neutrophils and lymphocytes). At both 600 and 1200 ppm, a statistically significant increase in post-implantation losses (early and late resorptions) and reduced number of live foetuses were observed. Post-implantation losses were: 0.30 ± 0.63 in control, 0.64 ± 1.00 at 600 ppm and 7.00 ± 3.94 at 1200 ppm. Two litters of 22 in the 600 ppm group and 14 litters of 21 in the 1200 ppm group consisted of implantation sites with no live fetuses, i.e., the litters consisted exclusively of resorptions. Reduced fetal body weights were also noted at 600 and 1200 ppm. In the 1200 ppm (3720 mg/m^3) group, increased incidence of hydrocephalus (2 in 2 litters). Hydrocephalus was also noted at 100 and at 300 ppm (1 incidence in 1 litter) but was not observed in the control group. Total incidence of malformation was noted at the top dose (right-sided esophagus, absent rish subclavian artery, swollen sublclavian artery, swollen sublingual salivary glangs, malformed stomach, small thyroid and parathyroid, abnormal caudal vertebrae, fused sternebrae and split sternebrae)with low incidences for each malformation (1 incidence in 1 litter).. The NOAEL for developmental and maternal toxicity in rabbit was 300 ppm (948 mg/m^3). Overt maternal toxicity was considered only at the top dose level of 1200 ppm (3792 mg/m^3). This NOAEL is supported by the negative study from Beliles *et al*. (1980) where no effects were seen up to 190-379 ppm (600-1200 mg/m^3).

Developmental delays, embryotoxicity, malformations and neurobehavioral effects in rats have been reported in the offspring of dams exposed at 3.2 ppm and above after exposure *in utero* over one or two generations (Tabacova *et al.* 1978, 1983; Tabacova and Balabaeva, 1980; Lehotzky *et al.,* 1985). These studies were not performed according to standard regulatory toxicity test guidelines and showed major deficiencies in regards to reporting and most importantly exposure levels. Nevertheless, although uncertainties were identified on exposure levels, results observed in these studies are of high concern and triggered further information on this endpoint.

The new requested EOGRTS, as reported in the study report, indicates that up to below 120 mg/kg (corresponding to ~ 300ppm or 948 mg/m^3 by inhalation), carbon disulphide did neither affect auditory startle response nor induce effects in motor activity. Nevertheless, the results are considered inconclusive as the DNT study on cohorts 2A and 2B had limitations (statistical analysis, no positive control, no historical control data).

A dose-related decrease in landing foosplay was noted in the FOB battery. Although neurotoxicant are usually increasing landing footsplay, both an increase and a decrease in

landing foot splay could be considered. Although the decrease in males and females were not statistically significant, they are potentially a trend for an effect was noted in the study. No historical control data are available for this effect, not allowing a definitive conclusion on the toxicological significance of the effect. The effect at 1.2 and 12 mg/kg may be treatment-related but may not be not adverse due to the very low magnitude of the effect at these dose levels.
Adverse effect on brain were also noted and were considered adverse at the top dose, including decreased brain weight and changes in the morphometric analysis. Nevertheless, the biological significance of the increase in the size of the caudate putamen in cohorts 2A and 2B females at the top dose is uncertain. Evaluating MSCA notes that this is the region involved in reward that is rich in dopaminergic neurons. At the top dose, evidence of slight neurotoxicity was also seen in parental animals(fresh brain weight, retinopathy). Fresh brain weight was slightly more severly affected in F1 compare to F0 animals at the top dose, suggesting an increasing sensitivity in pups. This higher sensitivity was not observed for retinopathy. In addition, in the study, small effects in terms of post-implantation losses, decrease number of implants and litter size were also noted at the top dose of 120 mg/kg (> 10%). The post-implantation losses were considered adverse by the study authors although not statistically significant and inside historical control. **In this study, no developmental effects were seen at the low and mid dose group neither in pups nor in parent**.

Oral route

Two guideline prenatal developmental studies have been conducted to assess the developmental effects of carbon disulphide resulting from oral exposure. Nevertheless, evaluating MSCA was not able to independently assess the quality and the reults of these studies as the study reports were not available. Notably, inconsistency was noted between the summary of these studies in different review papers.

Jones-Price *et al.* (1984a) investigated the effects of carbon disulphide on the development of rats following oral exposure of pregnant rats to 100, 200, 400 and 600 mg/kg bw/d during gestation days 6-15. Animals were sacrificed on day 20. The study showed retarded body weight gains at all dose levels in the dams. There was a dose-dependent reductions in foetal weight in the study at doses ≥ 200 mg/kg. There was no evidence of embryo or foetotoxicity in the study (ATSDR, 1996).

In a corresponding study in rabbits Jones-Price *et al.* (1984b) investigated the effects of carbon disulphide on development following oral exposure of pregnant rats to 0, 25, 75 and 150 mg/kg bw/d during gestation days 6-19. Animals were sacrificed on day 30. Retarded body weight gains and increased liver weights were measured in the dams at 75 and 150 mg/kg bw/d. Fetal malformations were statistically significantly increased in males following exposure to 150 mg/kg bw/d. Resorptions were seen at all dose levels. A LOAEC of 25 mg/kg was identified for the study (ATSDR, 1996).

The data from Jones-Price *et al*. studies, performed by the oral route, suggest that the rabbit fetus is more sensitive to carbon disulphide toxicity than the rat fetus.

Conclusion

- Fertility and sexual function

Overall, the new data obtained in the EOGRTS study confirm that a classification as at least Repr. 2, H361f is warranted. Category 1B cannot be excluded despite limitations noted in the EOGRTS study. Histopathology of the extended cohort 1B was not performed. In addition, dose-reponse is difficult to assess due to unclear dose selection. The interval between the top and the mid dose (12 and 120 mg/kg) is too large and the top dose could have been slightly higher.

- Developmental toxicity

Overall, carbon disulphide is embryotoxic and can induce malformations in rats, rabbits and mice. Malformations were seen only in presence of overt maternal toxicity in the key studies. Nevertheless, in the prenantal developmental inhalation rabbit study (Unpublished report, 1991), not considered during initial classification and labelling, increased resorptions were seen in absence of evident maternal toxicity. Increase in resorptions were

also noted in the absence of maternal toxicity in the rabbit oral developmental study (Jones-Price et al., 1984b).

By inhalation, a NOAEC of 200 ppm can be identified in rats and rabbits for this endpoint based on the study by Saillanfait *et al.* (1989) and the unpublished report from 1991.

By oral route, a NOAEL of 100 mg/kg in rats and a LOAEL of 25 mg/kg in rabbits was identified for developmental toxicity based on the studies by Jones-Price (1984a and 1984b). Nevertheless, evaluating MSCA had no access to these mentioned oral unpublished studies and the reliability of the studies and results was not assessed.

Overall, the data confirm that a classification as at least Repr. 2, H361d is warranted and that a stronger classification may be considered.

7.9.8. Hazard assessment of physico-chemical properties

Carbon disulphide is considered highly flammable.

Classification: Flam. Liquid 2 (Hazard statement: H225: Highly flammable liquid and vapour.)

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Three main critical effects were identified in the available database: cardiovascular disease, reproductive toxicity and neurotoxicity.

7.9.9.1. Long-term DNEL, systemic effects by inhalation

Carbon disulphide is in the list of indicative EU occupational exposure limit values established in the third list of directive 2000/39/EC amended directive 98/24/EC (protection of health and safety of workers from the risks related to chemical agents at work). **The indicative long-term occupational exposure limit value was set at 15 mg/m³ (5 ppm) based on neurotoxicity and cardiotoxicity**. A skin notation was also retained as dermal absorption is also a relevant route of exposure. This is based on a Scientific Committee on Occupational Exposure Limits (SCOEL) opinion published in 2008. SCOEL considered that the **value of 5ppm could be correlated to a biological limit value of 1.5 mg TTCA/g creatinine**. These values were used the risk assessment.

In addition, as discussed in 6.9 section, based on subtle neurological changes in workers observed in the study by Yoshioka *et al*. (2017) at 6 ppm, minor ECG abnormalities observed in Takebayahi *et al*. (1994) at 5 ppm and decreased T4 levels around 5ppm, **a LOAEC of 5 ppm can be considered for these effects**. There is a need to protect workers from subtle neurotoxic and cardiotoxic effects to also protect workers from potential fertility effects in male and females in these dose-range. The SCOEL in its opinion of 2008, insufficiently took into account the potential reproductive male and female effect in humans, as confirm by the recent EOGRTS. Moreover, perturbation of the oestrus cycle has been shown in women at exposure levels below 5 ppm (Zhou *et al*., 1988). Although uncertainties on exposure is acknowledged in this study, the current indicative value may not be protective enough. Therefore, **a systemic DNEL by inhalation of 1-2 ppm (considering an assessment/uncertainty factor (AF) of 3 for LOAEC to NOAEC) is considered appropriate to protect for neurotoxicity, cardiotoxicity but also reproductive toxicity. A DNEL of 2 ppm has also been used for risk assessment in this report.**

Indeed, in a recent study Yoshioka *et al*. (2017) revealed potential PNS effects at 6 ppm, with a measured urinary TTCA of 1.74 mg/g creatinine, just slightly above the current OEL.

A decrease in T4 level was shown in the new EOGRTS performed in rats in presence of slight neurotoxicity. This further supports that the decreased T4 level in human observed at the current OEL in the follow-up study by Takebayashi *et al*. (2004) should also be taken into account for the OEL setting.

SCOEL (2008) did not sufficiently take into the reproductive toxicity of carbon disulphide for the derivation of the OEL. Male and female reproductive toxicity (sperm, ovary) was seen in presence of only subtle neurological changes (slight brain weight decrease, retinal findings) in rats and support to protect workers from subtle neurological changes to also protect from potential reproductive effects. As seen in Zhou *et al*. (1988), women cycle disturbance already occur at 5 ppm. Although uncertainties on exposure is acknowledged in this study (H_2S), the current indicative value may not be protective enough.

It may also be noted that a lower OEL of 1 ppm was set by the American conference of governmental industrial hygienist (ACGIH, 2006) and 2 ppm was proposed by the Health council Netherlands (HCNL, 2011) based on clinically relevant (ischaemic symptoms) cardiovascular effects observed in humans (Takebayashi *et al*., 2004). Therefore, a lower systemic long-term DNEL for inhalation of 2 ppm would be relevant for risk assessment due to pulmonary absorption. In addition, as carbon disulphide may also be absorbed via dermal route (in addition to inhalation), a dermal DNEL would also be relevant for risk assessment.

Exposure assessment for inhalation was performed using Tier 1 tools (ECETOC TRA) or Tier II tools for some scenario (ART). For manufacture, measured data (air monitoring) was available. Only a qualitative assessment was performed for dermal exposure. Exposure assessment and exposure scenarios for human health are described in section 6.12.

7.9.9.2. Long-term DNEL, systemic effects by dermal route

The registrant considered that the derivation of a long-term occupational exposure dermal DNEL is superfluous to the inhalation DNEL that already provide a proxy of dermal exposure. Absorption of vapour by the skin is directly linked to exposure by inhalation.

Carbon disulphide penetrate the skin and is accumulating in the body (SCOEL, 2008). Thus, absorption of vapour by the skin may play a role in carbon disulphide exposure. In 2008, SCOEL concluded that the biomonitoring data using TTCA excretion levels in urine correlate well with total body load using carbon disulphide concentration in the air. SCOEL in 2008 concluded that "Recent studies have shown that a 8-h TWA inhalation exposure of 5 ppm (15 mg/m3) of carbon disulphide will correspond to a mean biological value of about 1.0 to 1.6 mg TTCA/g creatinine. Higher values may be indicative of excessive inhalation and/or dermal exposure."

According to the study by Kilo *et al*. (2015), although internal exposure of the employees correlated with the individual external exposure to carbon disulphide, several factors such as physical work load or dermal resorption can affect the uptake of carbon disulphide. Dermal resorption may be an important factor for internal exposure to carbon disulphide, particularly at low or medium ambient carbon disulphide levels (mean 1.6 ppm and 2.8 ppm). Moreover, they also noted that direct dermal contact to wet spinning spools (during manufacture of regenerated cellulose) result in an increase in relative internal exposure to carbon disulphide.

Overall, evaluating MSCA agrees that inhalation DNEL could be used as a surrogate to dermal DNEL. Nevertheless, at low exposure levels, **dermal exposure may be an important source of carbon disulphide uptake and total exposure may be underestimated using only an inhalation DNEL**.

7.9.9.3. Short-term DNEL, systemic effects

Based on a pragmatic default factor of 3, a short term DNEL of 45 mg/m^3 (15 ppm) is used for risk assessment.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

Carbon disulphide is an industrial chemical that has a **current Annex VI entry** in the CLP regulation (EC 1272/2008). Three main hazards were identified following exposure to carbon disulphide: neurotoxicity, cardiotoxicity and reproductive toxicity. Neurotoxicity and

cardiotoxicity effects are already covered by the classification of carbon disulphide as STOT RE 1 including specific concentration limits. Nevertheless, nervous sytem, cardiotoxicity would need to be indicated in the classification as a specific target organ.

The effects seen in the newly generated EOGRTS (Unpublished report, 2019), requested during the evaluation process, do not appear to require a revision of the current existing harmonised classification of the substance for reproductive toxicity. However, some effects reported in the new study e.g. a decrease in ovarian primary and primordial follicles (considered a marker of female reproductive toxicity) were observed in the study in cohort F1A. The borderline significance or the small magnitude of some of the effects observed in the study (e.g. post-implantation losses, decreased total number of implants, sperm) may have been due to the insufficient top dose concentration used in the EOGRTS. With regards to developmental toxicity, rabbit developmental toxicity studies not taken into account during initial TC C&L may indicate higher sensitivity of rabbits to carbone disulphide and the need of a stronger classification.

Therefore, since effects on both fertility and pre-/post-natal development are either confirmed or new, classification as Repr. 2 (H361df) is still warranted. There is no new human data available that would allow to classify the substance in category 1A. Nevertheless, category 1B for reproductive toxicity may need to be considered.
Additional classification of the substance as Acute Tox. 4; H332 may be warranted for carbon disulphide.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

7.10.1.1. Gathering relevant information

***In silico* and *in vitro* studies**

Regarding the current dataset for carbon disulphide provided on dashboard EPA, QSARs models do not indicate a concern and **carbon disulphide is considered as negative in all *in vitro* assays investigating endocrine activity**. See human health part, section 6.10.2, for more details.

In vivo assays providing data about endocrine mechanism and adverse effects

The registration dossiers do not contain any of the studies of the minimal data package according to the ECHA/EFSA guidance (2018) to consider the EATS modalities for non-target organisms other than mammals (for ED-activity: Fish short-Term reproduction assay OECD TG 229 and Amphibian metamorphosis assay OECD TG 231; for ED-adversity: LAGDA OECD TG 241 and Medaka EOGRT OECD TG 240). In the dossier, only one *in vivo* short term study conducted on fish early life stage (similar to OECD 212) is available. The NOEC value for hatching after 8 days exposure equals to 1 mg/L and is in the same order of magnitude as NOECs for survival and malformation (2.5 mg/L) and as LC_{50} from acute toxicity tests (see section 6.8.1). No other *in vivo* chronic test on non target organisms (non mammalian) is available in the registration dossiers.

Review of scientific open literature - Non-mammalian vertebrate species

A review of the literature was done in March 2021 using the SCOPUS® database with the following search terms without any temporal limits: (carbon disulfide AND 75-15-0) AND TITLE-ABS-KEY (endocrin* OR hormon* OR estrogen* OR androgen* OR steroid* OR thyroid*) AND (birds* OR fish* OR amphib*). No studies pertaining to endocrine activity or adverse effect of carbon disulphide on the environmental organisms were found.

7.10.1.2. Conclusion - Environment

According to the ECHA/EFSA guidance (2018) applicable to plant protection products and biocidal active substances, the data package of the registration dossiers of the REACH carbon disulphide substance for non target organisms other than mammals should be

considered as not sufficient to be conclusive. The available data for the assessment of the ED-activity or -adversity of carbon disulphide do not reveal any alerts on potential endocrine disruption properties of carbon disulphide. Under REACH Regulation, a concern leading to a potential risk needs to be identified to allow the eMSCA to request further data. In this case, it is considered that further testing is not scientifically justified for the following reasons.

No indications of ED-related activity or -adversity of carbon disulphide has been revealed in the literature on non target organisms and *in silico* data.

Given the high volatilization or high fugacity of carbon disulphide from water to air, carbon disulphide mainly partition to the atmosphere. In addition to the intrinsic physico-chemical properties of carbon disulphide as a gas which impair the testing with aquatic/amphibian organisms, it should be more relevant to test air breathing non target organisms as birds rather than fish or amphibian larvae. However, according to the ECHA/EFSA guidance (2018), only a limited number of standardised *in vivo* methods for birds are available, and little information can be gained from those guidelines concerning potential ED-related effects. Furthermore, their use has considerable animal welfare implications.

Finally, it could be underlined that carbon disulphide emissions in air also result from natural or biogenic activity. Then, organisms are permanently surrounded by a carbon disulphide-containing atmosphere and given the avaibility of robust data in mammalian organisms, no further testing with non-target non-mammalian species is needed.

Taking into account all these aspects in a weight of evidence approach, further tests with non-target non-mammalian species would not provide any robust data allowing to identify or exclude carbon disulphide as an endocrine disrupter and therefore should be avoided in terms of animal welfare.

7.10.2. Endocrine disruption - Human health

According to the WHO ED criteria, a substance shall be considered as having endocrine disrupting (ED) properties if it meets all of the following criteria:

- it shows an adverse effect in an intact organism or its progeny/non target organism, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
- It has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;
- The adverse effect is a consequence of the endocrine mode of action.

Adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as an endocrine disruptor.

In the section 7.10.2.1, parameters considered relevant to investigate ED properties of carbon disulphide are grouped into four categories: *in silico/in vitro* mechanistic, *in vivo* mechanistic, EATS-mediated, non-EATS mediated and sensitive to, but not diagnostic of EATS. As proposed in the EFSA/ECHA guidance document (2018), the information were reported and assessed in line of evidence for adverse effects and endocrine activity.
The analysis of the evidence and the mode of action analysis is performed in section 7.10.2.2. The conclusion is provided in section 7.10.3.

7.10.2.1. Available evidences

***In silico* and *in vitro* studies**

No receptor competitive binding assays, recombinant yeast assays or mammalian cell growth assay were identified in the registration dossier or in the literature.

Carbon disulphide was not found in the Danish QSAR database. carbon disulphide was screened in the endocrine disruptor screening program EDSP21 (US EPA) (https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID6023947#invitrod

b-bioassays-toxcast-data). The substance was negative in all performed *in vitro* high-throughput screening assays. Nevertheless, there may have been an issue with the quality control check as the samples are described as withdraw and the grade not determined. Consequently the results are rather considered inconclusive than inactive. The results of the screening test are summarised in table below.

The results reported below do not indicate that carbon disulphide interact with receptors that have been screened so far, in particular it does interact neither with thyroid hormone receptors nor with any of the receptors involved in EATS modalities. Nevertheless, due to potential issues on sample quality, the results are considered inconclusive.

In addition, as stated in the OECD ED TG 150 (OECD, 2018), in *vitro* assays are insufficient alone to exclude a possible endocrine disruption activity due to inherent ability limitations. For example, the available *in vitro* screening assays would not be able to detect mechanism not directly receptor-mediated, such as an interference of carbon disulphide with the hypothalamo-pituitary-gonadal axis (HPG).

Moreover, as described in section 0 (section related to toxicokinetics), carbon disulphide is actively metabolised and the potential activity of most of the metabolites is unknown. Only thiourea, one of the metabolites of carbon disulphide and classified in CLP for both developmental toxicity (Repr. 2, H361d) and carcinogenicity (H351), was screened in the EDSP21 screening program of US EPA. Thiourea was active in NCCT_TPO_AUR_dn screen assay, investigating the inhibition of Thyroxide peroxidase (TPO) in rat and NCCT_QuantiLum_inhib_2_dn assay investigating TPO enzyme activity in *e.coli* (T-modality). Moreover, thiourea has been reported in the literature to inhibit *in vitro* thyroxide peroxidase (TPO) (Davinson *et al*., 1979). Therefore, **there are positive evidence that one of the metabolite of carbon disulphide can inhibit thyroid peroxidase *in vitro***. The potential activity of other metabolites of carbon disulphide is unknown.

***In vivo* mechanistic studies providing information on endocrine activity**

No in vivo mechanistic studies, as described in the OECD Conceptual Framework for the Screening and Testing of Endocrine Disrupting Chemicals level 3, were identified in the registration dossier or in the literature. Nevertheless, hormonal levels have been investigated in several in vivo non-guideline assays and in human epidemiological studies as summarised in the table below. The reliability and the limitations of the animal and human data, presented below, are assessed in section 7.9 above.

Table 21: Summary of the screening assay results available with carbon disulphide (US EPA, EDSP21)

Assays	Activity	Modality*
Assays name: - TOX21_ERa_BLA_ Antagonist_ratio - TOX21_ERa_BLA_ Agonist_ratio - TOX21_ERa_LUC_VM7_Agonist - TOX21_ERa_LUC_VM7_Angonist Gene name: Human estrogen receptor 1 Organism : human kidney cell line Biological process target : regulation of gene expression	Inconclusive**	E modality
Assays name: - TOX21_ERa_BLA_Antagonist_viability - TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2_viability Organism : human kidney cell line Biological process target: cell proliferation	Inconclusive**	E modality
Assays name: - TOX21_AR_BLA_Agonist_ratio - TOX21_AR_BLA_Antagonist_ratio - TOX21_AR_LUC_MDAKB2_Agonist - TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881 - TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881 Gene name : human Androgen Receptor Organism : human kidney cell line Biological process target: Regulation of gene expression	Inconclusive**	A modality
Assays name: - TOX21_AR_BLA_Antagonist_viability - TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881_viability - TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881_viability Organism: human kidney cell line Biological process target: cell proliferation	Inconclusive**	A modality
Assays name: - TOX21_TR_LUC_GH3_Agonist - TOX21_TR_LUC_GH3_Antagonist Gene name: Thyroid hormone receptor alpha Organism: rat pituitary gland cell line Biological process target: Regulation of gene expression	Inconclusive**	Thyroid

Assays name : - TOX21_TSHR_Agonist_ratio - TOX21_TSHR_Antagonist_ratio - TOX21_TSHR_wt_ratio Gene name: Thyroid stimulating hormone receptor (TSHR) Organism: human kidney cell line Biological process target: Regulation of gene expression	Inconclusive**	Thyroid
Assays name: - TOX21_TR_LUC_GH3_Antagonist_viability Gene name: Thyroid hormone receptor alpha Organism: rat pituitary gland cell line Biological process target: cell proliferation	Inconclusive**	Thyroid
Assays name: TOX21_Aromatase_Inhibition Gene name: cytochrome P450, family 19, subfamily A, polypeptide 1 Organism: Human breast cell line Biological process target: Regulation of gene expression	Inconclusive**	S modality
Assays name: TOX21_Aromatase_Inhibition_viability Human breast cell line Biological process target: cell proliferation	Inconclusive**	S modality

*E, A, T, S: estrogen, androgen, thyroid, steroidogenic

** in the QC Data collection both CS_2 samples are marked as "sample withdrawn" and "grade – not determined". Considering volatility of the substance, the reliability of this dataset for this particular substance is questionnable.

Table 22: Lines of evidence for *in vivo* mechanistic studies

Species	Evidence	Study	Effective dose	Observed effect	Assessment of the line of evidence	Assessment of the integrated line of evidence	Modality *
Rat	Hormonal changes: males	Non-guideline 10-week male inhalation study, K2 (Zenick *et al.*, 1984)	NOAEC= 600 ppm (1896 mg/m^3)	No effects on LH, FSH, testosterone, no effects seen following HCG or GnRH challenge	Not supportive	Insufficient evidence by relevant route of exposure	E, A, S
Rat	Hormonal changes: males	Non-guideline 10- week male inhalation study, K2 (Tepe and Zenick, 1984)	NOAEC= 350 ppm (1106 mg/m^3)	No effects on plasma LH, FSH, testosterone values in unmated or sexually active rats	Not supportive		E, A, S
Rat	Hormonal changes: males	Non-guideline male inhalation study, K2 (Huang *et al.*, 2012)	LOAEC= 396 ppm (1251 mg/m3) NOAEC = 79 ppm (250 mg/m^3)	Decreased GnRH, LH, testosterone (not statistically significant) Increased FSH (statistically significant)	Supportive (non-standard study design, low number of animal (6/group), not statistically significant for LH and testosterone)		E, A, S
Rat	Hormonal changes: males	Non-guideline ip male toxicity study, K4 (Patel *et al*., 1999)	LOAEL = 25 mg/kg	Dose-related decrease in serum testosterone levels	Supportive (non-standard study design, ip route)		E, A, S
Rat	Hormonal changes: T4	Oral EOGRTS, OECD TG 443 (Unpublished report, 2019), K2	LOAEL= 120 mg/kg (~ eq. to 300ppm by inhalation) NOAEL = 12 mg/kg (equivalent to 30 ppm)	Decreased total T4 level in P0 males (By, 48.9%, p< 0.01, outside HCD) and P0 females (by 31%, p< 0.01, inside HCD), F1-cohort 1A males (by 26%, p<0.01, outside HCD)	Sufficient changes observed, top dose only in absence of general toxicity, in presence of indication of neurotoxicity in F0 and F1	Overall positive evidence for endocrine activity	T
Rabbits	Hormonal changes: T4	Non-guideline male 12-week study (Van Stee *et al.*, 1986)	LOAEC = 300 ppm (948 mg/m^3)	Decreased serum T4 levels	Supporting evidence (non-standard study design)		T

*E, A, S : estrogen, androgen, steroroidogenic or T thyroid modalities, HCD: historical control data

Only one human epidemiological study out of the most recent 4 studies on 3 independent cohorts, report effects on testosterone, LH and FSH at dose levels near the current OEL of 5 ppm. This study was a case-control study and is therefore of low weight.

Table 23: Summary of recent epidemiological studies on male hormone levels

Study type	Exposure levels (mean)	T	LH	FSH	Reference
Cohort (n=432)	13-yr, 1.28, 3.13 mg/g Creat.	No effect	No effect	No effect	Takebayashi *et al*., 1998
Cross-sectional (n=392)	19-yr, 0.98, 1.61, 1.94 mg/g Creat. (mean 3, 5 and 6 ppm)	No effect	No effect	No effect	Takebayashi *et al*., 2003
Case-control (n= 76)	10 yr, 0.03-14 ppm (3.1 ppm, 8h-TWA)	↓* (<N)	↑* (>N)	↑* (>N)	Guo *et al*., 2016
Cross-sectional (n=117)	>1yr, 0.3-95ppm*yr, >95ppm*yr	No effect	No effect	No effect	Vanhoorne *et al*., 1993

T: testosterone, LH: luteinising hormone; FSH: follicle stimulating hormone

In older studies, of lower quality and at higher exposure levels, inconsistent results were observed on gonadotropin levels. While higher level of gonadotropin were seen in Wägar *et al*., 1983, decreased levels were noted in Cirla *et al*., 1981 and Lancranjan *et al*., 1969.

Table 24: Summary of older epidemiological studies on male hormone levels

Study type	Exposure levels (mean)	Testosterone	LH	FSH	Reference
N=69	12y, 4-20 ppm (1960), 3-13 ppm (1970), 1-10 ppm (1980)	No effects	↑* (1-9 yr expo, age< 39 year)	↑*	Wägar *et al*., 1983
Cross-sectional N=50	3-12 years 3.2-8 ppm	No effects	No effects	No effects	Cirla *et al*., 1981
N=254 exposed, 54 controls	2-21 yr <19 ppm, 20-40, 40-77 ppm	No effects	↓* (58-77 ppm)	↓* (40-77 ppm)	Cirla *et al.,* 1978
N=33 exposed, 33 controls	7-42 month exposure 40-80 mg/m3	Decreased gonadotropin urinary excretions (immature mouse test) in exposed workers with polyneuritis compare to controls			Lancranjan *et al*., 1969

LH: luteinising hormone; FSH: follicle stimulating hormone

With regards to T4 level in human, decreased T4 levels has been observed in the cohort study by Takebayashi *et al*., 2003 and in Cirla *et al*., 1978. Cavalleri *et al*., 1975 and El-Sobkey *et al*., 1979 also found a decrease in thyroxin levels in exposed workers according to the review by Gelbke *et al*., 2009. Nevertheless, no effects were observed in the cross-sectional studies by Takebayashi *et al.,* 1998, in Cirla *et al*., 1981 and in the cohort study by Vanhoorne *et al*., 1993. No effect on TSH has been noted in humans.

Incoherent effects of carbon disulphide on prolactin are reported that might be explained either because of different parameters (sex difference for example), lack of adjustments or poor reporting: decreased prolactin levels have been observed in male workers in relation to exposure (Cirla *et al*., 1978). A decrease was also noted in the study by

Vanhoorne *et al*., 1993 but not following adjustment for potential confounders. In contrast, Wägar *et al*., 1983 did not find effects on serum prolactin. Based on abstract (article in Polish), plasma prolactin level was increased in menopaused women exposed to carbon disulphide compared to control in Pieleszek *et al*., 1997 (case-control study).

Potential effects on oxytocin or growth hormone have not been investigated in humans.

According to Pieleszek *et al*. (1997), daily excretion of adrenaline and noradrenaline in urine and concentrations of dopamine in plasma of menopaused women chronically exposed to carbon disulphide were statistically significantly lower ($p < 0.001$) and concentration of serotonin was higher compared to unexposed controls. Higher quality studies would be needed to confirm a potential effect of carbon disulphide on the urinary levels of adrenaline and noradrenaline hormones.

No changes in serum estradiol levels were noted in male workers in Wägar *et al.*, 1983. In Pieleszek *et al*., 1997, estradiol levels in menopaused women were decreased in carbon disulphide exposed group. Regarding the potential effect on progesterone levels: there is a lack of information on progesterone levels in men or women exposed to carbon disulphide.

Lancranjan *et al*. (1969 and 1972) noted that excretion of urinary 17-ketosteroid were decreased in occupational exposed men having sperm abnormalities and polyneuritis. Cavalleri's *et al*., 1969, also reported a decreased urinary excretion of 17 ketosteroid in male workers and suggested effects on adrenal function. In these studies, most of the patients had polyneuritis. In Yang *et al*. study (1998), levels of adrenocortical hormones in blood, urine and saliva were decreased in the workers exposed to high concentrations of carbon disulfide exceeding the national allowable standard (Yang *et al.*, 1998, article in Chinese).

No effect on Adreno Cortico Tropic Hormone (ACTH) has been reported in the cohort study by Takebayashi (1998, 2003).

Conclusion on in vivo mechanistic data:

Overall, there are **positive evidence for endocrine activity based on T4 level changes** for the thyroid modality.

In the recent EOGRTS (Unpublished report, 2019) a decreased T4 level has been noted in males and lactating females of the F0-generation and in males in the F1-generation (cohort 1A) at the top dose only (120 mg/kg bw/d) and in absence of general (maternal) toxicity. Nevertheless, at this dose, significant changes in brain absolute and relativeweight decrease was observed and retinal atrophy showing that the T4 effect can be accompanied with another toxicity that is not general toxicity. In this study, T4 levels were measured in F0-animals at necropsy (11 weeks in males and 16 weeks in females), in the F1 animals at PND 89-100 (Cohort 1A) and also in the following groups:
- F1 animals (Cohort 1B) at scheduled necropsy (LD21-23 in females and after mating in males),
- F1 culled pups on PND 4,
- F1 pups (Cohort 2B) and surplus pups on PND 21-22,
- F2 pups on PND 21-23.

No changes were reported in pups.

The effect on T4 levels was not secondary to excessive toxicity as no general toxicity was observed in the EOGRT study. Although not consistently seen, an effect on T4 levels has also been reported in human cohorts, supporting a human relevance for this effect. When measured either in animals or in humans, no changes in T3, TSH levels or UTBG have been reported.

There is insufficient evidence for endocrine activity based on changes in male reproductive hormone levels (testosterone, FSH, LH) although it has been investigated in several studies both *in vivo* in animals and in humans. The main uncertainties are:

- Effects on testosterone, LH, FSH were only assessed in non-guideline animal studies. Hormone levels were not assessed in EOGRTS or in 90-day guideline studies.
- In human, exposure was, in some studies, insufficiently characterized therefore some studies have low weigh due to the inherent design (case-control study, cross-sectional). Potential co-exposure was not taken into account, although there are known coexposure (e.g. dihydrogen sulfide (H2S)).
- There are only few studies available in women. However, transfer to babies of unmetabolized carbon disulphide via the milkis of concern (Cai and Bao, 1981).

There are insufficient evidence for endocrine activity based on prolactin changes due to insufficient data. **Decreased prolactin levels in males and increased prolactin levels in menopaused women have been reported in human epidemiological studies.** Nevertheless, in males only 3 studies investigated the prolactin levels and an effect was seen in one study where controls were not investigated for this parameter (Cirla *et al*., 1978). In female, only one study (Pieleszek *et al.,* 1997) is available but the results were published in Polish and a causality is difficult to establish due to the inherent limitation of case-control studies.

There are insufficient evidence for endocrine activity based on estradiol changes due to insufficient data. Estradiol levels were only investigated in workers in one study in males and one study in females. No effects were seen in males (low number of individuals) and effects were seen in menopaused females in Pieleszek *et al.,* 1997. As above for prolactin, the results were published in Polish only and a causality is difficult to establish due to the inherent limitation of case-control studies. Few epidemiological studies were available in women. No data in animals have been found investigating estradiol hormone levels.

With regard to effects of carbon disulphide on adrenocortical hormones (cortisol, ACTH), related ot non-EATS modalities, there are some evidence of effect on adrenocortical function based on decreased urinary levels in these hormones in some human studies. Nevertheless, the information is limited to old studies or studies not published in English. Due to potential bias in these studies and potential co-exposure, there are currently insufficient evidence due to insufficient data. No animal studies investigating adrenocortical hormone levels following carbon disulphide exposure have been found in the literature

***In vivo* data providing evidence of EATS-mediated parameters**

There are three 90-day inhalation studies in rats and mice conducted according to OECD TG 407 (Unpublished report, 1983a,b,c). A recent EOGRT study conducted in rats by oral route is also available (Unpublished report, 2019). Some *in vivo* studies investigating male sexual function were also retrieved in the literature. The table below summarized the observed effects in animals. The reliability and the limitation of the animal and human data presented below are assessed in section 6.9 above.

Table 25: Lines of evidence for EATS-mediated parameters

Species	Evidence	Study	Effective dose	Observed effect	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Rat	Sperm morphology	Oral EOGRTS, OECD TG 443 (Unpublished report, 2019), K2	LOAEL= 120 mg/kg (~ eq to 300ppm, inhalation) NOAEL=12 mg/kg (~ eq to 30 ppm, inh.)	At the top dose in F1-generation, increased cells with detached head in sperm, above historical control data	Sufficient change	Overall, positive evidence for adversity	E, A, S
Rat	Sperm morphology	Non-guideline 10-week male inhalation study, K2 (Huang *et al*., 2012)	LOAEC= 396 ppm (1251 mg/m3) NOAEC= 79 ppm (250 mg/m3)	Dose-related increased abnormal sperm, statistically significant	Supportive (non-standard study design, low number of animal (6/group))		E, A, S
Rat	Sperm morphology	Non guideline ip male study, K4 (Kumar *et al*., 1999)	LOAEL= 200 mg/kg	Increased sperm-head abnormalities	Supportive (non-standard study design, ip route)		E, A, S
Rat	Sperm numbers	Non-guideline male 10-week inhalation study, K2 (Tepe and Zenick, 1984)	LOAEC= 600 ppm (1896 mg/m3)	Statistically significant decrease in epididymal and ejaculated sperm count	Supportive (non-standard study design)	Overall, positive evidence for adversity at high dose only (maximum tolerated dose)	E, A, S
Rat	Sperm numbers	Non-guideline male 10-weel inhalation study, K2 (Zenick *et al*., 1984)	LOAEC = 600 ppm (1896 mg/m3)	Statistically significant decrease in sperm count in the epididymis, Decreased seminal plug weight	Supportive (non-standard study design)		E, A, S
Rat	Sperm numbers	Non guideline ip male study, K4 (Patel *et al*., 1999)	LOAEL= 200 mg/kg	Decreased sperm count	Supportive (non-standard study design, ip route)		E, A, S
Rat	Sperm numbers	Non-guideline 10-week male inhalation study, K2 (Huang *et al*., 2012)	LOAEC= 79 ppm (250 mg/m3) NOAEC= 16 ppm (51 mg/m3)	Dose-related statistically significant decrease in sperm concentration	Supportive (non-standard study design, low number of animal (6/group))		E, A, S
Rat	Sperm numbers	Oral EOGRTS, OECD TG 443 (Unpublished report, 2019), K2	LOAEL= 120 mg/kg (~ eq to 300ppm, inhalation)	No effects in sperm count	Not supportive		E, A, S
Rat	Mating	Non-guideline 10-week	LOAEC= 600 ppm	Decreased latency to ejaculation. No	Supportive	Sufficient	E, A, S

	behavior	male inhalation study, K2 (Zenick *et al.*, 1984)	(1896 mg/m3)	effects on mounts or intromissions	(non-standard study design)	evidence for ejaculation latency Observed in 2 independent experiments from the same laboratory but only one top dose tested, non-standard study design, low number of animal per groups.	
Rat	Mating behavior	Non-guideline male inhalation fertility study, K2 (Tepe and Zenick, 1984)	LOAEC= 600 ppm (1896 mg/m3)	Statistically significant decreased in ejaculation latencies after 4-week exposure and mount latency after 7-week exposure in sexual active rats	Supportive (non-standard study design)		E, A, S
Rat	Mating behavior	Oral EOGRTS, OECD TG 443 (Unpublished report, 2019), K2	LOAEL= 120 mg/kg (~ eq to 300ppm, inhalation)	No effect on precoital time	Not supportive		E, A, S
Rat	Organ weight: prostate	Non-guideline male inhalation study, K2 (Zenick *et al*., 1984)	LOAEC= 600 ppm (1896 mg/m3)	Statistically significant decrease in absolute prostate weight. Trend for relative weight	Supportive (non-standard study design)	Insufficient evidence	E, A, S
Rat, mice	Organ weight: prostate	90-day inhalation study, OECD 407 (Unpublished report, 1983a, b, c)	NOAEC= 800 ppm (2528 mg/m3)	No changes in prostate weight	Not supportive		E, A, S
Rat	Organ weight: prostate	Oral EOGRTS, OECD TG 443 (Unpublished report, 2019), K2	NOAEL= 120 mg/kg (~ eq to 300ppm, inhalation)	No changes in prostate weight	Not supportive		E, A, S
Rat	Organ weight: testis	Non-guideline 10-week male inhalation study, K2 Gao *et al*., 2014	LOAEC= 250 ppm (790 mg/m^3)	Marked statistically significant decrease in testis weight, not dose-related	Supportive (non-standard study design)	Insufficient evidence	E, A, S
Rat	Organ weight: testis	Non-guideline male 4-week inhalation study, K2 Guo *et al.,* 2015	LOAEC= 50 ppm (158 mg/m3)	Dose-related statistically significant decrease in absolute testis weight	Supportive (non-standard study design)		E, A, S
Rat, mice	Organ weight: testis	90-day inhalation study, OECD 407 (Unpublished report, 1983a, b, c)	NOAEC= 800 ppm (2528 mg/m3)	No changes in absolute testis weight.	Not supportive		E, A, S
Rat	Organ weight: testis	Oral EOGRTS, OECD TG 443 (Unpublished report, 2019), K2	NOAEL= 120 mg/kg (~ eq. to 300ppm, inhalation)	No changes in absolute testis weight	Not supportive		E, A, S

Rat	Organ weight: testis	Non-guideline male inhalation study, K2 (Tepe and Zenick, 1984)	NOAEC = 600 ppm (1896 mg/m^3)	No changes in testis weight	Not supportive		
Rat	Organ weight: testis	Non-guideline male 10-week inhalation study, K2 (Zenick *et al*., 1984)	NOAEC = 600 ppm (1896 mg/m^3)	No changes in testis weight	Not supportive		E, A, S
Rat	Histopathological changes: testis	Non-guideline 10-week male inhalation study, K2 Gao *et al*., 2014	LOAEC= 250 ppm (790 mg/m^3)	Deformed chromatin in Sertoli cells, disrupted membrane junction, vacuoles in spermatocytes	Supportive (non-standard study design)	Insufficient evidence	E, A, S
Rat	Histopathological changes: testis	Non-guideline male 4-week inhalation study, K2 Guo et al., 2015	LOAEC= 50 ppm (158 mg/m3)	Degenerative changes in seminiferous tubules (no incidence provided)	Supportive (non-standard study design)		E, A, S
Rat, mice	Histopathological changes: testis	90-day inhalation study, OECD 407 (Unpublished report, 1983a, b, c)	NOAEC= 800 ppm (2528 mg/m3)	No histopathological changes in testis	Not supportive		E, A, S
Rat	Histopathological changes: testis	Oral EOGRTS, OECD TG 443 (Unpublished report, 2019), K2	NOAEL= 120 mg/kg (~ equivalent to 300ppm, inhalation)	No histopathological changes in testis	Not supportive		E, A, S
Rat	Histopathological changes: testis	Non-guideline male 10-week inhalation study, K2 (Zenick *et al*., 1984)	NOAEC= 600 ppm (1896 mg/m3)	No histopathological changes in testis	Not supportive		E, A, S
Rat	Histopathological changes: testis	Non-guideline male inhalation study, K2 (Tepe and Zenick, 1984)	NOAEC= 350 ppm (1106 mg/m3)	No histopathological changes in testis	Not supportive		E, A, S
Rats	Organ weight: ovary	90-day inhalation study, OECD 407 (Unpublished report, 1983a)	LOAEC=800 ppm (2528 mg/m3)	Decreased absolute weight in presence of severe signs of neurotoxicity, decreased bw	Supportive (no effect on relative weight, high neurotoxicity)	Insufficient evidence (not seen in both rat studies, only in presence of mortality in mice)	E, A, S
Rats	Organ weight: ovary	90-day inhalation study, OECD 407 (Unpublished report, 1983 b)	NOAEC=800 ppm (2528 mg/m3)	No changes reported on ovary weight	Not supportive		E, A, S
Mice	Organ weight: ovary	90-day inhalation study, OECD 407 (Unpublished report, 1983c)	LOAEC=800 ppm (2528 mg/m3)	Decreased absolute and relative weight, in presence of mortality and decreased bw	Not supportive		E, A, S
Rat	Organ	Oral EOGRTS, OECD TG	NOAEL=120 mg/kg	No changes reported on ovary weight	Not supportive		E,A,S

	weight: ovary	443, K2 (Unpublished report, 2019)					
Rat	Oestrous cycle: prolongation	Oral EOGRTS, OECD TG 443, K2 (Unpublished report, 2019)	NOAEL =120mg/kg (~ 300 ppm, inhalation)	No changes in oestrous cycle	Not supportive	Insufficient evidence	E, A, S
Rat	Oestrous cycle: prolongation	Non-guideline female 4-month inhalation study, K4 (Acadzhanova *et al.,* 1978)	LOAEC = 3 ppm (9.5 mg/m3)	Significant prolongation of oestrous cycle	Supportive evidence (non-standard study design, secondary literature)		
Rat	Oestrous cycle: prolongation	Non-guideline female inhalation one-generation study, K2 (Nemec *et al*., 1993)	NOAEC = 500 ppm (1580 mg/m3)	No effect	Not supportive		

*E, A, S : estrogen, androgen, steroroidogenesis.

In men, various effects including effects on sperm count, morphology and decreased libido were reported. Already in 1969 and 1972 epidemiological studies (Lancranjan *et al.*) reported in men intoxicated with carbon disulphide (showing polyneuritis), effects on sperm morphology (e.g. teratospermia) and libido, ejaculation or orgasm complaints. After stopping the exposure for a few months, the sperm parameters returned to normal. No correlation with length of exposure was identified. In more recent cohort studies, at exposure levels around the current OEL, decreased semen quality was found in two studies (Ma *et al*., 2010 and Guo *et al*., 2016) from the same laboratory out of four studies. Decreased seminal round cell was also noted by Vanhoorne et al., 1994. According to Palermo et al. (2016), this findings may be a transient indicator of spermatogenic insult. Effects in semen morphology seen in animals is supported by the human data. Nevertheless, the effective dose in human is uncertain.

Decreased libido was reported in several human epidemiological studies, even in studies investigating dose levels around the current OEL (See table below for a summary). The possibility of a recall bias is mentioned by some authors, since exposure to carbon disulphide generally is associated with reduced libido and impotence. Another limitation is that in none of the studies potential co-exposure to other compounds such as H2S were investigated and taken into account. In animals, although not observed or investigated in guideline studies, effects on copulatory behavior (ejaculation latency) has been noted in two studies from the same laboratory *in vivo* in rats (Zenick *et al*., 1984, Tepe and Zenick, 1984) given positive evidence on mating behaviour effect.

Table 26: Summary of effects in sperm and libido in man epidemiological studies

Study type	Exposure duration/ levels (mean)	Semen quality	Other	Reference
Cohort (n=432)	13-year, 1.28, 3.13 mg/g Creat.	Not investigated	No effects on libido after adjustment with cofoundings	Takebayashi *et al*., 1998
Cross sectional (n=392)	19-year, 0.98, 1.61, 1.94 mg/g Creat. (mean 9.5, 15.8 and 19 mg/m3)	Not investigated	No effetcs on libido	Takebayashi *et al*., 2003
Cohort (n=175)	1-15 year, <6 to >32 mg/m3	No effects	-	Meyer *et al*., 1981
Cross-sectional (n=43)	12.5 year, 10 mg/m3	↓* (within normal WHO range)	↓* libido	Ma *et al*., 2010
Case-control (n= 76)	10 year, 0.03-300 mg/m3 (0.8 mg/m3, 8h-TWA)	↓* (motility, other p. within normal range)	↓SHGB, ↑* semen apoptotic cells, ↓* semen antioxidant capacity and abnormal structure chromatin	Guo *et al*., 2016
Cross-sectional (n=43)	>1year, 2.8-300 mg/m3*year, >300 mg/m3*year	No effects	↓* libido, impotence complaints ↓* seminal round cells	Vanhoorne *et al*., 1994

* Statistically significant, SHGB: Sex-hormone binding globulin

In women, in the retrospective cohort study by Zhou *et al*., 1988, a higher incidence of menstrual disturbance (irregular cycle) was found in workers exposed to carbon disulphide compared to non-exposed women workers (RR = 2, $p<0.01$). An exposure-response relationship was observed in the study, suggesting possible menstrual cycle disorders. According to Gelbke *et al*., 2009, the possible impact of shift work was unclear.

Conclusion on EATS-mediated parameters

Overall, there are **positive evidence for adversity on sperm count and morphology** in animal studies. These findings were supported by human data as there were reported in some studies, mostly at high dose levels. At lower dose levels, effects were not clear. Moreover, potential co-exposure was not investigated.

There is also **positive evidence on mating behaviour changes in male rodents** as decreased latency to ejaculation was seen in 2 independent studies from the same laboratory. With regard to uncertainties, there is no confirmation of the effect in a second laboratory. Moreover, no dose-response was investigated by the laboratory. An effect was only seen at the top dose level of 600 ppm. Mount latency was also seen as being decreased in sexually active males. Unfortunately, ejaculation or mount latency was not studied in the EOGRT study recently conducted. Time to mate was not considered affected in the EOGRTS but dose levels were lower than in Zenick *et al*. (1984) and in Tepe and Zenick (1984). These findings were supported by human data as several studies reported libido complaints.

There are insufficient evidence on effects on male or female reproductive organs. Despite some effects on organ weight have been reported in testis or ovary at high exposure levels, this was not consistently reported in rats and only at toxic doses (including mortality) in mice.

Effects on estrous cycle were reported in one old animal study from the secondary literature (rated use with care), but this effect was not confirmed in the EOGRT study or in the non-guideline one-generation study. Therefore, there were insufficient evidence in animals. Nevertheless, in a human epidemiological study, oestrous cycle change was noted (Zhou *et al*., 1988). Additional human data would be needed to confirm the observed effect taking into account potential confounding factors (co-exposure, shift-work...).

With regard to uncertainties, EATS mediated parameters have been investigated in studies conducted according to OECD TG 407 (Unpublished report, 1983a,b,c) and in an EOGRT study (unpublished report, 2019). Nevertheless, limitation were identified in the EOGRTS, notably the (top) dose selection which was not sufficiently justified. Dose-response may be difficult to assess due to the large interval between the mid and the top dose (12 and 120 mg/kg). In addition, higher dose levels may have been considered. Moreover, oral exposure was used in the EOGRT study for practical reasons. Although comparative TK data (AUC oral *vs.* AUC inhalation) were available (unpublished report, 2018), some differences between oral and inhalation exposure are still possible. Indeed, vapour may be directly distributed to brain via olfactory bulb during inhalation exposure. This nose-to-brain route is not possible following oral route of administration. In addition, the comparative TK study was only performed following acute exposure and in one sex i.e. males. Therefore, there are uncertainties on the converted values from oral to inhalation route for potential neurotoxic findings. Nevertheless, oral route of exposure was considered still acceptable to investigate reproductive toxicity potential of the substance.

***In vivo* experimental animal data providing evidence of Non-EATS mediated parameters or potentially sensitive to EATS parameters.**

The table below summarises the effects non assigned to EATS, non-EATS mediated or potentially sensitive to EATS endpoints.

Table 27: Lines of evidence for parameters 'non-EATS mediated' or 'potentially sensitive to, but not diagnostic of EATS'

Species	Evidence	Study	Effective dose*	Observed effect	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Rat	Number of ovarian follicles	Oral EOGRTS, OECD TG 443, K2 (Unpublished report, 2019)	LOAEL =120 mg/kg (~ 950 mg/m^3, inhalation)	At the top dose, decreased primary and primordial follicle count, above historical control data Decreased corporea lutea (not statistically significant)	Sufficient change	Positive evidence	N
Rat	Skeletal malformation	Prenatal developmental inhalation toxicity study, K2 Saillanfait *et al*., 1989	LOAEC =630 mg/m3	Increased club foot at maternal toxic dose	sufficient evidence, in presence of maternal toxicity		N
Rabbit	External and skeletal malformations	Prenatal developmental inhalation toxicity study, K2 Unpublished report, 1991	LOAEC = 3792 mg/m^3	Increased hydrocephaly and skeletal malformation in presence of maternal toxicity			N
Rat	Post-implantation losses	Oral EOGRTS, OECD TG 443, K2 (Unpublished report, 2019)	LOAEL =120 mg/kg (~ 950 mg/m^3, inhalation)	Increased post-implantation losses in both generation. Stronger effects in the F1 generation compare to F0 generation	Sufficient change	Positive evidence	N
Rabbit	Embryotoxicity	Oral prenatal developmental toxicity study, K2 Unpublished report, 1991	LOAEC = 1896 mg/m^3	Increased post-implantation losses, reduced number of live foetuses, no maternotoxicity at 1896 mg/m3			N
Rats	Liver weight	Guideline 90-day study (unpublished report, 1983a,b)	LOAEC=2528 mg/m^3	Relative weight increased	Sufficient changes at high dose only		N
Monkey	Biochemical changes: glucose tolerance	20-week inhalation study, K4 (Sperlingova *et al*., 1981)	LOAEC = 1200 mg/m^3	Reduced glucose tolerance	Supporting evidence (non-standard study design)	Sufficient changes. However happens at doses> liver effects	N
Rat	Biochemical changes:	Oral EOGRTS, OECD TG 443, K2	LOAEL= 120 mg/kg	Decreased blood glucose level in F1-generation males (statistically significant)	Sufficient changes		N

	glucose	(Unpublished report, 2019					
Rat	Adrenal weight	Oral EOGRTS, OECD TG 443, K2 (Unpublished report, 2019	LOAEL=120 mg/kg	Increased weight in F0 but not in F1	Not supportive	Insufficient evidence	N
Rat	Adrenal weight	10-day non-guideline study, K4 (Caroldi *et al*., 1985)	NOAEL = 2 mg/l	No changes in adrenal weight	Not supportive		N
Rat	Adrenal weight	Guideline 90-day study (Unpublished report, 1983a, c)	NOAEC = 800 ppm (Guideline 90-day study (Unpublished report, 1983a, b)	No changes in adrenal weight	Not supportive		
Rat	Brain weight	Guideline 90-day study (Unpublished report, 1983a, b)	LOAEC = 158 mg/m^3	Decreased absolute weight	Sufficient change	Positive evidence of an effect	N
Rat	Brain weight	Oral EOGRTS, OECD TG 443, K2 (Unpublished report, 2019)	LOAEL =120 mg/kg (~ 950 mg/m^3, inhalation)	Decreased weight in F0 and F1-generation	Sufficient change		N
Mice	Brain weight	Guideline 90-day study (Unpublished report, 1983c)	LOAEC = 950 mg/m^3	Decreased weight	Sufficient change		N
Rat	Brain histopathology	Non-guideline study, K4 (Goettfried *et al*., 1985)	LOAEC = 158 mg/m^3	Histopathological changes in neurons	Sufficient change	Positive evidence of an effect	N
Mice	Brain histopathology	Guideline 90-day study (Unpublished report, 1983c)	LOAEC=948 mg/m^3 NOAEC= 158 mg/m^3	Histopathological changes in neurons (axonal degeneration and swelling)	Sufficient change		N
Rat	Brain morphometry	Oral EOGRTS, OECD TG 443, K2	LOAEL = 120 mg/kg	Increased mean caudate putamen width in females, significant at all dose levels at PND21 and top dose at	Sufficient evidence, unknown biological	Positive evidence	N

		(Unpublished report, 2019)		PND 76-90, decreased corpus callosum thickness in males at the top dose only (120 mg/kg)	relevance		
Rat	Landing footsplay	Oral EOGRTS, OECD TG 443, K2 (Unpublished report, 2019)	LOAEL = 120 mg/kg	Decreased landing footsplay, not statistically significant but dose related in both males and females and above 20% difference compared to control	Sufficient evidence	Positive evidence	N

N: not assignable to a specific modality, * values in ppm were converted in mg/m3 using the standard conversion factor (1ppm =3.16 mg/m3)

Human studies providing evidence of Non-EATS mediated parameters or potentially sensitive to EATS parameters.

In human, early menopause was described in workers exposed to carbon disulphide compared to non-exposed women workers (Pieleszek *et al.,* 1997). Nevertheless, the study is limited as it is a case-control study and as it was not published in English.

In human, some studies have reported potential effects of carbon disulphide on abortion as in Hemminki and Niemi (1982). Nevertheless, none of the studies were robust enough to make a firm conclusion. It may be noted that a transfer of carbon disulphide to the newborn has been shown with the presence of carbon disulphide in milk and urine of babies of women workers (Cai and Bao, 1981).

Effects on blood glucose metabolism have been described in human but not consistently across the studies. Moreover, glucose tolerance has not been analysed in reliable studies, leaving uncertainties about the relevance of these observations in humans.

Effects on lipid metabolism (mainly increased LDL cholesterol) were consistently reported across the human studies and there are positive evidence of an effect in human. Fatty deposit formation has been demonstrated in rats, mice and rabbits (Lewis *et al*. 1999; Van stee *et al*., 1986) at high concentrations (≥ 500 ppm). A link between changes in LDL and fatty deposits has been hypothesized in the literature as discussed in the mode of action section. In human this has been hypothesized to be involved in the cardiovascular toxicity of the substance in addition to its direct cardiovascular toxicity.

Carbon disulphide is a neurotoxicant in animals and in human. As described in section 6.9.4, exposure to carbon disulphide induces neuropathy for myelinated axons (axonal swelling with accumulation of neurofilament proteins in distal motor and sensory nerve tracts). Such effects were reported in workers and consisted notably of nerve conduction velocity reduction, polyneuropathy and impaired performance in psychomotor testing. Autonomic nervous system effects were also reported in humans. Retinopathy and effect in vision has also been reported in human and animals.

Conclusion on in vivo parameters sensitive to EATS

In animal or human studies, there is positive evidence that carbon disulphide is a neurotoxicant, cardiotoxicant and is embryotoxic and foetotoxic and can induce effects on ovary follicle counts. In addition in human, increased LDL cholesterol have been consistently seen in several studies, including cohorts. Glucose levels were also affected by carbon disulphide.

Main uncertainties on the available database on carbon disulphide

The main uncertainties are that the reproductive effects analysed in the EOGRTS were not enough investigated due to insufficient top dose level.

Moreover, male hormonal levels were only investigated in non-guideline studies, having some limitations. Confirmation of the presence or absence of effects on male hormone levels in guideline studies was not available. Other hormones such as GHRH, prolactin or oxytocin were not investigated leading to uncertainties on potential MoA.

7.10.2.2 Analysis of the evidences and Mode of Action (MoA)

In order to conclude wether ED criteria are met, after considering the adverse effects and endocrine activities observed in the above section, the plausible link between endocrine activity and observed effects that are relevant to human shall be investigated. To this aim, a mode of action analysis has been done.

With regard to carbon disulphide MoA leading to toxicity, the substance can react directly with sulfhydryl- and amino groups of proteins and amino acids and catechol amines (e.g. dopamine) following the formation of reactive intermediate (e.g. sulfur) during oxidative

metabolism via Cyp 450. This can lead to the disturbance of various biochemical parameters. In addition, during oxidative metabolism of carbon disulphide, the formation of metabolites such as thiourea (i.e. a clear CMR substance) can also interfere with enzymes (e.g. thyroxine peroxidase). Besides, the formation of thiocarbamates can also form complexes with metals such as Zn or Copper and inactivate certain enzymes (e.g. dopamine-beta-hydroxylase);

a) Sperm effects

Sperm effects (decreased count, increased morphological changes) have been reported in animals and the relevance of the effect is supported by human data.

Although no effect on fertility has been observed in the EOGRT study, the low doses used in the study do not allow to draw a firm conclusion.

The observed adverse effects were **not seen in presence of excessive dose/toxicity**. Indeed, in the EOGRTS, no general toxicity was seen in animals at the top dose where sperm effects were reported. The effect is thus considered as potentially adverse and **not a non-specific secondary consequence of general toxicity**. Nevertheless, in the EOGRTS, at the top dose, some findings suggestive of neurotoxicity (decreased brain weight, retinopathy) were observed but no brain histopathological findings were noted. Sperm effects could therefore **be secondary to a specific toxicity**.

Some studies suggested potential effects on FSH, LH or testosterone and thus a potential interaction with hypothalamic-pituitary-gonadal axis (HPG) cannot be excluded. Nevertheless, data were insufficiently robust to conclude on an EATS-mediated MoA.

In that sense, one could hypothesise another specific neurotoxicity MoA affecting this axis but there is no specific data to support this hypothesis. Moreover, carbon disulphide is able to produce neurological damage to both the central and the peripheral nervous system, there is no clear evidence of a specific neurotoxicity on this axis.

Another hypothesis tested is that carbon disulphide may interfere with steroid synthesis pathway as the substance can alter cholesterol homeostatis. There is no evidence that carbon disulphide would modify the transformation of cholesterol to pregnolone, which is a limiting step for steroid hormone synthesis. In addition, as carbon disulphide increased LDL cholesterol, it may be hypothetised that carbon disulphide could increase cholesterol bioavailability. As circulating cholesterol is not a limiting step for steroidogenesis, no link between increased LDL cholesterol, steroid synthesis alteration and sperm alteration could be established.

Analysis of alternative MOAs than ED explaining sperm effects:

There are mechanistic data in the literature suggesting non-ED MoA for this effect. These non-ED MoA are summarised below. Some recent studies have investigated specific mechanism in male gonads exposed to carbon disulphide.

*Excessive oxidative damage

Carbon disulphide is reported to be associated with the production of reactive oxygen species. Luo *et al*. (2011) also recently showed that in human, oxidative stress may contribute to carbon disulphide-induced toxicity.

In Guo and Ma (2016), semen antioxidant capacity (measurement of total antioxidant capacity in seminal plama) was lower in exposed group compared to control. However, the results should be considered with care as it was a case-control study. This was also observed by the same authors in rats.

*Nitric oxidase pathway
In the study published by Huang *et al.* (2012), changes in sperm number and mobility was observed. The authors explored the role of nitric oxide (NO) in carbon disulphide toxicity. Sperm effects were partially reversed following co-administration of carbon disulphide and a NOS inhibitor (sodium nitroprusside). The authors reported that NO is an intracellular

and intercellular messenger that plays an important role in controlling several cellular functions, and may regulate the HPGA and sex hormone secretion. It has also been reported elsewhere that the level of NO in testis may affect spermatogenesis. As carbon disulfide can react directly with sulfhydryl- and amino groups of proteins, the authors suggest that carbon disulphide may directly interfere with NO synthase. The formation of ROS in tissue may also decrease the concentration of NO by consuming it directly. Overall the study showed that NO pathway may be involved in carbon disulphide toxicity. There are some uncertainties in the results as low number of animals per groups were used (n=6).

*DNA damage
With regard to sperm abnormalities on morphology, an hypothesis is that DNA damage may be responsible of the observed effects.
This is supported by increased DNA damage in male reproductive organs observed in several comet assay in several studies (See 6.9.5 section concluding that the available database does not warrant classification concerning genetic toxicity according to CLP criteria). It is also suggested that "*DNA damage on germ cell has been observed from comet assay in several studies […] may be secondary to the oxidative stress produced by the substance*".)

It may be hypothesised that oxidative stress and increased DNA strand breaks may lead to increase apoptosis and reduced sperm count.

*Apoptosis
Three studies from the same laboratory investigated testicular injury in rats induced by carbon disulphide.
Gao *et al.* (2014), showed that carbon disulphide exposure can impair ultrastructure of germ cells, increase the numbers of apoptotic germ cells, accumulate intracellular level of calcium, increase ROS level, and increase activities of certain respiratory chain complexes. In this study, rats were exposed 10-week and cell culture was performed. carbon disulphide decreased the mitochondrial transmembrane potential and levels of ATP and membrane permeability transition pore opening. The authors suggested that carbon disulphide can cause damage to testicular germ cells via this mitochondrial apoptotic pathway.

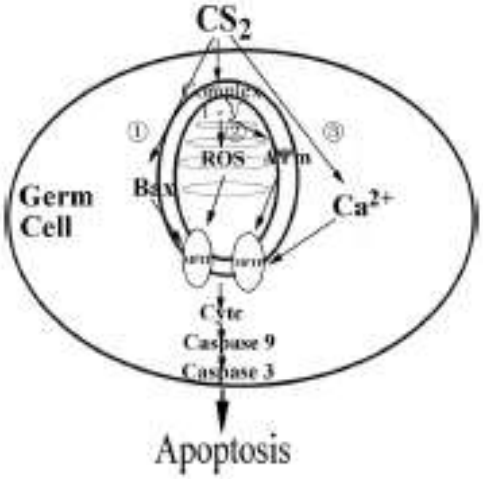

Figure 2: Graphical abstract of CS_2-induced apoptosis (Gao *et al.*, 2014)

Guo *et al.* (2015) found that following 4-week exposure to carbon disulphide, loose structures of seminiferous tubules and disordered cell arrangements were observed by light microscopy. Ultrastructural lesions, deformed chromatins and vacuoles formed from swollen endoplasmic reticulum were observed by electron microscopy. After primary culture of Sertoli cells, a dose-dependent increased apoptosis was found. The authors suggested that endoplasmic reticulum apoptotic pathway played an important role in carbon disulphide-induced Sertoli cell impairment.

In Guo *et al*. (2016), the authors observed that mitochondrial dysfunction may also play a role in the effects observed in human sperm quality following carbon disulphide exposure. Nevertheless, there are some limitations in this study as it was a case-control study.

Conclusion

Possible link between an ED mode of action and adverse effect notably on sperm endpoints could not be formally evidenced due to lack of convincing data. By contrast, a plausible non ED MoA was identified. As described above, carbon disulphide is a reactive substance that is able to induce oxidative stress and damages, potentially leading to cell death and apoptosis. As for the ED-MOAs, there is some uncertainties on this non-ED MoA, as the available studies had some limitations, as described in section 6.9. However, evaluating MSCA considers it plausible. **On this basis**, **the substance does not meet the ED criteria for this effect**.

b) Male behavioral changes

An adverse effect on mating behavior (ejaculation latency, mount latency) has been identified in the studies from Tepe and Zenick (1984) and Zenick *et al*. (1984) at the high dose of 600ppm. The effects were not secondary to excessive toxicity. At 600 ppm, the maximum tolerated dose was not exceeded as body weight changes were around 10% compared to controls. Nevertheless, at this dose, strong neurotoxicity is expected.

Tepe and Zenick (1984) hypothesised that the effects on male copulatory behaviour may be due to alteration of brain monoamine levels, in particular of brain dopamine (due to decreased brain norepinephrine concentrations, probably through the inhibition of dopamine-beta-hydroxylase activity) as increased dopamine concentration stimulate mating behaviour. As ejaculation is controlled by the sympathetic nervous system, one hypothesis is that carbon disulphide could alter the availability of neurotransmitters leading to incomplete ejaculations.

The formation of thiocarbamates during carbon disulphide metabolism can complex metals such as Zn or copper and inactivate certain enzymes such as dopamine beta-hydroxylase (DBH) (DeMartino *et al*., 2017). Reversible dopamine beta-hydroxylase activity inhibition by carbon disulphide has been demonstrated in animals in vitro. DBH converts dopamine to norepinephrine*. In vivo*, exposure of rats for 4 hours per day during 10 days at 642 ppm showed decreased noradrenaline levels and increased dopamine levels in the brain (Magos and Jarvis, 1970). In exposed workers, Homovanillic acid and vanillylmandelic acid, two end products of dopamine metabolism, have been found to be reduced following chronic exposure (Yang *et al.,* 1996).

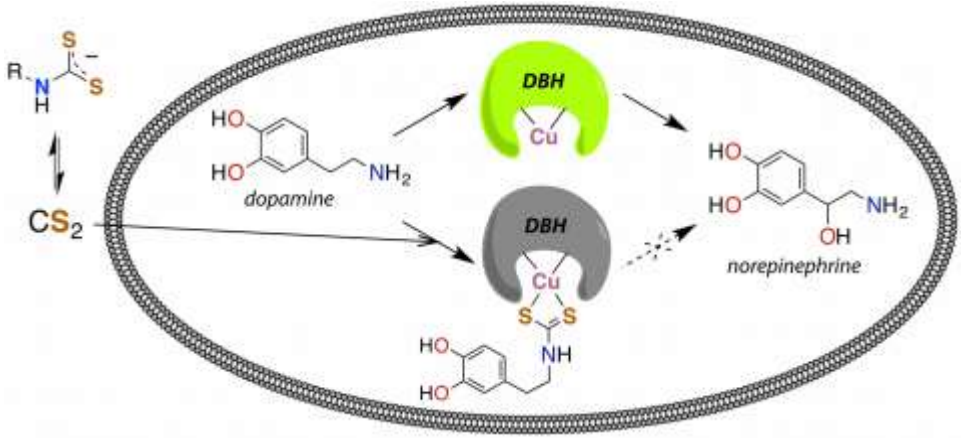

Figure 3: Desactivation of DBH by CS_2 (DeMartino *et al.,* 2016)

Decreased dopamine concentration and dopamine beta-hydroxylase activity may be related to alterations in the mesolimbic system (region where dopamine is synthesised and secreted), which is involved in the regulation of mating behavior and may be a relevant non-specific non-ED MoA.

In humans, decreased libido has been noted quite consistently in several studies. Nevertheless, some authors pointed out that "recall" bias (i.e. when participants in a study are likely to recall and relate previous information) may have occurred in these epidemiological studies since potential sexual effects of carbon disulphide were well known to viscose rayon workers. An effect on dopamine may also be a potential hypothesis for the observed effect.

Although no AOP has been developped, the proposed MoA hypothesis is discussed in the table below.

Table 28: MoA hypothesis and supporting evidence for male behavioral changes

Description of events	Supporting evidence
Non-specific inhibition of dopamine-beta-hydroxylase (DBH) activity	In vitro data suggest that CS_2 can inhibit DBH activity.
Altered brain dopamine levels	Inhibition of DBH can alter dopamine brain levels. Reduced end-product of dopamine was seen in worker's urine exposed to CS_2, suggesting potential effects of CS_2 on dopamine levels. Brain dopamine levels alteration was seen in vivo in rats.
Alteration of limbic system	Effects on ejaculation, libido seen following CS_2 exposure in human or animals. It is known that dopamine and norepinephrine are distributed in the limbic system that is involved in the regulation of male behaviour.

It may also be noted that in the EOGRTS, an increased width of caudate putamen has been observed in pups. This effect is of unknown biological relevance but may support an effect on dopamine levels as this region is rich in dopaminergic neurons.

With regard to dose levels, neuropathology already occurred at 50 ppm in non-guideline studies. In contrast, male rat behavioural changes were only noted at 600 ppm. This supports that the effects were seen in presence of neurotoxic findings. Nevertheless, one uncertainty is that only one dose was tested in the study and that the NOAEL for the effect is unknown. Libido alteration in human was already reported in recent studies at a concentration close to the current OEL. In that case again, the "*potentially endocrine-related adverse effects can be considered as secondary to other (non-endocrine) toxicities*" as substantiated by the abovementionned MOA. In this case, the ED-guidance (ECHA/EFSA guidance document, 2018) clearly states that the substance should not be considered as an ED.

Nevertheless, in the limbic system, hormones such as oxytocin can also regulate and enhance sexual behaviour in male rodents. Central administration of oxytocin facilitates erections and reduces the latency to ejaculation in male rats. There is no available data on oxytocin levels following exposure to carbon disulfide (either in animals or in humans). Therefore, an alternative MoA, related to a direct effect of carbon disulfide on oxytocin cannot be excluded.

Furthermore, it is important to remind that testosterone is the main hormone involved in libido in men and expression of sexual behavior in rodents, through genomic actions upstream the neurotransmitters dopamine and serotonin, or neurohormones such as oxytocin. The studies of Zenick *et al*. (1984) showed no changes in testosterone levels, but potential effects of carbon disulphide on male behavior through modifications of the neural expression of sex steroid receptors mediating testosterone-dependent activation of this behavior cannot be excluded either.

Conclusion

Possible link between an ED mode of action and adverse effect notably on behavior could not be formally evidenced due to lack of convincing data. By contrast, a plausible non ED MoA was identified. Decreases in dopamine levels and subsequent changes in the limbic nervous system may alter male behaviour. Carbon disulphide is able to interact **non-**

specifically with enzymes and was shown to inhibit dopamine hydroxylase activity and reduce brain dopamine levels. Although the inhibition of neurotransmitter synthesis should be taken into account in general in the ED MOA, the non-specific interaction suggested here for carbon disulphide and neurotransmitter enzymes should be considered as a non-ED MOA.

Overall, changes in behaviour do not meet the ED criteria as the effects observed, though involving neurotransmitters and potentially neurohormones and hormones, are impacted *via* a non-ED specific early key event.

c) Thyroxin level changes

A marked decrease in T4 levels has been noted in the EOGRTS performed in males and lactating females of the F0-generation and in males in the F1-generation (cohort 1A) at the top dose only (120 mg/kg). In this study, T4 level changes were only observed in adults and not in pups on PND 4 or PND 21-23.

T4 decrease has also been described previously in rabbits and in human. No change in T3 nor in TSH has been noted in both experimental animals or in human. Inconclusive data on thyroid transactivation reporter assay, cell proliferation assays has been obtained (Toxcast data) due to quality control check deficiencies. Nevertheless, the anti-thyroid effect of metabolites of carbon disulphide has not been sufficiently investigated. In the literature, some authors believed that the antithyroid action of carbon disulphide is a consequence of the formation of a variety of potentially antithyroid metabolites. Among these metabolites, thiourea is quantitatively the most important and has been found to inhibit TPO in a screening assay (EDSP21 program). Data on other potential metabolites of carbon disulphide has not been found.

Several hypotheses have been postulated between endocrine activity (decreased T4 levels) and evidence of an adverse effect. Although endocrine activity (decreased T4 levels) was observed in animals, no changes in thyroid weight or thyroid histopathology (e.g. thyroid hyperplasia, thyroid hypertrophy) were noted in the available rodent data. Therefore, AOPs including thyroid follicular cell tumors as an adverse outcome linked to chemical inhibition of thyroperoxidase (TPO) or odium Iodide Symporter (NIS) in rodents are not substantiated.

Nevertheless, thyroid hormone levels are also critical for maturation and function of the CNS. Three postulated MoAs are discussed below based on AOPs (under development), included decrease T4 level as a key event, and neurodevelopmental toxicity as an adverse outcome. The AOPs were found in the OECD AOP Knowledge Base.

The tables below summarise the key events (KEs) and the supporting evidence (i.e. the lines of evidence that support each KE).

Table 29: AOP on inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals

	Brief description of key event (KE)	**Supporting evidence**	**Uncertainties**
Molecular Initiating Event (MIE)	Thyroxyperoxidase (TPO), Inhibition	Thiourea, one of CS_2 metabolite inhibit TPO. Analogy between PTU and CS_2 (Neal and Halpert, 1982) support potential effect on TPO.	Only screening data available on CS_2. No data on metabolites except thiourea.
KE 1	Thyroid hormone synthesis, Decreased	T4 serum levels was decreased in animals and humans.	No effects on T3 or TSH
KE 2	T4 in serum, Decreased	T4 levels were decreased in both human and adult animals.	In the EOGRTS, T4 levels were not altered in pups. However, T4 levels during critical window such as PND4-15 were not investigated in vivo mechanistic studies.

KE 3	T4 in neuronal tissue, Decreased	No data	/
KE 4	Hippocampal gene expression, Altered	No data	/
KE 5	Hippocampal anatomy, Altered	No effects observed in pups in the EOGRTS study.	Low dose levels and large interval between mid and top dose used in the study raised some uncertainties on the results. Hippocampal anatomy alteration can be consequent to other MIE such as excessive oxidative damage. Hippocampal neuron apoptosis was found in adult rats (Wang *et al.* 2016). Biochemical changes in hippocampus and CNS (increased beta-glucuronidase) were also reported in adult rats by Opacka *et al*., 1986, suggesting that the effects may be related to an other AOP.
KE 6	Hippocampal Physiology, Altered	Insufficient data.	/
Adverse Effect (AE)	Cognitive Function, Decreased	Lehosky *et al.* (1985) (rated as unreliable mostly due to exposure uncertainties) found potential disturbance of learning ability in pups at around 600 ppm. Decreased landing footsplay was noted in the study in both sexes.	Insufficient investigation of cognitive function in developmenting animals. Learning and memory tests were not performed in the EOGRTS. Auditory startle and motor activity was not affected but some methodology deficiencies noted. Decreased landing footsplay was not statistically significant and historical control were not available. Landing footsplay is not specific of T-modality or hippocampal alteration. Decreased cognitive performance were also reported in adult rats (Wang *et al.* 2016) suggesting that the effect may be due to an other AOP. Memory impairment has also been noted in human adult workers.

Table 30: AOP on sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals

	Brief description of key event (KE)	**Supporting evidence and uncertainties**
Molecular Initiating Event (MIE)	Inhibition, Na+/I-symporter (NIS)	No data.
KE 1	TH synthesis, Decreased	Blood T4 levels was decreased but not T3 levels.
KE 2	Thyroidal Iodide, Decreased	No data
Adverse Effect (AE)	Cognitive Function, Decreased	Lehosky *et al.* (1985) (rated as unreliable mostly due to exposure uncertainties) found potential disturbance of learning ability in pups at around 600 ppm. Effects on motor activity in the EOGRTS cannot be excluded due to study methodology deficiency. Decreased landing footsplay was noted in the EOGRTS in both sexes. Uncertainties: Insufficient investigation of cognitive function in developmenting animals. Learning and memory tests were not performed in the EOGRTS. Decreased cognitive performance were also reported in adult rats (Wang *et al.* 2016) suggesting that the effect may be due to an other AOP. Memory impairment has also been noted in human adult workers.

Table 31: AOP on nuclear receptor induced TH catabolism and developmental hearing loss

	Brief description of key event (KE)	**Supporting evidence**
Molecular Initiating Event (MIE)	Activation, Pregnane-X receptor	NOT SUPPORTIVE No data on CS_2 or its metabolites. Mice exposure results in Cyp 450 inhibition which is not in line with the proposed mechanism (Jarviso *et al.* 1977)
KE 1	induction, Upregulation of glucuronyltransferase activity	NOT SUPPORTIVE: UDP-glucuronyltransferase was not affected or decreased *in vivo* by CS_2 (ATSDR, 1996 review)
KE 2	Increase, Biliary excretion TH glucuronide	No data
KE3	Thyroxine (T4) in serum, Decreased	Observed in animals and in human
KE 4	Thyroxine (T4) in neuronal tissue, Decreased	No data
KE 5	Hippocampal gene expression, Altered	No data
KE 6	Hippocampal anatomy, Altered	Hippocampal neuron apoptosis found in adult rats (Wang *et al.* 2016). Biochemical changes in hippocampus and CNS (increased beta-glucuronidase) reported in adult rats by Opacka *et al.*, 1986. Nevertheless, not effects in pups were observed in the available EOGRTS study. Nevertheless, low dose levels used in the study raised some uncertainties on the results.
KE 7	Hippocampal Physiology, Altered	No data
Adverse Effect (AE)	Loss, Cochlear function	No effect on acoustic startle response in DNT cohort of the EOGRTS (but some study deficiencies in the methodology was noted). Low dose exposure does not allow a conclusion at higher dose levels (insufficiently investigated in this study and not investigated in other animal studies). Hearing function has been found to be altered in human workers following CS_2 exposure.

Interaction of carbon disulphide with the hypothalamus pituitary thyroid axis via a non-ED MoA has also been identified. Wang *et al.* (2017) found direct apoptotic effects of carbon disulphide on neurons in the hippocampus, in adult rats due to oxidative damages, supportive of an non-ED MoA. Guo *et al.*, 2008 also found that in rat hippocampus, calcium-dependent constitutive nitric oxide synthase (cNOS) was decreased, including neuronal NOS. The authors hypothetised that the reduction was due to direct reaction of carbon disulphide with nucleophilic groups. Carbon disulphide also altered neuronal NOS gene expression in the study that is, according to the authors likely to be a potential molecular mechanism of the effect of carbon disulphide on learning and memory. **Therefore, the effect of carbon disulphide on hippocampus was suggested to be related to cNOS effects that was not selective to the hippocampus as it may have been observed in other parts of the brain.**

There is no data available on carbon disulphide suggesting that neuroendocrine cells (e.g. in the hypothalamus) would be more sensitive to carbon disulphide than other nervous tissue cells. However, it is noted that as some hypothalamic neuroendocrine cells, in particular those located near the median eminence, are not protected by the classical non-fenestrated blood brain barrier, these cells may be more exposed than other neural cell types.

No data are available to support a link between T4 level decrease and embryotoxicity or malformation (hydrocephalus, skeletal malformation). Moreover, malformation were mostly observed in presence of maternal toxicity.

According to the review published by Wagner et al., 2008, there are clear evidence that thyroid hormone is an important hormonal regulator of testicular development and function. Effects on spermatogenesis has been noted in F1 generation in the EOGRTS study at the same dose level as the observed T4 level decrease. As no AOP has been well established, and despite the above-mentioned publication, the biological link been thyroid T4 level and sperm effect was not further investigated. Indeed, direct unspecific cytotoxic MoA are likely to intervene on the sperm toxicity. Therefore, if T4 modulations could participate to sperm toxicity, it is not considered as the most plausible MoA.

Some authors (Damiano *et al*., 2017, Duntas and Brenta, 2018) suggested an association between the lower serum thyroxine levels and increased serum cholesterolemia. Cholesterol is transported through the circulation by lipoproteins. Among lipoproteins, LDL is atherogenic, susceptible to oxidation and may be involved in coronary heart diseases. Thyroid hormone is the main regulator of lipid metabolism by stimulating the mobilisation and degradation of lipids as well as *de novo* fatty acid synthesis in the liver. T3 actions are mediated via modulation of gene expression and cell signalling pathways, while cholesterol synthesis is mediated by the sensing of intracellular cholesterol in the endoplasmic reticulum via sterol regulatory element binding proteins −1 and−2, the transcription factor that regulates the expression of LDL receptor and cholesterol synthesis (Damiano *et al.*, 2017, Duntas and Brenta, 2018). The figure below from Damiano *et al.* (2017) illustrates the biological link between dyslipidemia, LDL and atherosclerosis.

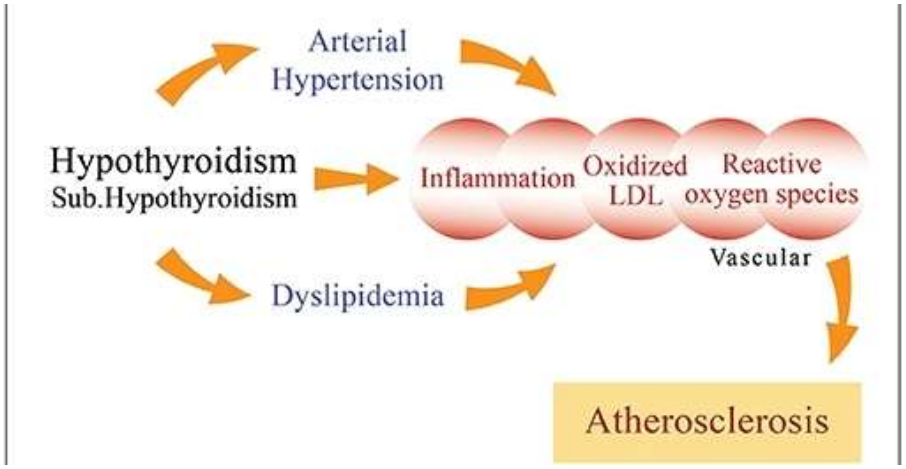

Figure 4: Biological link between dyslipidemia, LDL and atherosclerosis (Damiano *et al.,* 2017)

Nevertheless, no link has been found between decreased T4 levels in rabbits and potential cardiac lesions as measured by atherosclerosis (Van Stee *et al*., 1986). In addition, in case of LDL hypercholesterolemia due to hypothyroidism, increased in TSH and decreased T3 levels would have been expected. TSH values are considered good predictor of cardiovascular disease. This was not observed following carbon disulphide exposure. In addition, in the EOGRTS study, although decreased T4 level was noted at 120 mg/kg, no change in cholesterol was observed at this dose level, suggesting no correlation between the effects.

In contrast, there are supportive evidence of an non-ED MOA. Laurman *et al*., 1989 found that carbon disulphide interacts in *vitro* with LDL, resulting in an increase electrophoretic mobility of particles, due to a decrease in free amino groups of apolipoprotein B-100. Carbon disulphide modification decreases the ability of LDL to down-regulate sterol synthesis and to stimulate cholesterol esterification in fibroblast. Wronska-Nofer *et al.* (1996) also found that, in vitro, carbon disulfide can modify LDL and increase its cytotoxicity.

Exact chemical reactions between carbon disulphide and LDL *in vivo* is not fully elucidated. Chemical modification, such as oxidation of low-density lipoproteins (LDL), is tightly associated with increased LDL uptake by macrophages and the development of arterial fatty streaks.

Overall, carbon disulphide can impair hepatic cholesterol homeostasis via oxidative or protein metabolism disturbance, which is a non-ED MoA. There are too many gaps to fit the sequence of key events between hypothyroxinemia and hypercholesterolemia leading to atherogenesis. The other main uncertainty is that no AOP has been established for hypothyroidism inducing atherosclerosis and that no effects on T3 levels were seen. Additional uncertainty is that the increased in LDL cholesterol can be consequent of both a non-ED MoA (direct interaction with LDL cholesterol) and ED MOA (hypothyroxinemia).

Conclusion:

It is considered that the available information support a biological link between T4 level decrease and an adverse ED-related effect (neurodevelopmental outcome)but that they might be side effects of other toxicities (e.g. direct neurotoxicity).

Neurodevelopmental outcomes may have been the consequence of MoA, ED or not ED related. Nevertheless, the hypothesis of a thyroid-mediated neurodevelopmental alteration and maternal thyroid disruption can not be fully ruled out.

The main uncertainties identified in the AOP is the lack of data on the potential TPO or NIS inhibitory activities of carbon disulphide and all its metabolites (having in mind that these data exist for thiourea, a carbon disulphide metabolite) and the insufficient data on pup cognitive function. Most of all, a non-ED MoA (via excessive oxidative damages) has been identified for the observed effects on the hippocampus.

Similaly, with regard to atherogenesis, both ED-mediated MoA or not ED related MoA were identified. The direct toxic effect of the substance via the formation of reactive sulfur or dithiocarbamates, leading to neuropathology or oxidized LDL already occur at very low dose levels and may explain the lipid deposit. Nevertheless, it is not possible to fully exclude an additionnal direct thyroid-mediated effect.

Overall, carbon disulphide does not meet the ED criteria as the effects observed, though involving T4 hormone activity actions are impacted *via* a non-ED specific toxicity .

d) Non-EATS endocrine parameter

Metabolic syndrome, which is a cluster of three or more conditions occurring together among abdominal obesity, high blood pressure, hyperglycemia, high serum triglycerides, and low serum high density lipoprotein (HDL), is increased in workers intoxicated to carbon disulphide (Jhun *et al*., 2009; Vanhoorne *et al.,* 1992, Luo *et al.,* 2003). Consistently increased serum LDL cholesterol has been observed. In addition, Increased TG, decreased HDL cholesterol, increased blood pressure, increased apolipoprotein A1 and blood oxidative stress were observed in some studies. Interference of blood glucose has also been reported in some studies.

Functional and morphological changes of the heart which extend to necrosis of the myocardium is due to a direct effect on the heart and the increased incorporation of cholesterol and lipoproteins into the heart vessels and thus arteriosclerosis (BUA, 1993).

Decreased somatotropic function may lead to increase body fat, abnormal lipid profile, atherosclerosis, interference with thyroid function and heart diseases.

No biological link between hypothyroxinemia and hypercholesterolemia has been demonstrated following carbon disulphide exposure (See above).

There are insufficient data to conclude on a potential link between cholesterol disturbance, glucose tolerance and an activity on the somatotropic axis.

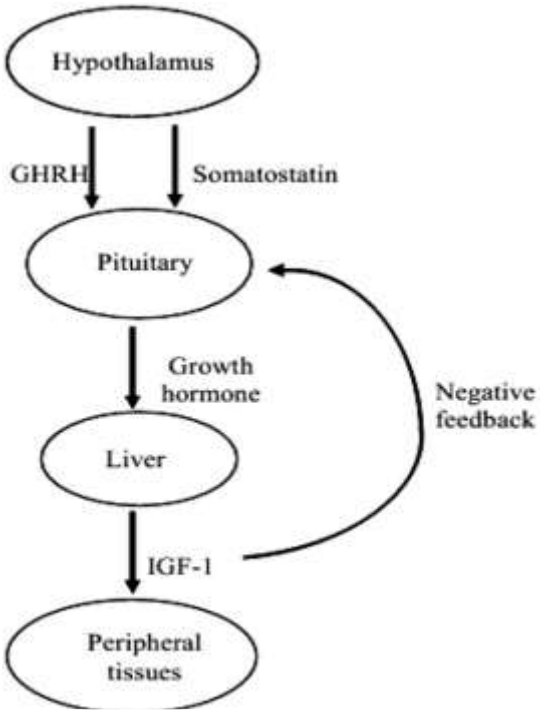

Figure 5: Activity on somatotropic axis (Figure from Manibusan and Touart (2018), adapted from LeBlanc *et al*., 2012)

In the liver, growth hormone regulates enzymes involved in steroid metabolism and in the production of Insulin-like Growth Factor (IGF) levels, that may be a determinant in LDL cholesterol concentration. Modulation of GHRH or somatostatin in the hypothalamus has not been investigated. There is no data on potential changes in liver hepatic growth hormone and IGF-1 levels, which is a somatotropic axis biomarker. There is insufficient data on potential effects on insulin growth factors. The weight of the fetuses or pups were not increased in the developmental studies.

With regard to non-ED MoA, the major precursor of cholesterol synthesis in the liver is acetyl coenzyme A by glycolysis or fatty acid oxidation. Acetyl-CoA gives rise to hydroxyl methylglutaryl-CoA. The rate limiting step in the cholesterol biosynthetic pathway is the conversion of HMG-CoA to mevalonic acid (MVA) by hydroxyl methylglutaryl -CoA reductase. In addition to being synthesized, cholesterol can also be taken up through receptor-mediated endocytosis by hepatocytes. LDL receptor plays an important role in cholesterol homeostasis since it binds plasma LDL particles, thus lowering plasma cholesterol levels. The cholesterol pool obtained from *de novo* synthesis by hepatocytes can be enzymatically esterified by Acyl-CoA- cholesterol Acyl transferase and incorporated into apolipoprotein B. Cholesterol synthesis in the peripheral tissues or transfer from intestine (food) also contributes to the hepatic cholesterol pool through the transfer of cholesterol to the liver in a process mediated by HDL particles.

The formation of reactive intermediate (e.g. carbon sulfuryl) during oxidative metabolism via Cyp450 of carbon disulphide in liver is a probable non specific mode of action of carbon disulphide toxicity; carbon disulphide inactivate Cyp450 in liver. In mice, intermediate-duration inhalation exposures at a concentration of 482 ppm (1523 mg/m3) for up to 23 days (4 hours a day, 5 days a week) have resulted in a marked reduction in cytochrome P-450 and cytochrome c-reductase content after 2-3 days (Jarvisalo *et al*., 1977).

Conclusion

Analysis of the MoA was conducted to investigate the biological plausibility between the adverse effects (metabolic syndrome) and non-EATS endocrine activity. Carbon disulphide can impair hepatic cholesterol homeostasis via oxidative or protein metabolism disturbance, which is a non-ED MoA. As no biological link was found between adverse effect and endocrine activity, the ED criteria are not met for the analysed parameter.

e) Effects 'sensitive to but not diagnostic EATS parameters'.

- *Effect on primary follicles*

Regarding the adverse effects, a decrease in the number of primary follicles was seen in the ovaries in female rats of the F1-generation at the top dose in the EOGRTS. No other effects on fertility or reproduction was noted at this dose in this study. The only effect observed in ovary in other studies was a decreased weight in mice, in presence of mortality at 800 ppm. In view of the severe toxicity observed, the weight effects may not have been treatment–related.

At or around the time of birth, depending on the mammalian species, the ovary contains its maximum quota of primordial follicles, which constitute the true ovarian reserve of the female germ line. The size of the ovarian reserve varies between species and also between individuals of the same species or strain. After birth, no new oocytes are formed during adulthood.

Effects on the ovarian reserve may result in an important effect on fertility and reproduction. This was not observed in the EOGRT study.

As follicles are composed of different types of cells; it implies a lot of possible mechanisms of action and targets. These mechanisms have not been investigated with carbon disulphide.

No link has been found between this effect and an endocrine MoA. In the absence of link, the substance does not meet the ED criteria based on the available data.

- *Developmental toxicity*

With regard to developmental toxicity, malformations (hydrocephalus, skeletal malformation) were noted in both rats and rabbits at maternal toxic doses. Resorptions were also observed at lower dose levels than malformations in both species.

Regarding a potential ED mode of action, Tsai *et al.* (2000) found that pregnant uterus of rat is sensitive to carbon disulphide. In this study, *in vivo* rat exposure to carbon disulphide (10-days during gestation) augmented the frequency of oscillatory contractions induced by KCl, carbachol and a calcium ionophore but not by oxytocin. The authors suggested that *in vivo* exposure to carbon disulphide may sensitise contraction machinery that might be due to the increase of calcium influx. The authors cited an earlier reference showing that carbon disulphide is able to modulate activity of calcium/calmodulin-dependent kinase II in nerve tissue, which might be responsible of carbon disulphide toxicity.

Sun *et al*. (2013) found that carbon disulphide exposure at peri-implantation disrupts embryo implantation by decreasing β3 integrin expression in the uterine tissue of mice. In addition, carbon disulphide would be able to induce embryo loss in mice by perturbing the expression of mTOR signaling pathway, which is involved in decidualization and autophagy in the mouse uterus (Huang *et al.*, 2018 a&b, Liu *et al*., 2019). However, the MOA or potential involvement of nuclear receptors in these modulations have not been reported so far.

Wang *et al. (*2015) reported that single intraperitoneal injection of carbon disulphide in mice during gestation at very high dose (614 mg/kg), produced changes in progesterone and estradiol levels and in the expression of leukemia inhibitory factor, involved in embryo implantation. The authors suggested that these findings may be important factors for implantation disorders. Nevertheless, these effects would need to be confirmed following physiological route of exposure.

Independently of ED potential MoAs, some authors investigated the effects of oxidative stress and DNA damage on embryo implantation in mice. Several studies indicated that DNA damage and apoptosis of endometrial cells might be one of the mechanisms mediated carbon disulphide embryotoxicity (Zhang *et al*., 2014; Yang *et al.,* 2014).

Overall, a plausible biological link has not been sufficiently demonstrated between developmental toxicity and an endocrine activity or adverse endocrine effect induced by carbon disulphide.

7.10.2.3 ED database

According to DEDuCT database (Database of endocrine disrupting chemicals and their toxicity profile), a category III was assigned to carbon disulphide supporting evidence from only in vivo rodents experiments.

The following endocrine-mediated endpoints are reported in the database: Reduced sperm count, affected sperm motility, increased number of abnormal sperms, decreased LH levels, increased and decreased FSH levels, decreased testosterone levels, decrease in T4 levels, changes in morphology of thyroid gland, increased weight of adrenal glands, affects neuronal signaling. Literature identified corresponds to the publications of Kumar *et al*., 1999; Guo *et al*., 2008 and Huang *et al*., 2012. All these findings have been discussed in the section above.

Carbon disulphide is in the TEDX list of potential endocrine disuptors. Additional references provided as supporting evidence were Caroldi *et al*., 1985, Opacka *et al*., 1986 and Tsai *et al*., 2000. These studies were taken into account in the ED-assessment.

7.10.3. Conclusion on endocrine disrupting properties

Human health

The available data show that carbon disulfide can adversely affect male behavior and sperm. Although endocrine activities are reported (T4 levels), the observed effects on male mating behaviour (described above) seem most probably related to the non-specific disturbance of the central and peripheral nervous systems, in particular of neurotransmitter and neurohormone levels, via inhibition of key enzymes involved in neurotransmitter synthesis or directly through neuronal apoptosis processes (e.g. in the hippocampus) involving excessive oxidative damage, reactive sulfur and the formation of reactive dithiocarbamate. Indeed, carbon disulfide can form reactive sulfur or dithiocarbamate, that may be responsible for inactivation of enzymes and induction of oxidative damage already at very low dose levels (e.g. neurotoxicity). Similarly, effects on sperm seem most probably related to non-specific effects via oxidative stress and damages, potentially leading to cell death and apoptosis. It is therefore difficult to identify in this context any specific ED-MoA, given the wide range of toxic effects of carbon disulfide.

In that sense, it was shown that carbon disulfide can alter thyroid hormone concentrations (T4 levels) and neural anatomy and functions most probably by impairing non specifically enzymes or proteins. Similarly, hypothyroxinemia and hypercholesterolemia leading to atherogenesis may be due to direct tissular/cellular toxicity (e.g. direct effect on LDL cholesterol) of carbon disulfide leading to hormonal impairement. Ultimately, the fact that these effects are observed at similar dose levels was an important parameter to conclude that carbon disulfide should not be considered as an ED in a regulatory point of view.

Lipid metabolism homeostasis has been shown to be impaired following carbon disulfide exposure. Cardiopathy have been observed in workers. Nevertheless, data were insufficient to establish a biological link between an endocrine activity notably on the somatotropic axis and the adverse effect. In contrast, carbon disulfide is able to modulate non specifically key enzymes of lipid metabolism.

It is difficult to distinguish a specific adverse effect manifested as a consequence of an ED MoA between the numerous non-specific toxicological effects caused by a substance as reactive and toxic as carbone disulfide. Many pathways are disturbed. Alterations in endocrine function secondary to CS2 exposure appear to be limited but may play a part in the atherogenic and reproductive effects of CS2. It is however doubtful that CS2 induces an endocrine specific key event. In other words, the endocrine MoA observed and

participating to these effects are considered unspecific consequence of other toxicities. Indeed, a a detailed evaluation of the database shows that:

- The endocrine activity observed does not appear at doses lower than those inducing neurotoxicity (althought this is not a prerequisite for being an ED).
- The high cytotoxic potential of the substance is not selective to hormone producing cells.
- The adverse effects that may have arise via an endocrine MoA, were observed along with neurotoxicity;
- The endocrine activities observed could be indirect as a result of other non- endocrine mediated systemic toxicity.

Therefore, although adversity and endocrine activities were observed, many different plausible biological links were described. It is plausible that the effects are secondary to other non endocrine mediated systemic toxicity. Therefore, the ED criteria are not met as carbon disulfide does not fulfill the WHO definition as implemented in EU regulation.

Environment

According to the ECHA/EFSA guidance (2018) applicable to phytopharmaceutical and biocidal active substances, the data package in the registration dossiers of the REACH substance should be considered as not sufficient to be conclusive. The available data for the assessment of the ED-activity or -adversity of carbon disulfide do not reveal any alerts on potential endocrine disruption properties of carbon disulfide on non target organisms other than mammals. Based on a weight of evidence approach, further tests with non-target non-mammalian species would not provide any robust data allowing to identify or exclude carbon disulfide as an endocrine disrupter and therefore should be avoided in terms of animal welfare (see section 6.10.1).

7.11. PBT and VPVB assessment

The substance is readily biodegradable and its bioaccumulation potential is low (BCF <60). Based on the assessment described in the subsections above, carbon disulfide is not a PBT/ vPvB substance in accordance with Annex XIII to Regulation (EC) No 1907/2006.

7.12. Exposure assessment

7.12.1. Human health

Worker

Table 32: Worker exposure scenarios

ES n°	Exposure Scenario (ES) name	PROC	Sector of end-use
Manufacture			
1	Manufacture of Carbon Disulphide	1	-
Use at industrial sites			
2	Manufacturing of regenerated cellulose	1, 2, 4, 8a, 8b, 15, 19	Manufacture of textiles, leather fur, Scientific research and development Technical function: intermediate (precursor); solvent
3	Industrial use as an intermediate, use in the manufacture of plant protection products and biocides	1, 2, 3, 4, 8b, 15	Manufacture of bulk, large scale chemicals, scientific research and development Technical function: intermediate (precursor)
4	Industrial use as a solvent	1, 8b, 15	Manufacture of bulk, large scale chemicals, scientific research and development Technical function: solvent

Inhalation exposure

For all the exposure scenario, modelling with either ECETOC TRA 3.0 or ART v1.5 was used. For manufacture of carbon disulphide, a measured value from personal samplers was provided in the tank loading area (PROC 1). Exposure value provided was more than 50-fold higher than the proposed value obtained by TRA Workers. An explanation for the difference between the measured and the modelled values was not provided. According to ECETOC TRA, 2018 technical report, TRA does allow to assess exposures to very volatile liquids without upper bound set on vapour pressure. Nevertheless, the report noted that the estimate is really likely relevant for PROCs that do not describe open conditions of use. The underestimated value obtained from the model compared to air data observed for PROC1 leads to uncertainties on exposure assessment and safety concern in case of open condition.

The table below provide the range of exposure values provided for the four exposure scenarios.

Table 33: Worker exposure ranges available in the registration dossier

ES no.	EXPOSURE RANGES	
1	1.55 mg/m^3 (0.50 ppm)*	Measured
2	0.032-11.89 mg/m^3 (0.01-3.77 ppm)*	Modelling
3	3.17- 13.32 mg/m^3 (1-4.2 ppm)*	Modelling
4	0.032- 4.76 mg/m^3 (0.01-1.5 ppm)*	Modelling

*converted based on 1 mg/m3 = 0.317 ppm

Dermal exposure

No measurement or modelling of dermal exposure was performed by registrants. The lead registrant only proposed a qualitative way to assess dermal exposure and required the use of gloves only in case of exposure to liquid form of carbon disulfide (mainly from accidental exposure).

The lack of dermal exposure data modelling or human biomonitoring data provide uncertainties on exposure assessment based on inhalation modelling only. The modelled exposure values (TRA) based on inhalation only may underestimate the fact that carbon disulfide vapour may be readily absorbed via the skin.

Literature data

Although a comparison is difficult (e.g. differences in OC & RMMs, no direct comparison to PROC), the literature (published period: 2010-2020) does not contradict that high exposure to worker may occur for some of the uses. Indeed, in the viscose rayon industry, mean values near to the current indicative OEL were observed and median TTCA levels around or slightly above BLV (BLV = 1.5 mg TTCA/g creatinine) were noted (Goen *et al.*, 2014).

Table 34: Worker exposure range available in the litterature

Use	Source	Air exposure level (ppm*)	TTCA levels (mg/g creatinine)
CS_2 in viscose process to manufacture of basic consumables	Domergue *et al.*, 2016		Factory 1 Mean: 0.383-0.833 (2006), 0.05-0.158 (2013 following LEV revision) Factory 2 Mean: 0.067-2.067
Rayon Viscose industry (spinning of textile rayon, technical rayon, washing of textile rayon spools, post-treatment, rayon ageing and filter cleaning departments)	Goen *et al.*, 2014	In 2009: <0.2-20.9 ppm (individual air values), median 2.48 ppm Large variation over time (1992-2008) and departments	In 2009: <0.10 and 5.27 mg/g (median 1.63 mg/g; 65 measurements), spinning of technical rayon department
Viscose production plants	Kilo et al., 2015	2.77 (0.46-20.87) ppm	0.93 mg/g (0.16-5.27 mg/g)

*1 ppm = 3.16 mg/m3

Consumer

No consumer use identified in the registration dossiers. No subsequent service life scenarios were proposed.

7.12.2. Environment

Tonnage approach

Table 35: environmental exposure scenario – Release factors

ES n°	Exposure Scenario name	ERC	Source of release factors considered
Life Cycle Stage (LCS) M: Manufacture			
1	Manufacture of Carbon Disulphide	1	Annual site tonnage = confidential data Daily amount used at site = confidential data Release times per year = 300 days/year **Release fraction to air** = 0% On-site treatment of off-air: Excess sulphur is condensed and recycled to the reactor. The sulphur-free vapours are cooled down below boiling point of CS_2 (46 °C) to separate a liquid CS_2 phase from the gaseous H_2S phase. Left CS_2 vapours in the gas phase are removed in an oil absorption column and the pure H_2S can be used as intermediate for H_2S derivatives while the rest of the H_2S is treated in a so called Claus Unit to form Sulphur again, which is recycled to the reaction section. When recycling is not possible due to low concentrations, air/gas is flared. Air explanation: Gas is completely burned with an efficiency of 100%. **Release fraction to wastewater** = 0% No water used in manufacturing process and cleaning of reactors. **Release fraction to soil** = 0% Systems are closed, therefore no release to soil

Life Cycle Stage (LCS) IS: Use at industrial sites			
2	Manufacturing of regenerated cellulose	6b	Annual site tonnage = confidential data Daily amount used at site = confidential data Release times per year = 365 days/year **<u>Release fraction to air</u>** = 0.049% Local release rate: confidential data Calculated from monitored air concentrations by downstream users over a period of 1 year at three worst case locations on site on 1-2 m height. **<u>Release fraction to wastewater</u>** = 0.065% Local release rate: confidential data Calculated from monitored effluent concentrations by downstream users daily or weekly at the outlet of the STP. Levels measured from monitoring data are always below the limit of detection of 0.1 mg/l. The release fraction is therefore set in such a way that it reflects the release described above with maximum effluent concentration of 0.1 mg/L (worst case assumption). **<u>Release fraction to soil</u>** = 0% No soil contamination during the process. Fraction used at main source = 1 Fraction tonnage to region = 1 STP = no (on-site STP) Discharge rate of STP = confidential data (*maximum volume discharged to the sewer*). River flow rate = 1 730 000 m^3/day *Lowest average river flow rate of 20 m3/s (worst case) indicated by all downstream users* Application of the STP sludge on agricultural soil: No (incineration)
3	Industrial use as an intermediate, use in the manufacture of plant protection products and biocides	6a	Annual site tonnage = confidential data Daily amount used at site = confidential data **<u>Release fraction to air</u>** = 0% Closed system. **<u>Release fraction to wastewater</u>** = 0% Closed system. **<u>Release fraction to soil</u>** = 0% Closed system.
4	Industrial use as a solvent	6b	Annual site tonnage = confidential data Daily amount used at site = confidential data **<u>Release fraction to air</u>** = 0% Closed system. **<u>Release fraction to wastewater</u>** = 0% Closed system. **<u>Release fraction to soil</u>** = 0% Closed system.

Additional relevant details for exposure scenario:

Table 36: Fate and distribution in the local environment – input parameters

Input parameters for calculating the fate and distribution in the local environment		
Input	**Value**	**Unit**
Molecular weight	76.14	$g.mol^{-1}$
Vapour pressure	27400	Pa [25°C]
Henry's law constant	384[1]	$Pa.m^3.mol^{-1}$ [20°C]
Water solubility	2900	$mg.l^{-1}$ [20°C]
Organic carbon/water partition coefficient (Koc)	34	$l.kg^{-1}$
Octanol-water partition coefficient	2.7	[log10]
Biodegradability	Readily biodegradable	[-]
Soil-water partition coefficient	1.24	$[m^3.m^{-3}]$
Total rate constant for removal from agricultural top soil	0.104	d^{-1} at 12°C
Total rate constant for removal from grassland top soil	0.184	d^{-1} at 12°C

[1] Calculated from SimpleTreat V4.

Table 37: Calculated fate and distribution in the STP – SimpleTreat V4

Compartment	Percentage [%]
Air	33.67
Water	4.518
Sludge	0.308957
Degraded in STP	61.51

Table 38: Exposure scenario for Carbon Disulphide

Inputs					
	ES1	**ES2**	**ES3**	**ES4**	**UNIT**
	Manufacture of Carbon Disulphide	**Manufacturing of regenerated cellulose**	**Industrial use as an intermediate, use in the manufacture of plant protection products and biocides**	**Industrial use as a solvent**	
TONNAGE / year	confidential data	confidential data	confidential data	confidential data	**[Tonnes/year]**
TONNAGE / day	confidential data	confidential data	confidential data	confidential data	**[Tonnes/day]**
N days per year	300	365	300	300	**[days/year]**
Fmainsource	1	1	1	1	**[-]**
F regional source	100	100	100	100	**%**
Fwater	0	confidential data	0	0	**%**
Application of the STP sludge on agricultural soil	NR	No	NR	NR	**[-]**
Discharge rate of STP	NR	confidential data	NR	NR	**[m^3/day]**
Receiving surface water flow rate	NR	1.73E+06	NR	NR	**[m^3/day]**
Elocalwater	NR	1.43E+02	NR	NR	**[kg/day]**

NR: not relevant

7.13. Risk characterisation

7.13.1 Human health

Using the current indicative OEL of 5 ppm (15 mg/m^3), risk ratios were below 1 for all modelled exposure scenarios. Only modelling data were available for these scenarios. Thus, there are uncertainties in the exposure estimates. Although Tier I tool ECETOC TRA may be conservative, this was not confirmed by the available measured data on the manufacture of the substance. Indeed, higher values were obtained from air measured data than from modelling data for this exposure scenario raising concern on potential underestimation of worker exposure for other PROC. Moreover, as discussed above, dermal exposure may also be underestimated by TRA modelled estimates for inhalation only.

Using a more conservative inhalation systemic DNEL of 2 ppm (6.32 mg/m^3), a risk characterisation ratio (RCR) > 1 is clearly identified for workers for:
- Industrial use of carbon disulphide for the manufacture of regenerated cellulose:
  - PROC 4 "chemical production where opportunity of exposure arises"
  - PROC 19 "Manual activities involving hand contact"
- Industrial use of carbon disulphide as an intermediate in the manufacture of PPP and biocides:
  - PROC 4 "chemical production where opportunity of exposure arises"

Table 39: Summary of risk characterisation for carbon disulphide uses

Identified use	**Process Category (PROC)**	**RCR** ($DNEL_{inhal}$=2 ppm)	**RMM**	**Conclusion on risk**
Manufacturing of regenerated cellulose	**PROC 4** (indoor, process temp. ≤ 40°C, 8h/d)	**RCR =1.5 (TRA**)	Enhanced general ventilation (70% effectiveness) LEV, 90% effectiveness Dermal protection 95% effectiveness	Risks may not be sufficiently controlled. Additional RMMs such as RPE could be considered but the feasibility is unknown
	PROC 8b (Indoor, process temp. ≤ 25°C, 0.25h/d)	RCR ~1 (ART 1.5)	None	There are remaining options for additional RMMs to be applied on PROC 8b. Therefore, risks may be adequately controlled if specific RMMs and/or OCs are applied
	PROC 19 (Indoor, process temp. ≤ 40°C, 8h/d)	**RCR=1.9** (TRA)	Enhanced general ventilation (70% effectiveness) Dermal protection, 95% effectiveness Respiratory protection, APF20, 95% effectiveness No LEV	As hand contact is expected for this use, dermal exposure may not be negligible compare to inhalation exposure. Risks may not be sufficiently characterised and controlled. LEV has not been taken into account and could reduce dermal exposure.
Industrial intermediate use	PROC 2; (indoor, temperature process ≤ 40°C, 8h/d)	RCR = 1.3	LEV, 90% effectiveness, Dermal protection 95% effectiveness	Additional RMMs are possible to be applied on PROC 2. Therefore, risks may be adequately controlled if further RMMs and/or OCs are considered

Identified use	Process Category (PROC)	RCR ($DNEL_{inhal}$=2 ppm)	RMM	Conclusion on risk
	PROC 4 (indoor, temperature process ≤ 40°C 4h/d)	**RCR = 2.1** (TRA)	General ventilation, 30% effectiveness LEV, 90% effectiveness Dermal protection, 95% effectiveness	As dermal exposure is expected for this use, dermal exposure may be underestimated Risks may not be sufficiently characterised and controlled. LEV implementation could be considered to reduce dermal absorption. No RPE is proposed, feasibility of wearing RPE during 4h/d is unknown
	PROC 8b (Indoor, process temp. ≤ 25°C, 0.25h/d)	RCR ~1 (ART 1.5)	None	Additional RMMs could be applied on PROC 8b. Therefore, risks may be adequately controlled if further RMMs and/or OCs are applied
Industrial use as a solvent	PROC 8b (Indoor, process temp. ≤ 25°C, 0.25h/d)	RCR ~1 (ART 1.5)	None	Additional RMMs could be applied on PROC 8b. Therefore, risks may be adequately controlled if further RMMs and/or OCs are applied.

Based on these recent literature data, annual 95e percentile in 2009 was above current indicative TTCA levels of 1.5 mg/g creatinine for all rayon viscose departments (Goen *et al.,* 2014). In this study, annual 95e percentile from personal assessments were also above he current indicative OEL of 5 ppm in some departments (spinning of textile rayon or technical rayon, post-treatment). It is thus unclear whether the companies are currently able to comply to the current BGV of 1.5 mg/g creatinine in all departments and thus with the current OEL.

Conclusion on risk assessment for workers

Potential risk has been identified for PROC 4 for industrial intermediate use and in the manufacture of regenerated cellulose and PROC 19 in the manufacture of regenerated cellulose using the systemic DNEL of 2 ppm but also possibly using the current indicative OEL.

7.13.2 Environment

Evaluating MSCA conclusion of the environmental risk assessment for each exposure scenario of Carbon Disulphide is presented below.

REGIONAL ASSESSMENT

The exposure estimates have been obtained with EUSES 2.1.2.

Table 40: Risk characterisation - regional environment

Risk characterisation - regional environment		
Compartment	**REGIONAL PEC**	**Risk characterisation**
Freshwater	3.81E-05 mg/l	RCR < 0.01
Sediment (freshwater)	5.24E-05 mg/kg_{wwt}	RCR < 0.01
Marine water	3.07E-06 mg/l	RCR < 0.01

Sediment (marine water)	4.21E-06 mg/kg_{wwt}	RCR < 0.01
Agricultural soil	4.25E-07 mg/kg_{wwt}	RCR < 0.01
Man via environment - Inhalation	Concentration in air: 3.42E -06 mg/m^3	RCR < 0.01
Man via environment - Oral	Exposure via food consumption 4.01E-06 mg/kg bw/day	RCR < 0.01

LOCAL ASSESSMENT

Table 41:

Risk characterisation - local environment		
Compartment	**LOCAL PEC**	**Risk characterisation**
Life Cycle Stage (LCS) M: ES 1 Manufacture of Carbon Disulphide		
Acceptable risks **RMM** - On-site treatment of off-air with an efficiency of 100% (by recycling or burning). - No use of water in manufacturing process and cleaning of reactors		
Life Cycle Stage (LCS) IS: ES 2 Manufacturing of regenerated cellulose		
STP	0.1 mg/l	RCR = 0.77
Freshwater	3.62E-03 mg/l	RCR = 0.36
Sediment (freshwater)	5.51E-03 mg/kg_{wwt}	RCR = 0.36
Marine water	1.00E-03 mg/l	RCR = 1
Sediment (marine water)	1.52E-03 mg/kg_{wwt}	RCR = 1
Agricultural soil	NR considering the RMM	NR considering the RMM
Groundwater	NR considering the RMM	NR considering the RMM
Acceptable risks **RMM** - STP on-site with a maximum effluent concentration of 0.1 mg/l - The dilution factor for sewage from STP emitted to a freshwater environment must be above 10. - No application of the STP sludge on agricultural soil (incineration) - No factories located within relevant distance to marine environments or the dilution factor for sewage from STP emitted to a marine water environment must be above 100		
Life Cycle Stage (LCS) IS: ES 3 Industrial use as an intermediate, use in the manufacture of plant protection products and biocides		
Acceptable risks **RMM** Closed system		
Life Cycle Stage (LCS) IS: ES 4 Industrial use as a solvent		
Acceptable risks **RMM** Closed system		

CONCLUSION FOR ENVIRONMENT

Environmental risk assessment shows acceptable risk for the manufacture and the use of carbon Disulphide. Nevertheless the **risk mitigation measures** listed below per emission scenario are essentials.

ES 1 Manufacture of Carbon Disulphide
- On-site treatment of off-air with an efficiency of 100% (by recycling or burning).
- No use of water in manufacturing process and cleaning of reactors

ES 2 Manufacturing of regenerated cellulose
- STP on-site with a maximum effluent concentration of 0.1 mg/l
- The dilution factor for sewage from STP emitted to a freshwater environment must be above 10.
- No application of the STP sludge on agricultural soil (incineration)
- No factories located within relevant distance to marine environments or the dilution factor for sewage from STP emitted to a marine water environment must be above 100

ES 3 Industrial use as an intermediate, use in the manufacture of plant protection products and biocides
- Closed system

ES 4 Industrial use as a solvent
- Closed system

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7.15. Abbreviations and acronyms

ACGIH:	American Conference of Industrial Hygienist
ACTH:	Adreno Cortico Tropic Hormone
AEGL:	Acute exposure Guideline level
ANSES:	*Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du travail* [French Agency for Food, Environmental and Occupational Health & Safety]
AOP:	Adverse outcome Pathway
AR:	Androgen receptor
ART:	Advance Reach tool
ATP:	Adenosine triphosphate
ATSDR:	Agency for Toxic Substances and Disease Registry
AUC:	Area under the curve
BCF:	Bioconcentration Factor
BLV:	Biological limit value
BMI:	Bosy Mass Index
BOEL:	Binding occupational exposure Level
BUA:	Beratungsgremium für Umweltrelevante Altstoffe
BW:	Body weight
CLP:	Classification, Labelling, Packaging
CMR:	cancerigen, mutagen and reprotoxic
CNS:	Centeal Nervcous system
CoRAP:	Community rolling action plan
CS_2:	Carbon disulphide
CSR:	Chemical Safety Report
CV:	Cardiovascular
CYP:	Cytochrome
DBH:	Dopamine-beta-hydroxylase
DFg:	Deutsche Forschungsgemeinschaft
DNA:	Deoxyribonucleic acid
DNEL:	Derived no effect level
DMEL:	Derived minimum effect Level
DNT:	Developmental neurotoxicity
EATS:	Estrogen, Androgen, Thyroid, Steroidogenic
ECETOC:	European Center for Chemical Safety Assessment
ECHA:	European Chemical Agency
ECG:	Electrocardiogram
ED:	Endocrine disruption
EDSP:	Endocrine Disrupting Screening Program
ELOC:	Equivalent Level of Concern
EOGRTS:	Extended one generation reproductive toxicity study
ER:	Estrogen Receptor
ES:	Exposure scenario
EU:	European Union
EUSES:	European Union System for the Evaluation of Substances
FR-MSCA:	France-Member State Competent Authority
FSH:	Follicle Stimulating Hormone
GD:	Gestation day
GLP:	Good Laboratory Practice
GnRH:	Gonadotropin releasing hormone
H_2S:	Dihydrogen Sulfide
HCD:	Historical control data
HCG:	Human chorionic gonadotropin
HCNL:	Health council of Netherland
HDL:	High Density Lipoprotein
HPG:	hypothalamo-pituitary-gonadal axis
IGF:	Insulin-like Growth Factor
IPCS:	International Programme on Chemical Safety-

IRIS:	Integrated Risk Information System
KE:	Key Events
LAGDA:	Larval Amphibian Growth and Development Assay
LD:	Lactation day
LC50:	Lethal concentration 50%
LD50:	Lethal Dose 50%
LDL:	Low density lipoprotein
LEV:	Local Exhaust Ventilation
LH:	Luteinizing hormone
LLNA:	Local Lymph Node Assay
LOAEC:	Lowest observed adverse effect concentration
LOAEL:	Lowest observed adverse effect level
LOD:	Limit of Detection
LSC	Life stage cycle
MCV:	Motor nerve conduction velocity
MIE:	Molecular initiating event
MoA:	Mode of Action
MSCA:	member state competent authority
MTD:	maximum Tolerated dose
NIOSH:	National Institute for Occupational Safety and Health
NIS:	Sodium Iodide Symporter
NMDA:	N-methyl-D-aspartate receptor
NO:	Nitric oxide
NOAEC:	No observed Adverse Effect concentration
NOAEL:	No observed adverse effect level
NOS:	Nitric oxide synthase
NTP:	National Toxicology Program
OC:	Operating condition
OECD:	Organisation for Economic Co-operation and Development
OEL:	occupational exposure limit
OR:	Odd ratio
PBT:	Persistent, bioaccumulable and Toxic
PND:	Postnatal Day
PNEC:	Predicted no effect concentration
PNS:	Peripheal nervous system
PROC:	Process category
QSAR:	Quantitative structure-activity relationship
RCR:	Risk Characterisation Ratio
REACH:	Regulation (EC) No 1907/206 of 18/12/06 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACh)
RMM:	Risk Management Measures
RR:	Risk ratio
RPE:	Respiratory protective equipment
SCE:	Sister Chromatid Exchange
SCL:	Specific Concentration Limit
SCOEL:	Scientific Committee on Occupational Exposure Limits
SCV:	Sensory nerve Conduction Velocity
SD:	Standard Deviation
SHBG:	Sex-hormone binding globulin
TSHR:	Thyroid stimulating hormone receptor
STOT RE:	Specific target organ toxicity – repeated exposure
STP:	Sewage treatment Plant
SVHC:	Substance of Very High Concern
T:	Testosterone
TBG:	Thyroxin Binding protein
TG:	Technical Guidance
TGD:	Technical guidance document
TH:	Thyroid Hormone
TK:	Toxicokinetics
TPO:	Thyroxide peroxidase

TRA:	Targeted Risk Assessment
TSH:	Thyroid stimulating hormone
TTCA:	thiazolidine-2-thione-4-carboxylic acid
TWA:	Time Weight Average
UDP:	Phophate Uridyl Transferase
US EPA:	United State Environmental Protection Agency
UTBG:	Unsaturated thyroxin binding protein
vPvB:	Very Persistent, very Bioaccumulable
WHO:	World Health organisation