SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Triclocarban

EC No 202-924-1

CAS No 101-20-2

Evaluating Member State(s): France

Dated: 10 September 2020

March 2015

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2019

Member State concluded the evaluation without any possibility to ask more information from the Registrant(s) under Article 46(1) decision, due to a downgrading of tonnage from 100-1000 tpa at the start of the evaluation to a cease of manufacture from all Registrant(s) in November 2019.

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

Triclocarban was originally selected for substance evaluation in order to clarify concerns about:

- Suspected reprotoxicity
- Potential endocrine disruption
- Wide dispersive use
- Microbial resistance.

During the evaluation also another concern was identified. The additional concern was:

- Use as a biocide but not approved for such use.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

The completed and ongoing processes other than CoRAP are presented below.

Table 1

OTHER PROCESSES / EU LEGISLATION			
Process	MSCA	Status	Outcome
Risk Management Option Analysis (RMOA) Concern: endocrine disruption	France	Completed	Conclusion document (dated March 2018) published on 09/08/2018[2]. Conclusion: Appropriate to initiate regulatory risk management action. The proposed regulatory action is Substance Evaluation. As indicated in the RMOA conclusion document: "*(…) In the current state of the knowledge and with regard to the guidelines of the OECD (OECD, on 2012) for the evaluation of PE, it is considered that on the basis of the supplied toxicological and ecotoxicological data, there is not enough data to identify potential ED effects. The result suggests that TCC* [triclocarban] *has an endocrine disruptor character with an important level of evidence. Nevertheless, due to the lack of information and in the absence of known adverse effects, it is not possible to conclude on the ED properties of this substance.* *In order to have information to link the mode of action observed in vitro with adverse effects, a reprotoxicity assay could be recommended to clarify uncertainties about ED properties. We also recommend to clarify the promoting effect of TCC on the bacterial resistance.* *A substance evaluation on TCC is therefore considered as the most suitable option.".*
Dossier Evaluation (Compliance check)	ECHA	Follow-up ongoing	A Decision[3] was sent to the lead Registrant of triclocarban on 21/03/2018. The deadline for dossier update was 28/03/2019. The information requested were:

[2] RMOA triclocarban: https://echa.europa.eu/rmoa/-/dislist/details/0b0236e180b26399, accessible at https://echa.europa.eu/rmoa/-/dislist/details/0b0236e180b26399.

[3] Decision on a Compliance Check, 21 March 2018: https://echa.europa.eu/documents/10162/a0e2d66b-cbd6-1940-5bf9-79a46a738a64.

Scope: comprehensive			1. *In vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1,; test method: Bacterial reverse mutation test, EU B.13/14. /OECD TG 471) [using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102] with the registered substance; 2. *In vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or *in vitro* micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance; 3. If the above two tests are negative, then *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance; 4. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rat or rabbit), oral route with the registered substance. Requests 3 and 4 have not been fulfilled by the Registrant and therefore a SONC-FTR (Statement of Non-Compliance-Failure to Respond) has been issued to the Registrant on 18 November 2019.
Regulation (EU) 528/2012 (Biocidal Product Regulation)	European Commission	Completed	Not approved. Regulation (EC) 1451/2007[4]: triclocarban is listed in Annex I (active substances identified as existing) and Annex II (active substances to be examined under the review programme) for product-types 1, 2, 4. Decision 2008/809/EC[5]: triclocarban is not to be included in Annex I, IA or Ib to Directive 98/08/EC [i.e. triclocarban is not approved as a biocidal active substance] for product-types 1, 2 and 4. This is because no complete dossier was received and no person or Member State indicated an interest in taking over the role of participant for the substances and product-types concerned.
Regulation (EC) No 1223/2009 on cosmetic products	European Commission	Completed	Triclocarban is listed under reference number 23 in Annex V and reference number 100 in Annex III of regulation (EC) No 1223/2009 on cosmetic products. It is allowed for use: - as a preservative: maximum concentrations in ready for use preparation 0.2% (V.23) - other than as a preservative: maximum concentrations in ready for use preparation 1.5% in rinse-off products (III.100) For both uses the purity criteria are <=1ppm of 3,3',4,4'-tetrachloroazobenzene and 3,3',4,4'-tetrachloroazoxy-benzene. A Scientific Committee on Consumer Products (SCCP) opinion has been adopted for triclocarban on 1 June 2005 for other cosmetic uses than as a preservative (SCCP/0851/04). It has been concluded that use of

[4] COMMISSION REGULATION (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

[5] COMMISSION DECISION of 14 October 2008 concerning the non-inclusion of certain substances in Annex I, IA or IB to Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

			triclocarban for non-preservative purposes in cosmetic rinse-off hand and body care products up to a maximum concentration of 1.5% does not pose a direct risk to the health of the consumer. However, the opinion did draw the Commission's attention to the possible effects of triclocarban to the environment and, subsequently, on human health from such environmental contaminations. A call for data[6] on ingredients with potential endocrine-disrupting properties used in cosmetic products was published from 16 May 2019 to 15 October 2019. In the framework of the cosmetic regulation, triclocarban was included in Group A (substances that should be treated with higher priority for assessment as they are undergoing substance evaluation (SEV) under REACH for ED concerns or the SEV has already confirmed ED concerns). "Upon receipt of sufficient data, the Commission will mandate the SCCS to evaluate the substances as soon as possible. If needed, the Commission will then take appropriate action to prohibit or restrict the use of the different substances in cosmetics."

Additionally to EU processes, the evaluating Member State (eMSCA) points out the following other processes:

In France:

- The decree of 7 August 2015 (modifiying the decree of 25 January 2010 which sets the monitoring programmes for the implementation of the Water Framework Directive) establishes that triclocarban has to be monitored in surface water and sediment from 2019.

In the USA:

- The triclocarban consortium submitted in 2002 to the U.S. EPA High Production Volume (HPV) Challenge Program data on physical-chemical properties, human health, and environmental toxicity. In 2010, triclocarban was being considered for inclusion in the California Environmental Contaminant Biomonitoring Program (CECBP) by the Scientific Guidance Panel (SGP) Biomonitoring California. It was not included.
- FDA final rule 81 FR 61106 of 6 September 2016 (effective 6 September 2017): Over-the counter consumer antiseptic wash products containing triclocarban can no longer be marketed. Safety during long-term daily use and effectiveness were not demonstrated. Consumer hand sanitizers, consumer wipes, and antibacterial products used in health care settings are not covered by this rule.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the eMSCA to the following conclusions, as summarised in the table below.

[6] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic-products_en

Table 2

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

The eMSCA concludes that more data is required to clarify the ED concern. However, as this substance no longer has any active registrations, the evaluation is terminated with the open concern.

If, in future, the currently inactive registrations are re-activated, or if there are new registrants for the substance, authorities shall consider including the substance again in the CoRAP for obtaining the information which is considered important to clarify the concern related to this substance. In such a situation, the potential registrants are recommended to take note of these conclusions and make appropriate testing proposals to ECHA, where relevant under Article 12(1)(d) and (e) of the REACH Regulation.

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

The available data show that triclocarban may affect the reproductive function of male (fertility and/or male sexual behavior) and female (pre-implantation and/or implantation development) and cause adverse effects on the offspring *via* lactation. Considering the numerous observation of adverse effects on reproductive function in male (testicular alteration) and female (preimplantation loss) due to triclocarban, the potential exposure by lactation (high triclocarban levels in plasma and in maternal milk), this substance is considered to have a potential for reproductive toxicity.

Thus, a C&L process should be foreseen to update the actual harmonised classification and classify triclocarban as Reprotoxic (fertility and developmental effect *via* lactation).

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

Not applicable.

4.1.3. Restriction

Not applicable.

4.1.4. Other EU-wide regulatory risk management measures

Enforcement is the recommended follow-up. The National Enforcement Authorities are invited to take note of the following considerations:

[1] The Registrants have declared a cease of manufacture just before and during the evaluation of the substance (evaluation dates: 19 March 2019 to 19 March 2020). In details:

- The joint submission member has ceased manufacture and switched its registration status to “inactive” on 6 February 2019
- The lead Registrant has reduced its tonnage to 1-10 tpa in its update of 27 March 2019, then has ceased manufacture and switched its registration status to “inactive” on 19 November 2019.

Consequently, there is no active registration on triclocarban in the EU.

The National Enforcement Authorities are invited to check that triclocarban is not placed on the market anymore at a tonnage above 1 tonne per year per company. This tonnage includes the amount of substance used in mixtures (manufactured or imported), including cosmetics and excluding medicines, and contained in articles (produced or imported) according to REACH Article 5, 6(1) and 7(1).

[2] The eMSCA noted that, based on the information in the REACH registration of triclocarban, there was a breach with the Biocidal Products Regulation (Regulation (EU) 528/2012). Based on the information provided in the registration dossier and confirmed by the lead Registrant, triclocarban was registered for uses in mixtures/articles as antibacterial and antifungal agent in a number of mixtures and articles (adhesives; air care products; coatings and paints, thinners, paint removes, fillers; putties, plasters, modelling clay; finger paints; ink and toners; washing and cleaning products; stone, plaster, cement, glass and ceramic articles). This suggests a mis-use of triclocarban as product type (PT) 6 (preservatives for products during storage) and possibly PT 7 (film preservatives) and PT 10 (construction material preservatives) depending on the exact role of triclocarban. However, triclocarban is not listed in the BPR review programme under PT 6, 7 or 10 and accordingly, these uses are not allowed in the European Union. In addition, triclocarban had been identified for biocidal uses in PT 1 (human hygiene), PT 2 (disinfectants and algaecides not intended for direct application to humans or animals)and PT 4 (food and feed area) under Regulation (EC) No 1451/2007[7] but has not been approved (Decision 2008/809/EC). Therefore, there could be a breach with the Biocidal Products Regulation. The National Enforcement Authority of the Member State where the lead registrant is located have been informed. National Enforcement Authorities are invited to check that triclocarban is not placed on the market as a biocidal active substance (preservative, antibacterial, antifungal agent).

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable.

5.2. Other actions

Not applicable.

[7] COMMISSION REGULATION (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

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

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 3

FOLLOW-UP		
Follow-up action	Date for intention	Actor
• Enforcement to check that triclocarban is not placed on the market for any use at a tonnage above 1 tonne per year (including in cosmetics) • Enforcement to check that triclocarban is not placed on the market as a biocidal active substance (preservative, antibacterial, antifungal agent)	Continuous	National Enforcement Authorities
CLH (fertility and developmental effect via lactation)	2021	France (tbc)
Evaluation by the SCCS (Scientific Committee on Consumer Safety) for any cosmetic uses that would still be compliant with REACH (see above)	tbc	SCCS (already planned)

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Triclocarban was originally selected for substance evaluation in order to clarify concerns about:

- Suspected reprotoxicity
- Potential endocrine disruption
- Wide dispersive use
- Microbial resistance.

During the evaluation also another concern was identified. The additional concern was:

- Use as biocide but not approved for such use.

Table 4

EVALUATED ENDPOINTS	
Endpoint evaluated	Outcome/conclusion
Reprotoxicity	Triclocarban may affect the reproductive function of male (fertility and/or male sexual behavior) and female (pre-implantation and/or implantation development) and cause adverse effects on the offspring *via* lactation. Thus, a C&L process should be foreseen to update the actual harmonised classification and classify triclocarban as Reprotoxic (fertility and developmental effect *via* lactation)
Endocrine disruption	It could be plausible that low doses of triclocarban induce hepatic steatosis *via* endocrine disrupting mode of action. No clear conclusions on the environmental ED properties of triclocarban can be drawn in absence of appropriate studies. More data are needed to conclude on this endpoint.
Wide dispersive use	The Registrant(s) had initially declared wide-dispersive uses in their registration dossiers. Before the end of the evaluation period, registrants changedtheir registration status to “inactive” because ceased import or manufacture at a tonnage above 1 tonne per year per company. Therefore wide dispersive use are not relevant anymore.
Microbial resistance	Not assessed. Not relevant anymore as microbial resistance would be related to wide dispersive use which are not relevant anymore (see above).
Use as biocide	The lead Registrant informally confirmed a use as biocide, but triclocarban is not approved nor included the review programme to be used as a biocide. The National Enforcement Authority of the country where the lead Registrant (only one active at that time) was located has been informed of the breach with the Biocidal Products Regulation.

7.2. Procedure

Triclocarban was assessed in the context of a RMOA (Regulatory Management Option Analysis) to address a concern on endocrine disruption in the framework ofthe French National Strategy on Endocrine Disruptors. The intention was notified on 8 February 2016 and the conclusion published on 9 August 2018. It was concluded that, in the current state of the knowledge and with regard to the guidelines of the OECD (OECD, on 2012) for the evaluation of endocrine disruptors, the available toxicological and ecotoxicological data suggested that triclocarban has an endocrine disruptor character with an important level of evidence. However, the available information was not sufficient to determine to which extent nor to envisage SVHC identification under Article 57(f) (equivalent level of concern), in particular because adverse effect was not demonstrated. Hence, substance evaluation was recommended as a follow-up in order to obtain information to link the mode of action observed *in vitro* with adverse effects. It was recommended to consider requesting a reprotoxicity assay. It could also enable to clarify the promoting effect of triclocarban on bacterial resistance.

A Dossier Evaluation (Compliance check, CCH) has been performed by ECHA and a Decision[8] was sent to the lead Registrant of triclocarban on 21 March 2018. The deadline for dossier update was 28 March 2019. The information requested were mutagenicity studies and a pre-natal developmental toxicity study. Initially, an Extended one generation reprotoxicity study (EOGRTS) was requested as well, but the Member State Committee (MSC) decided that this request should be addressed under Substance Evaluation.

In parallel to the CCH, triclocarban was included in the Community Rolling Action Plan (CoRAP) for Substance Evaluation under REACH regulation by the eMSCA due to concerns related to suspected reprotoxic effect, potential endocrine disrupting effects along with wide dispersive use and microbial resistance. The justification document was dated March 2018[9]. The Substance Evaluation under REACH regulation started on 19 March 2019 (i.e. just before the CCH deadline). The evaluation of triclocarban is based on the previous work done in the RMOA and completed with:

- updated information in the registration dossier (2019),
- full study reports provided by the Registrant(s),
- informal exchanges with the Registrant(s) (2019),
- open literature sources (until January 2020 for human health and until August 2019 for the environment[10]).

On 6 February 2019, the joint submission member changedts registration status to “inactive” (cease of manufacture/import).

On 27 March 2019, the lead Registrant updated its registration dossier with new data, in response to the CCH requests, (the requested prenatal developmental toxicity study was not provided). The National Enforcement Authorities of the country of the lead Registrant have been informed. Furthermore, the registrant reduced the tonnage from 100-1000 tpa to 1-10 tpa.

This change prevented FR-MSC from making formal request(s) of information *via* a substance evaluation decision therefore FR-MSC issued a conclusion document.

In the framework of the French National Strategy on Endocrine Disruptors, it was decided to update the assessment of the endocrine disruption effects as new published literature

[8] Decision on a Compliance Check, 21 Match 2018: https://echa.europa.eu/documents/10162/a0e2d66b-cbd6-1940-5bf9-79a46a738a64.

[9] https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1820e17bb.

[10] Environment: Scopus with the combination of different key words as trichlorcarban, trichlorocarbanilide, triclocarban, bird*, mallard, duck, quail, bobwhite, anas*, colinus*, collembol*, macro organism, folsomia, springtail, mite*, hypoaspis, fish, amphib*, fathead, stickleback, medaka, xenopus, zebrafish, estro*, andro*, steroido*, thyroid*, vtg, vitellogenin.

became available. The literature published after the completion of the RMOA was therefore incorporated.

On 19 November 2019, the lead Registrant changed its registration status to “inactive” (cease of manufacture/import).

Therefore, at the time of this substance evaluation there is no active registration dossier for triclocarban under REACH.

Legally speaking, a cease of manufacture/import is not a reason not to fulfil a formal request that was issued before the announcement of a cease of manufacture/import. This cease of manufacture/import had not been announced by the lead Registrant during the formal commenting steps during the Dossier Evaluation. The eMSCA highlights that beyond the legal compliance requirements, the data on reprotoxicity requested under compliance check was needed to clarify the potential hazards of the substance (reprotoxicity and endocrine disruption), which raised the concerns justifying the inclusion in the CoRAP.

On 3 February 2020, the lead Registrant provided a study report which had been requested by the eMSCA. Together with this report, several other reports of studies were provided and the eMSCA found that these studies were never included in the registration dossier. These studies are : a reprotoxicity/teratogenicity study on rats and rabbits *via* oral route, a reprotoxicity/teratogenicity study on rats and rabbits *via* intracutaneous injection, a repeated toxicity study on rats (12 months) and a 3-generations study on rats.

The eMSCA notes that should the registration dossier have been complete with this existing data, as it is requested under REACH, the prioritisation of the substance, the involvement of the eMSCA and ECHA (including resources spent) and the conclusions at each step (RMOA, CCH and SEV) might have been different. These data has been evaluated together with the other existing data.

Nevertheless, the eMSCA concludes that more data is required to clarify the concerns. However, as this substance no longer has any active registrations, the evaluation is concludedwith several unclarified concerns. As triclocarban no longer has any active registrations, it is not allowed to be placed on the EU market anymore, including in cosmetics, at a tonnage above 1 tonne per year per company. This tonnage includes the amount of substance used in mixtures (manufactured or imported), including cosmetics and excluding medicines, and contained in articles (produced or imported) according to REACH Article 5, 6(1) and 7(1).

Thus, it is up to the National Enforcement Authorities to decide whether to enforce the CCH and to control the placing on the market (including import) of products and articles containing triclocarban.

If, in future, the currently inactive registrations are re-activated, or if there are new registrants for the substance, authorities shall consider including the substance again in the CoRAP for obtaining the information which is considered important to clarify the concerns related to this substance. In such a situation, the potential registrants are recommended to take note of these conclusions and make appropriate testing proposals to ECHA, where relevant under Article 12(1)(d) and (e) of the REACH Regulation.

This evaluation has been performed on the basis of information available at the time of evaluation. As knowledge evolves and new data may become available, the evaluation and its conclusions may evolve as well.

In the context of action 3 of the second French National Strategy on Endocrine Disruptors, triclocarban will be subjected to further methodological work, which aim will be to determine precisely if it should be considered as a “presumed”, “suspected” or “recognised” endocrine disruptor. The conclusions of this work will be published on Anses website.

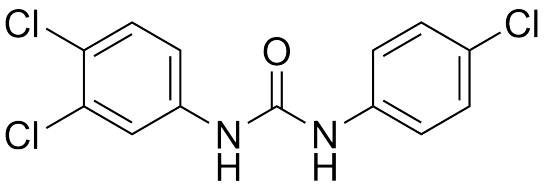
7.3. Identity of the substance

Table 5

SUBSTANCE IDENTITY	
Public name:	Triclocarban
EC number:	202-924-1
CAS number:	101-20-2
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	$C_{13}H_9Cl_3N_2O$
Molecular weight range:	315.582 g/mol
Synonyms:	1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)urea (IUPAC name) Urea, N-(4-chlorophenyl)-N'-(3,4-dichlorophenyl)- 1-(3',4'-Dichlorophenyl)-3-(4'-chlorophenyl)urea 3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)urea 3,4,4'-Trichlorocarbanilide

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



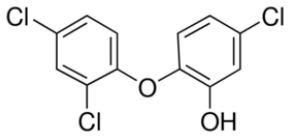
The composition submitted by the Registrant(s) is considered as monoconstituent according to REACH guidance for identification and naming of substances (see confidential annex).

Analytical informations are provided (UV/VIS, IR, NMR and GC chromatograms) to confirm the purity and the structure of the substance. No data have been provided on the identity of impurities.

7.3.1. Similar substance: Triclosan?

Triclocarban has a dichlorinated phenyl ring connected through an urea bond to a monochlorinated phenyl ring, and shares limited structural similarity to triclosan, i.e. both substances have two chlorinated phenyl rings. But while the phenyl rings in triclocarban are connected by an urea bond, in triclosan they are connected by an ether bond. Furthermore, triclosan contains and additional hydroxyl group, and the chlorines are located at different positions compared to triclocarban. Moreover, triclocarban is an halogenated diphenylurea while triclosan is an haloganated phenol derivative.

Table 6

SUBSTANCE IDENTITY (Triclosan)	
Public name:	Triclosan
IUPAC name	5-chloro-2-(2,4-dichlorophenoxy)phenol
EC number:	222-182-2
CAS number:	3380-34-5
Index number in Annex VI of the CLP Regulation:	604-070-00-9
Molecular formula:	$C_{12}H_7Cl_3O_2$
Structural formula	

Considering these informations the eMSCA concludes that the comparison of triclosan with triclosan by read across is not relevant.

7.4. Physico-chemical properties

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	Value
Physical state at 20°C and 101.3 kPa	Key value: White solid (fine plates) *Data is available in peer reviewed handbooks (Merck Index 2006 and CRC Lide 2005-2006), data is also available from an experimental study wich gives a consistent result.*
Melting point	Key value: mp 245.3 °C at 101.3 kPa *Melting point was determined in accordance with the test method OECD Guideline 102 (EU test method A.1; differential scanning calorimetry). Data is available in peer reviewed handbook (Merck Index 2006 and CRC Lide 2005-2006), which gives consistent result.* *Differences may be due to difference of purity or method.*
Boiling point	Key value: bp >250 °C at 101.3 kPa *Boiling point was determined in accordance with the test method OECD Guideline 103.*
Relative density	No reliable data
Particle size distribution	Key value: The particle size distribution was determine to be in the range of 150 µm to 75 µm. *Particle size distribution was determined according to the gravimetric method with rotap sieve shaker.*
Vapour pressure	No reliable data
Water solubility	Key value: 0.624 mg/L at 25 °C

	Water solubility was determined according to the test procedure OECD Guideline 105.
Partition coefficient n-octanol/water (Log Kow)	Key value: Log Kow 3.633 at 25 °C *Partition coefficient was determined according to the test procedure OECD Guideline 117 (EU test method A.8; HPLC method).*
Surface tension	In accordance with column 2 of REACH Annex VII, Surface tension (required in section 7.6.) does not need to be conducted because water solubility is below 1 mg/L at 20°C.
Flash point	In accordance with column 2 of REACH Annex VII, Flash point (required in section 7.9.) does not need to be conducted as the substance is a solid.
Auto flammability	No reliable data
Flammability	No reliable data
Explosive properties	Key value: Not explosive *According to the REACH guidance on information requirements and chemical safety assessment, chapter R7a, triclocarban does not contain any chemical groups associated with explosive properties. Thus, according to REACH annex VII, column 2, a study does not need to be conducted.*
Oxidising properties	Key value : No oxidising properties *According to the REACH guidance on information requirements and chemical safety assessment, chapter R7a, triclocarban does not contain any chemical groups associated with oxidising properties. Thus, according to REACH annex VII, column 2, a study does not need to be conducted.* *Based on the structure, triclocarban does not contain oxygen, fluorine and/or chlorine chemically bonded to other element than carbon or hydrogen*

7.5. Manufacture and uses

The quantities and uses listed in this section reflect the information at the time of the evaluationon ECHA dissemination website (accessed 19 December 2019). However, they are not relevant anymore, as all Registrants have switched their registration status to “inactive”.

It should be noted that the information has changed between the time when the RMOA and the CCH were performed, and the Substance Evaluation was started. The eMSCA highlights (for the Registran(s)’ consideration), that inappropriate information leads to inappropriate prioritisation and waste of public resources.

7.5.1. Quantities

Table 8

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	☒ > 1000,000 t	☐ Confidential

As all registrations are inactive, the tonnage should be set to < 1 tonnes per annum and therefore the table above is not filled in.

7.5.2. Overview of uses

The uses that were declared in the active registration dossiers are given below for information purpose.

Before the registration became inactive, the eMSCA found that the names of the scenarios were unclear and asked informally the Registrants to clarify their meanings. However, clarifications were not received, therefore these scenarios remain unclear.

At that time, the lead Registrant has declared and confirmed that triclocarban was used in mixtures/articles as antibacterial and antifungal agent. However, antibacterial and antifungal agent are biocides, which must be approved under the Biocidal Products regulation to be placed on the market. However, triclocarban is not approved as a biocidal active substance. Triclocarban is listed in Annex I (active substances identified as existing) and Annex II (active substances to be examined under the review programme) for product-types 1, 2, 4 in Regulation (EC) 1451/2007[11]. Decision 2008/809/EC[12] states that triclocarban is not to be included in Annex I, IA or Ib to Directive 98/08/EC [i.e. triclocarban is not approved as a biocidal active substance] for product-types 1, 2 and 4. This is because no complete dossier was received and no person or Member State indicated an interest in taking over the role of participant for the substances and product-types concerned. It is also not approved for any other product-types. The uses indicated in the registration dossier would likely correspond to uses as preservative in mixtures, such as product-types (PT) 6 (preservatives for products during storage), PT 7 (film preservatives) and potentially PT 10 (construction material preservatives)). Therefore, this is a breach with the Biocidal Products Regulation. The National Enforcement Authorities have been informed.

However, these uses are not relevant anymore, as all Registrants have changed their registration status to “inactive” which means that triclocarban is not allowed to be placed on the market for any of the uses listed below (nor any others) at a tonnage above 1 tonne per year per company.

[11] COMMISSION REGULATION (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

[12] COMMISSION DECISION of 14 October 2008 concerning the non-inclusion of certain substances in Annex I, IA or IB to Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

Table 9

FORMER USES	Former use(s)
Uses as intermediate	*Yes (see below, industrial)*
Formulation	*Manufacture of construction chemicals* *Formulation of:* - *PC 9a: Coatings and paints, thinners, paint removes* - *PC 9b: Fillers, putties, plasters, modelling clay* - *PC 9c: Finger paints* - *PC 18: Ink and toners* - *PC 29: Pharmaceuticals* - *PC 35: Washing and cleaning products* - *PC 39: Cosmetics and personal care products*
Uses at industrial sites	*Industrial use in cosmetics and personal care products:* - *ERC4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article)* - *PROC 4: Chemical production where opportunity* - *PROC 5: Mixing or blending in batch processes* - *PC 39: Cosmetics and personal care products* *Industrial end-use:* - *ERC6a: Use of intermediate* - *PROC 4: Chemical production where opportunity for exposure arises* - *PC 19: Intermediate* *Industrial end-use:* - *ERC4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article)* - *PROC 4: Chemical production where opportunity* - *PROC 5: Mixing or blending in batch processes* - *PC 39: Cosmetics and personal care products* - *SU 10: Formulation [mixing] of preparations and/or re-packaging (excluding alloys)* *Automated application of water-borne adhesive industry:* - *ERC4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article)* - *PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions* - *PROC 7: Industrial spraying* - *PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities* - *PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing)*
Uses by professional workers	*None*
Consumer Uses	*Consumer use:* - *ERC8a: Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)* - *PC 9a: Coatings and paints, thinners, paint removes* - *PC 9b: Fillers, putties, plasters, modelling clay* - *PC 9c: Finger paints* - *PC 18: Ink and toners* - *PC 29: Pharmaceuticals* - *PC 35: Washing and cleaning products*

	Consumer use in cosmetics and personal care products: - *ERC8b: Widespread use of reactive processing aid (no inclusion into or onto article, indoor)* - *PC 39: Cosmetics and personal care products* *Consumer use:* - *ERC8a: Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)* - *PC 39: Cosmetics, personal care products* *Consumer use in coatings and paints:* - *ERC8f: Widespread use leading to inclusion into/onto article (outdoor)* - *PC 9a: Coatings and paints, thinners, paint removes* *Use in air fresheners:* - *ERC8a: Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)* - *PC 3: Air care products* *Consumer use:* - *ERC8d: Widespread use of non-reactive processing aid (no inclusion into or onto article, outdoor)* - *PC 9a: Coatings and paints, thinners, paint removes*
Article service life	*Service life of cured/installed construction chemicals:* - *Articles used by consumers* - *ERC10a: Widespread use of articles with low release (outdoor)* - *AC 4: Stone, plaster, cement, glass and ceramic articles*

Considering that the Registrants have ceased manufacture/import, the eMSCA checked other sources of information to know whether triclocarban is found on the EU market and compare this results to the Registrants' information.

In France:

- As of 23 December 2019, 3 medicinal products for human use containing triclocarban were authorised for sale in France (date of authorisation: June 1994, May 1996 and December 1997) including one that is not sold anymore. The two others are a local antiseptic powder for dermal application (Cutisan 1%) and a local antiseptic solution (Solubacter 1 pour cent).
- In the French National products Database (where products are declared for the poison centers use) there have been no new product containing triclocarban since 2013. Between 1988 and 2013, only 5 products were declared (2 cosmetics and 3 medicinal products for human use)
- In response to a request of January 2019, the Directorate-General for Competition, Consumer Affairs and Prevention of Fraud (DGCCRF) in France responded it has not detected triclocarban in non-targeted tests in consumer products for more than 10 years. Targeted analysis of cosmetics (for the purpose of compliance with the Cosmetics regulation) detected triclocarban in 5 soaps since 2016.

In other Member States / in EU:

- Triclocarban is not approved for use as a biocide (see above).
- Triclocarban is listed under reference number 23 in Annex V and reference number 100 in Annex III of regulation (EC) No 1223/2009 on cosmetic products. It is allowed for use:

  - as a preservative: maximum concentrations in ready for use preparation 0.2% (V.23)
  - other than as a preservative: maximum concentrations in ready for use preparation 1.5% in rinse-off products (III.100)

- Triclocarban is not approved as a food or feed additive.
- In its report on technical-economical data on triclocarban (INERIS, 2016), INERIS (French National Institute for Industrial Environment and Risks) concludes that the uses of triclocarban are very narrow in Europe as they could find only a few soaps and antiseptic medicines on the market. However, the authors note that triclocarban is persistent in the environment and can still be found in sediments in surface water. They suggest that emissions can occur from uses of triclocarban to treat imported textiles. The eMSCA points out that the importation of articles treated with triclocarban is not allowed in the EU, as the substance is not approved as a biocide under PT9 (fibre, leather, rubber and polymerised materials preservatives).
- Triclocarban is listed in the SPIN database[13] (under the name 3,4,4'-trichlorcarbanilid) with exposure "use index" of 3 and 4 (max. 5) in Norway for surface water, air, soil, waste water, consumer and occupational, "range of use" of 1 (max. 5) and "article index" of 1 (max. 3); however the tonnages and number of preparations are down to zero.
- No information on potential uses could be found on medicines for human use, in the Kemi database[14], HERA database[15], EU food additives database, Cordis[16].

In the USA:

- In the US Department of Health & Human Services Household Products Database[17], a few products are listed (only soaps and deodorants). This is consistent with triclocarban being used in the USA.
- Triclocarban is not authorised for sale in over-the-counter consumer antiseptic wash products (FDA final rule 81 FR 61106 of 6 September 2016, effective 6 September 2017). Consumer hand sanitizers, consumer wipes, and antibacterial products used in health care settings are not covered by this rule.

Overall, the very narrow ranges of uses in EU until 2019 is confirmed in the other sources of information. Now that all REACH Registrants have declared a cease of manufacture/import and switched their registration status to "inactive" (respectively 6 February 2019 and 19 November 2019), there shouldn't be any more triclocarban placed on the EU market, neither as such, in mixtures nor in articles, including cosmetics, at a tonnage of the substance above 1 tonne per year per company.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

None available.

[13] http://www.spin2000.net/spinmyphp/?pid=101202. Accessed 23 December 2019.

[14] http://webapps.kemi.se/flodesanalyser/FlodesanalyserSok.aspx. Accessed 23 December 2019.

[15] https://www.heraproject.com/RiskAssessment.cfm. Accessed 23 December 2019.

[16] http://cordis.europa.eu/projects/home_en.html. Accessed 23 December 2019.

[17] https://householdproducts.nlm.nih.gov/index.htm. Accessed 23 December 2019.

7.6.2. Self-classification

- In the registration(s):

Aquatic Acute 1, H400: Very toxic to aquatic life.

Aquatic Chronic 1, H410: Very toxic to aquatic life with long lasting effects.

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory (accessed 19 December 2019):

Skin Irrit. 2, H315: Causes skin irritation

Eye Irrit. 2, H319: Causes serious eye irritation.

7.7. Environmental fate properties

7.7.1. Degradation

Not assessed (not targeted by this Substance Evaluation).

7.7.2. Environmental distribution

Not assessed (not targeted by this Substance Evaluation).

7.7.3. Bioaccumulation

Not assessed (not targeted by this Substance Evaluation).

7.8. Environmental hazard assessment

Refer to section 7.10.1 on the assessment of endocrine disruption.

7.9. Human Health hazard assessment

The RMOA[18] (published 2018) investigated the endocrine effects of triclocarban. The recommendation for a follow up was Substance Evaluation, as a way to obtain more information to be able to conclude whether triclocarban could be identified as a SVHC for endocrine disruption effects under Article 57(f) (equivalent level of concern). However, no new data could be acquired, neither under Compliance Check (as the lead Registrant did not fulfil the requirements) nor under Substance Evaluation (as all Registrants changed their registration status to “inactive”, which prevent the eMSCA from making formal request *via* a Decision).

For the sake of giving an up-to-date evaluation, the literature published after the completion of the RMOA was therefore incorporated. The human hazard properties presented below are based on available data from the chemical safety report (CSR, 2019) of triclocarban and additional literature (Pubmed and Scopus).

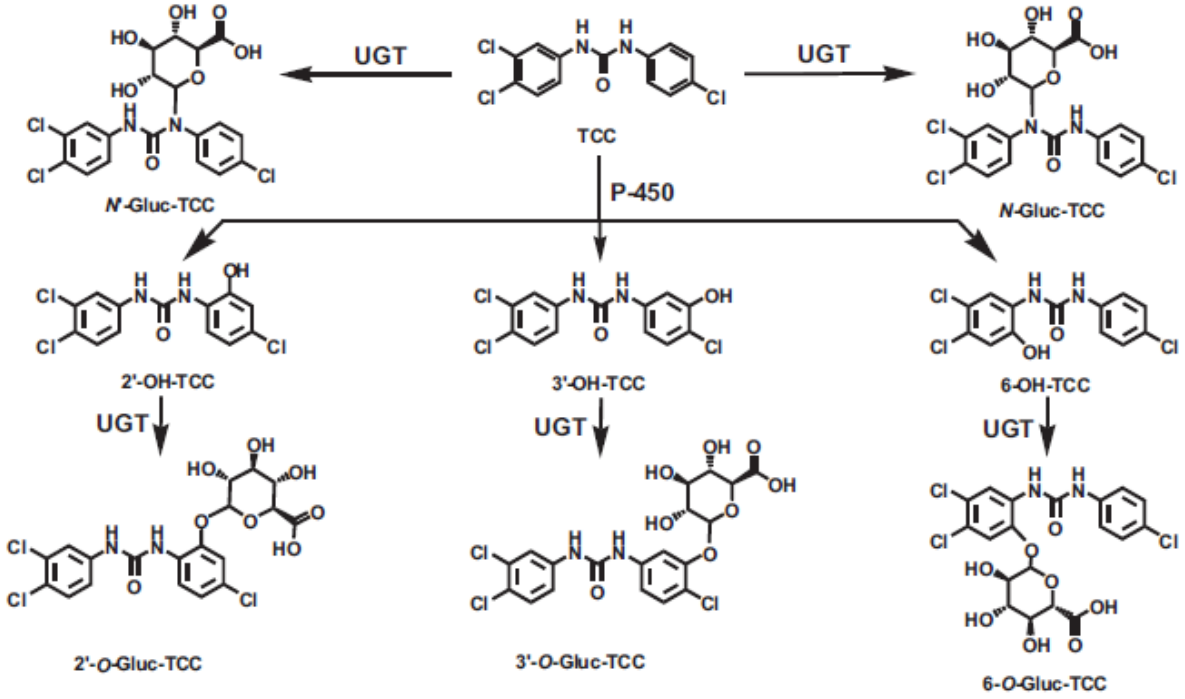
[18] RMOA triclocarban: https://echa.europa.eu/rmoa/-/dislist/details/0b0236e180b26399, accessible at https://echa.europa.eu/rmoa/-/dislist/details/0b0236e180b26399.

7.9.1. Toxicokinetics

- Toxicokinetics (absorption, distribution, metabolisation and elimination).

Triclocarban is moderately absorbed by the oral route and, depending on the vehicle and exposure conditions, poorly to moderately absorbed by dermal application (Hiles, 1978). The dermal absorption of 14C-labeled triclocarban was investigated in *in vitro* skin cell systems using full thickness human newborn and adult as well as a monkey skin (SCCP, 2005). At 37 °C, 2.5% of the applied dose was absorbed in human newborn foreskin, 0.60 % in human adult foreskin, 0.29 % in human infant abdominal skin, 0.26 % in human newborn abdominal skin and 0.23 % in human adult abdominal skin. In the monkey adult abdominal skin model 0.25 % of the applied dose was absorbed.

Biotransformation appears to be rapid but did not appear to involve splitting of the basis structure.
In all species investigated, triclocarban was widely metabolized to compounds that were more water soluble (triclocarban has a water solubility of 0.624 mg/L at 25 °C, see section 7.4). Thus, metabolites are more readily excreted than parental compounds. Concerning metabolites, hydroxylation followed by conjugation were observed. The principal metabolites common to all species were the sulphate and glucuronid conjugate of 2', 3' and 6-Hydroxy-triclocarban (Hiles, 1978).



The eMSCA conclude that based on the metabolisation and on the elimination of triclocarban, the bio-accumulation of triclocarban is unlikely.

7.9.2. Acute toxicity

Not assessed (not targeted by this Substance Evaluation).

7.9.3. Eyes irritation and corrositivity

Not related to the initial concern, no additional concern identified.

7.9.4. Skin irritation and corrositivity

Not related to the initial concern, no additional concern identified.

7.9.5. Sensitisation

Not related to the initial concern, no additional concern identified.

7.9.6. Repeated dose toxicity

The data available in the registration dossier and additional literature are described below.

- Female and male Sprague-Dawley rats were treated orally with 500 mg/kg and 1000 mg/kg of triclocarban 5 days per week during 30 days (Monsanto report ; 2011 cited in SCCP, 2005). Each dose and control group contained 10 rats per sex and the subchronic oral gavage was performed with 25% aqueous solution of triclocarban. Food consumption and weight gain were recorded weekly and observations were made for outward symptoms of toxicity such as reduced activity and non-grooming. At the end of the 30 days period, the viscera of the 1000 mg/kg and control groups were examined microscopically. Macroscopic tissue examination was made for liver, kidneys, gonads, adrenals, brain, heart and lungs.

The Registrant concluded that no macroscopic adverse effect in male and female rats over an exposure period of 30 days was found at the dose of 1000 mg/kg body weight of triclocarban.

The eMSCA was not able to conclude due to lack of raw data on the effects described in the registration dossier.

- Triclocarban was administered to three groups of 35 Sprague-Dawley rats in their diet at concentrations equivalent to 25, 75 and 250 mg/kg bw/day for 8 weeks (Monsanto report, 1985; cited in SCCP, 2005). The animals were observed twice daily for morbidity and mortality and once daily for clinical signs. Body weight, food consumption and detailed clinical signs were recorded weekly. Blood samples were taken from 5 animals per group every two weeks for evaluation of blood levels of triclocarban. No necropsy was performed at the end of the study. There were no signs of toxicity or treatment-related mortalities throughout the study. Mean body weight and food consumption were lower in the highest dose group. The Registrant noted that no statistical analysis can be performed due to the absence of control group in the study.

The Registrant concluded that no compound-related pathological or histopathological findings were noted.

The eMSCA was not able to conclude due to lack of raw data on the effects described and absence of control group (no statistical analysis performed) in the registration dossier.

- The Registrant reported the study of Wright *et al*., (1975). It is a chronic toxicity study in which rats were fed with a diet containing doses of triclocarban of 100, 300 and 1000 mg/kg/day resulting in a degeneration of the germinal epithelium lining of the seminiferous tubules, atrophy of the tubules, and oligospermia after 6 months of exposure at 300 and 1000 mg/kg/day. No testicular lesions were present in rats fed 100 mg/kg/day. No other gross, biochemical, haematological, central nervous system or histopathological effects related to triclocarban were observed in the course of this study.

The eMSCA considered that there was not enough information available to evaluate the quality of the study, and to conclude due to lack of raw data on the effects described in the registration dossier. Meanwhile, a degeneration of the germinal epithelium lining of the seminiferous tubules, atrophy of the tubules, and oligospermia were described, these observations needed to be consolidated by further analysis.

- The Registrant reported a two-year chronic triclocarban feeding study (NTRL, 1981, cited in the registration dossier (2019)) in Sprague-Dawleys rats (age: 46 days) exposed to 0, 25, 75 or 250 mg triclocarban/kg bw/day (once week preparation: appropriate levels of triclocarban were mixed with standard diet on the basis on body weight). At the study termination, all animals were subjected to complete necropsy and pathological examination. There were no treatment-related clinical signs or mortality throughout the study.

At the 16th to 19th months of treatment, many males, and to a lesser extent females, died because of a respiratory infection; animals received tetracyclin for 3 months to control the epidemia, and the observations at the 18th month has been delayed at the 20th month.

No difference between any groups was observed by regarding food consumption, ophthalmic observation or urine analysis. The mean body weights of the 250 mg/kg bw/day dosed males and the 75 and 250 mg/kg bw/day dosed females were lower than the controls. However, differences from control never exceeded 9 and 12% in the females and 6% in the males. Anemia occurred in males treated by 75 and 250 mg/kg bw/day and in females treated with 250 mg/kg bw/day; blood chemistry showed slight but statistically significant increases in alkaline phosphatase, urea nitrogen, glucose and total bilirubin at various time points for the high dose in males. Significant increases of organ relative weights occurred according to triclocarban dose and sex: 75 and the 250 mg/kg bw/day for the liver in both sexes, 75 (males) and 250 mg/kg bw/day (males and females) for the spleen, 25, 75 and 250 mg/kg/day (females) and 250 mg/kg bw/day (males) for the adrenal, 250 mg/kg/day for the heart in both sexes. Compound-related pathological changes were seen grossly in the 250 mg/kg bw/day males only and, microscopically in the 75 and 250 mg/kg bw/day dose males and females. Grossly, flaccidity and size decrease of the testes were in a large number of 250 mg/kg bw/day dose males. Males and females from the 75 and 250 mg/kg/day treated groups exhibited microscopic changes (testes/epididymis, spleen, liver, kidneys, bone marrow and mesenteric lymph nodes).

The data did not reveal strong specific effect on female reproductive organs, but some females had uterine polyps and nodules and ovarian cysts later in life (12 month and more) at the high dose. Male anomalies concerned mainly microscopic pathological changes in testes/epididymis (degeneration of seminiferous tubules, enlargement of epididymal secretory epithelium) coupled to a decrease or absence of sperm in epididymal ducts which contained numerous cell debris. In both sexes, other organs displayed histological anomalies (hepatocellular hypertrophy; brown pigment in Kupffer‘s cells; cholangiofibrosis; brown pigment in cytoplasm of proximal convoluted tubules; splenic congestion; hypercellularity and congestion of bone marrow).

Testicular degeneration was observed in the 250 mg group for most of the study. This effect was observed in the 75 mg/mg/day group during the last 11 months. No such effects were observed with 25 mg/kg/day.

- Additionally, the Registrant reported a study (US department of commerce, 1992, cited in the registration dossier (2019)) where CD strain male albino rats were treated for 30 or 60 days with triclocarban at 2500 or 5000 mg/kg/day pharmacological-doses, with and without recovery period. Treatment-related lesions were seen in testes and epididymis and are related to impairments in spermatogenesis, degeneration of the germinal epithelium of the seminiferous tubules and inflammatory responses of seminiferous tubules and epididymal ducts to cellular debris and non-viable spermatozoa. These changes were found in animals treated with 2500 or 5000 mg test chemical/kg bw for 30 days, with 2500 mg test chemical/kg bw for 60 days and in animals allowed 30 or 60 days of recovery. Based on the observations of the study, the Registrant considered the LOAEL to be of 2500 mg/kg/day in CD strain albino rats exposed for 30 or 60 days by oral route of exposure.

- The Registrant provided a study where male albino rats (25 rats per dose) were treated *via* diet for 12 months with triclocarban at dose levels of 0, 300, 1,000, 3,000, 10,000

ppm. (Monsanto 1979, not cited[19] in the registration dossier (2019)), corresponding to approximately, 0, 30, 100, 300 and 1,000 mg/kg bw/d respectively (on the basis of a theoretical 100g food intake /kg bw). This study investigated the histopathology of many organs at the end of the experiments.

Triclocarban did not affect neither body weight nor body weight gain at 300 ppm and 1,000 ppm, but the 3000 ppm dose slightly decreased the body weights of males at 6 months of age and a significant low decrease was found on body weights and body weight gain at the 10000 ppm dose at various weighting interval. There were no findings regarding food consumption, mortality rate, reactions and organ weights and ratio which were related to treatment.

As a particular result, histopathological examination revealed a treatment-related change among 3000 ppm and 10000 ppm groups on male reproductive organs, but not on other organs. This concerned one or both testes and epididymis and the lesions consisted in focal to multifocal degeneration of the germinal epithelium, affected tubules which involved the formation of spermatocytic-spermatidic giant cells within lumina of tubules, a decrease in diameter of tubules (atrophy), and spermatic granulomas resulting from the stasis of non-viable sperm within seminiferous tubules. The above lesions were linked to a diminution of sperm in the ducts of ipsilateral epididymis in the affected gonads. The degree of tubular involvement and relative severity of the degenerative lesions were greater among animals exposed to 10,000 ppm.

eMSCA conclusion on repeated dose toxicity:

Overall, , the eMSCA concludes that the available data, which are of limited quality (in the 24 months study, males and females showed respiratory infection; animals received tetracyclin for 3 months to control the epidemia), on repeated dose toxicity show some effects on male reproductive organs or functions (such as testes, epididymis and spermatozoa). Some results show that 75 mg/kg/day of triclocarban is toxic for male reproduction. However, these studies have a number of limitations such as the absence of sperm counts, no observation of the motility and the morphology of sperms, no weighing of androgenic-dependent sex organs (seminal vesicles and prostate) and no hormonal assay performed. Therefore these studies do not allow for a conclusion to be

Furthermore, althoughalterations of non-reproductive function or organs were observed at 75 mg/kg/day (hepatocellular hypertrophy; brown pigment in Kupffer‘s cells; cholangiofibrosis; brown pigment in cytoplasm of proximal convoluted tubules; splenic congestion; hypercellularity and congestion of bone marrow), it remains unknown whether the reprotoxic effect of triclocarban observed here is the result from its effect on the reproductive system or from a general toxicity at this dose.

Some others results clearly show specific alterations of male reproductive function in rats exposed to 300 and 1,000 mg/kg/d. This suggests that lower doses (30 and 100 mg/kg/d) do not affect these functions. However, the results presented in these reports provide substantially less information than what is needed to show the safety of these doses, for the following reasons:

1) in the testis, only major histological alterations were taken into consideration: germinal epithelium degeneration, atrophy of tubules, formation of granuloma and giant cells, which are ultimate degradation of spermatogenesis. A correct evaluation of spermatogenesis implies morphometric analyses such as the number of seminiferous tubules, the diameter of these tubules, the seminiferous epithelial height, the stages of spermatogenesis cycle.

2) in the epididymis, only a qualitative histological observation was performed. Such an analysis allows only important deterioration to be detected. A correct evaluation of quality and quantity of sperms implies to collect sperm in the epididymis and to count the number of spermatozoa, to analyse their morphology and to measure their motility.

[19] See 7.2 on Procedure. The data has been provided directly but not via the registration dossier.

7.9.7. Mutagenicity

In vitro: Not related to the initial concern, not assessed.

In vivo: No studies are available for *in vivo* genotoxicity of triclocarban.

It should be noted that the registration dossier was incompliant regarding this endpoint and ECHA requested data in a Compliance Check (CCH) (see part A section 2): *in vitro* gene mutation study in bacteria using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102]; *in vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study; if the above two tests are negative, then *in vitro* gene mutation study in mammalian cells with the registered substance.However, the information requested via CCH has been fulfilled by the Registrant.

7.9.8. Carcinogenicity

Not related to the initial concern, no additional concern identified.

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

It should be noted that the registration dossier was incompliant regarding this endpoint and a prenatal developmental toxicity study has been requested by ECHA in a Compliance Check (CCH) (see part A section 2). Howerver, this study has not been provided by the Registrant.

The data available in the registration dossier and additional literature are described below.

Human data : Triclocarban and pregnancy outcomes

In a study on the PROTECT cohort in Puerto Rico including 922 pregnant women, Aker *et al.*, (2019) reported that triclocarban maternal levels were associated with a non significant 2-day decrease in gestational age. No association was observed for other outcomes: preterm birth, birthweight and birthweight z-scores. Geer *et al.*, (2017) in a Caribbean immigrant community in Brooklyn (NY) investigated the relationship between maternal exposure to triclocarban during pregnancy (n = 185) and birth outcomes. No associations were observed between maternal exposure and gestational age, birthweight, body length and head circumference at birth. When triclocarban levels were measured in cord blood (n = 38), a significant inverse association was observed with gestational age, but not with other birth outcomes. Because one potential mechanism between phenols and pregnancy outcomes may be through increased maternal oxidative stress, Ferguson *et al.*, (2019) examined in the LIFECODES cohort (USA) the relationship between triclocarban exposure and urinary oxidative stress biomarkers. Exposure and outcome biomarkers (8-hydroxydeoxyguanosine and 8-isoprostane) were measured at 4 time points in pregnancy and no association were observed.

The eMSCA concluded that although some pieces of information suggest an association between maternal and/or fetal triclocarban exposure and decreased length of gestation, data are still inconsistent to draw conclusions.

Non human data :

Oral route

- The Registant reported two studies from Nolen and Dierckman (1979), where the tested substance is a mixture containing triclocarban and not the registered substance. Not assessed by the eMSCA.

- The Registrant reported a three generation study (Monsanto, 1983 cited in HPVIS 2011 or 1983) performed to investigate the effects of triclocarban on fertility in rat by oral exposure at 250 ppm (25 mg/kg), 500 ppm (50 mg/kg), 1000 ppm (100 mg/kg), 3000 ppm (300 mg/kg). The mean number of live pups at birth was lower than controls for both litter intervals only for the F1 generation at the high dose group (3000 ppm). The Registrant proposed a NOAEL for both the maternal and fetal population of 300mg/kg/day. The eMSCA considered that there was not enough information available to evaluate the quality of the study. This study was described twice by the Registrant in the registration dossier (2019), and results are dissimilarly described including errors in the dose range, dose descriptors identified (NOAEL) and effects observed on fetal parameters.

- The Registrant provided a three-generation study (Monsanto, 1980, not cited[20] in the registration dossier (2019)) performed to evaluate the effects of triclocarban on fertility, reproductive performance, survival index in rat by oral exposure at 300 ppm, 1000 ppm, 3000 ppm.

However, the following shortcomings r about the experimental design are as follows:

- For each generation, the first litter of the dams has been discarded and only the second litter (F1b, F2b,) has been selected to make the groups

- Mating period was not similar in F1 and F2 generations, F2 generation mating being extended on six months. The mating tests were not synchronized between the treatment groups and observations on mating were performed on a long duration of time.

- Animals have been selected on the second litter of the dams.

Males and females fed with 3000 ppm (300 mg/kg bw) triclocarban only displayed mean body weights lower than those of control group males during F0 and F1 generations. Additionally, some early reproductive toxicity appeared in F0 and F1 males, leading to a lower number of pups and, consequently, a decrease of rat number per group in the F3 generation

The studied parameters of reproductive performance were similar for the control, 300 ppm (30 mg/kg bw) and 1000 ppm (100 mg/kg bw) groups, but they were impaired in the 3000 ppm (300mg/kg bw) triclocarban group during F0 and F1 generations. The calculated percentage for 21-day pup survival for the F1a, F2a and F3b from the 3000 ppm groups were lower than control groups. Importantly, the PND4 and PND21 survival indices were also lower for the F3b 1000 ppm group. Histopathologic studies showed varying grades of degenerative testicular lesion among males of the 3000 ppm group in the F0 and F1 generations. Triclocarban effects reported were restricted to testis and epididymis, with focal degeneration of the germinal epithelium, which involved seminiferous tubules in one or both gonads. The testicular lesions were also described by spermatic granuloma, diminution of sperm in the ispsilateral epididymal ducts. No testicular lesion was found in control rats. No treatment-related testicular deterioration was found in 300 and 1000 ppm groups.

Concerning the other organs, and more specially reproductive organs of females, the Registrant provided little data and did not report strong histological anomalies in ovaries and uterus, related to triclocarban intake, even in animals dosed at 3000 ppm.

The eMSCA considered that this study clearly shows specific alterations of male reproductive function in rats exposed to 300 mg/kg/d. It suggests that lower doses (30 and 100 mg/kg/d) do not affect these functions. However, the present data provide

[20] See 7.2 on Procedure. The data has been provided directly but not via the registration dossier.

substantially less information than what is needed to document the safety of triclocarban at these doses, for the reasons detailed in conclusions of the one-year oral toxicity study of triclocarban on rat (cf. 7.9.6 Repeated dose toxicity).

- The Registrant reported reproductive and developmental studies following long-life pharmacological exposure to triclocarban in two animal models: one was performed on rodents (albino rats) to investigate the reproductive impact and teratogenicity of 500 and 1000 mg/kg bw/day perinatal exposure, the other one was performed on rabbits to investigate teratogenic effects only, at 30 and 100 mg/kg bw daily exposure (Monsanto 1971 and 1990, not cited[21] in the registration dossier (2019)).

First, fertility, reproductive performance and perinatal / lactational effects were studied in Albino rats (10 males / 20 females per doses) exposed to triclocarban at daily oral gavage doses of 500 mg/kg bw/d and 1000 mg/kg bw/d.

Males were administered triclocarban from 40 days of age (i.e. 2 months before mating) until the end of the mating period (10 animals per group) , whereas females were dosed from 86 days of age (i.e two weeks prior to mating) up to the end of the experiment (20 animals per group). Animals from the control group received corn oil alone. At 100 days of age, female rats were caged in pairs and, mated with one male from the same group.

In these conditions, triclocarban did not affect food intake, body weight or body weight gain of male rats. A low decrease of body weight gain was noted in treated females over the first two weeks of treatment. However, the body weight in both groups was similar to that of the control group throughout the lactation untill the end of the study. No deaths observed in control or treated groups during the study.

Fertility was lower among the triclocarban groups: general fertility (defined as the number of pups born viable) was 121, 100 and 72 in control, 500 and 1000 mg groups respectively. Male fertility index (defined as the number of males impregnating one or more female/total number of male) was 10/10, 9/10 and 8/10 for the 3 groups respectively. Lastly, the female fertility index (defined as the number of pregnancy/total number of mating observed) was 20/20, 20/21, 18/21 for the 3 groups respectively. Both male and female mating indexes were dose-dependently reduced. Mating index (defined as the percentage of mating of one male in presence of one female) during 5 days was 69, 43 and 31% for the 3 groups respectively. Histopathological examination of non-fertile animal failed to detect any anomalies. Gestation time and incidence of pregnancy were similar among groups.

About female fertility, triclocarban decreased in a dose dependent manner the number of implantation sites (% of corpora lutea): control (95/100), 500 mg/kg bw/d (83/100), 1000 mg/kg/bw/d (62/100). Triclocarban did not affect the number of both viable fetuses per 100 implantation sites and resorption sites, and did not induce abnormalities in females.

The number of live-born pups and the number of pups delivered was fewer only at the 1000 mg/kg bw/d by comparison to controls. The progeny survival indices (Number of viable pups divided by number of viable pups born X 100) and the progeny body weight at various point of lactation were similar among treatement groups and control. All pups delivered were without any abnormalities at birth and at weaning.

Concerning perinatal and lactational effects, female body weights were similar in 500 mg/kg bw/d and 1000 mg/kg bw/d treated groups. The number of delivered pups and viable pups as well as the progeny survival indices and body weights were similar in treated and control group at weaning and during lactation. All delivered pups were free of gross abnormalities and did not display abnormal general behavior. These observations are consistent with a lack of effect of triclocarban during gestation and lactation.

In the first teratogenicity study, female rats (17 to 18 females/groups) were exposed from GD6 to GD15 to the vehicle or triclocarban at 500 and 1000 mg/kg bw/d. Results showed no significant difference between treated and control female body weight at any time of

[21] See 7.2 on Procedure. The data has been provided directly but not via the registration dossier.

gestation. There was no death noted. Indicative modifications of preimplantation losses were observed.

At the GD20 sacrifice, females were free of gross uterine pathology and gestational defects by considering the number of implantation sites, resorption sites, corpora lutea, and viable fetuses, body weight of fetuses and sex-ratio, all being similar among groups. Fetuses were without apparent external or internal abnormalities related to treatment among groups. No treatment-related skeletal abnormalities were observed among groups. Internal examinations failed to reveal particular abnormalities compared to controls.

In the second teratogenicity study, female rabbits were exposed from GD6 to GD18 to vehicle or triclocarban at 30 and 100 mg/kg bw/d or to thalidomide as a positive control for teratogenicity, through oral capsule. Again indicative modifications of preimplatatory losses (decreased number of implantation sites/ corpus luteum without changes in the number of fetuses/implantation sites) have been reported. The author claimed that this cannot be treatment-related as implantation is effective at GD6. However, in rabbit, the implantation just starts at GD6 but it is achieved only toward GD8 and 9 (Nishimura, Congenital anomalies, 2001, 41:198-203). Thus, a possible effect of treatment on the implantation process cannot be excluded.

As in the rat, triclocarban did not change the body weight of dams during the gestation, and it induced neither pup death nor abnormal maternal behavior. Nevertheless, the number of implantation sites (% of corpora lutea) was lower in both, females treated with 30 mg/kg bw/d (57/100) or females treated with 100 mg/kg/bw/d (70/100), by comparing to control (86/100). The number of viable fetuses per 100 implantations for the control, 30 mg/kg bw/d and 100 mg/kg/bw/d, was 93, 98, 93, respectively. Fetuses were without apparent external abnormalities related to triclocarban. The weight of rabbit progeny was similar among the groups.

The prenatal treatment with triclocarban did not affect the 24h-survival of the progeny. The rabbit progeny was free of internal gross abnormalities. Triclocarban treatment did not induce skeletal abnormalities in this study.

Teratogenic effect could be evidenced neither with triclocarban, nor with thalidomide.

The eMSCA considered that at 500 mg/kg/day triclocarban considerably reduces fertility of both male and female rats. However, investigated end-points were largely too limited in number and in sensitivity. For male, it is highly regrettable that weighting of testes and sex organs, counting of sperms, observation of the motility and the morphology of sperms, testicular histology and hormonal assay were not performed on all the animals. For female, the results of these studies suggest that triclocarban can induce preimplantatory losses in two different species. In the oral study in rats and the dermal study in rabbit, this appears to be related to adverse effect on fertility and viability of offspring. Given the fact that no endocrine data is provided, that there was no real histology evaluation of the ovaries, it is difficult to conclude on a possible endocrine mode of action. Preimplantatory losses can indeed be related to endocrine mechanisms as well as other mechanisms such as oocyte cytotoxicity or non-endocrine-related epigenetic reprogramming.

- In animal studies, Chen *et al*., (2008) was the first to investigate if an ED mode of action could be responsible for the possible reproductive effect of pharmalogical dose of triclocarban (0.25% in diet = 250 mg triclocarban/kg/j) on castrated male rats exposed for 10 days. The aim was to investigate a possible androgenic effect through androgen receptor (AR) (Hershberger experimental model design). Triclocarban given alone increased ventral prostate weight but did not exert androgenic effect on the other sex organs. However, triclocarban in combination with testosterone potentialized the inducing effect of testosterone on all sex organs. According to Duleba *et al*., (2011), triclocarban led to a significant increase of body weight (+ 5,1%) in intact treated animals compared to intact control animals, and increased the weights of liver (13,3%), seminal vesicles (42%), ventral prostate (37%), LABC (levator ani-bulbocavernosus muscle, 136%), and glans penis (35%), suggesting an androgenic effect. Nevertheless, no effect was found on

the LH and testosterone blood levels, or on the AR expression in these tissues whereas the *in vitro* study performed in parallel on LnCaP cells showed a triclocarban potentialisation of androgens that could be inhibited by the bicalutamide AR inhibitor.

- The study of Kennedy *et al*., (2014) aimed to investigate the effect of pharmacological doses (triclocarban 0.2 and 0.5% of the diet) and to identify a period of critical exposure during the gestation and/or the lactation period (GD5-PND21) on the basis of the developmental observations of the offspring (up to PND54). This study highlights the reduced offspring survival occurring when offspring were exposed during the lactation (13% of pups survived beyond weaning in 0.2% w/w triclocarban treated dams and none of the offspring raised by 0.5% w/w triclocarban treated dams did). Because there were no effect on fertility, embryonic implantation, anogenital distance, vaginal opening, and no embryonic malformations, this mortality did not result from an endocrine disruption.

The eMSCA conclude that there is no effect of triclocarban on fertilization, embryonic setting-up of the ano-genital distance, vaginal opening, and no embryonic malformations. On the other hand the presence of stomach ulcers, leading to the death of pups, is associated with the ranges of triclocarban plasma concentrations and in the maternal milk (X4 vs blood levels), showing an acute toxicity of triclocarban *via* lactation.

- Two recent studies investigated the effect of triclocarban at relevant environmental doses.

A recent publication from Enright et al., (2017) using CD-1 mice was analysed by the eMSCA. This study used an environmental relevant concentration of triclocarban. The exposure was performed by adding 130 nM 14C-triclocarban (purity 98.8 %) in drinking water i.e. a concentration 5-fold higher than the highest concentration of triclocarban found in tap water. As 30% of triclocarban was adsorbed on the walls of the bottle and 150 mL/kg/day is the average daily water drinking by a mouse, it can be estimated that the mice were given 4.7 µg/kg/day in this study.

The first aim of this study was to determine whether triclocarban is transferred from mother to offspring through the placenta and *via* maternal milk. A passage of triclocarban through the placenta and *via* the milk was observed as triclocarban was recovered both in fetuses at GD 18 after dam exposure from GD0 to GD18 and in 10 day-old neonates after dams exposure from PND0 to PND10.

The second aim of this study was to investigate the long term effects of neonatal exposure. Here again, triclocarban (130 nM) was administered through the drinking water to the dams from PND0 to PND10.

Weightings of offspring were performed throughout development from PND0 to PND56. From birth throughout weaning (PND21), both sexes were grouped and the rate of body gain was statistically higher in the triclocarban group than that in the control one. From PND21 to PND56, the weights of offspring exposed to triclocarban were significantly greater compared to controls in both male and female groups. At PND56, the body weight of offspring exposed to triclocarban was increased by 9 % in females and 6 % in males.

Organ weights referred to body weight were analysed in female (control: n=30, triclocarban: n=27) and male (control: n=9, triclocarban: n=24) offspring at PND56.

Relative brain weight was statistically reduced by 9% in both sexes. No other changes in relative weight was observed in all other organs examined in males.

In females:

- uterine relative weight was reduced by 14 %

- inguinal and retroperitoneal fat relative weights were increased by 23 % and 40 %, respectively
- thymus relative weight was increased by 14%

No statistically significant changes were observed in other organs examined (heart, liver, lung spleen, kidney, gonadal fat, muscle, colon, adrenal, gastro intestinal tract, gonads).

The tissue distribution of triclocarban (or its metabolites) was studied in offspring at PND42 using Accelerator mass spectrometry (AMS)(n=5 from 5 different litters). ^{14}C was found in all organs examined. Higher concentrations were detected in brain tissue compared to other tissues in both sexes. Significantly higher ^{14}C concentrations were detected in female offspring's gonadal fat, gonads, adrenal, muscle and heart compared to littermate males.

Lipid metabolism

Dams were exposed through drinking water from offspring birth to PND10 and associations between triclocarban exposure and alterations in lipid metabolism were investigated in male and female offspring.

- Hepatic triglycerides levels

At PND56, a three-fold increase in hepatic triglycerides level was found in triclocarban-exposed female offspring (94 and 24 mg/dL in triclocarban and control groups respectively) whereas no change was observed in males (n = 4-5 from 4-5 different litters).

- Gene expressions

The expression of genes was evaluated by RT-qPCR in liver and adipose tissue (origin not specified) from both sexes at PND42 (n = 5 from 5 different litters).

In liver, levels of mRNA for Peroxisome proliferator-activated receptor alpha (PPAR alpha), carnitine acyltransferase 1A (CPT1A) and carnitine acyltransferase 2 (CPT2), which are all involved in ß-oxidation of fatty acids, were decreased by half in triclocarban female group compared with controls.In male offspring, The expression of these genes was unchanged..

In adipose tissue, the expressions of *Lep* and *Adipoq* encoding leptin and adiponectin, respectively were decreased by half in triclocarban group compared with controls in females but unchanged in males.

This studypresents some limitations on the methodology, the limited number, more specifically for measurement of the body weight at PND10 with n=2 in male control litter. Moreover, dealing with the adverse effects of triclocarban on female body weight, the various organs selected for weighting represents about 20 % of the body weight. Thus, it remains unknown which organs were responsible for the increase in body weight.

In the same way, no selected organ exhibited a weight increase in triclocarban-exposed males whereas body weight increased; thus, it remains unknown which organ were responsible for thebody weight increase.

Plasma levels of leptin, adiponectin and insulin were not measured therefore does not enable to clarify the mode of action initiated by exposure to triclocarban.

The eMSCA concludes that although this study contains limitations, it demonstrates that maternal exposure to triclocarban induces deleterious changes on various endpoints in offspring at adulthood. Some of them could be explained by changes in lipid accumulation in the liver and the expression of genes either encoding adipokines or involved in lipid metabolism. The data show the following long term deleterious effects of triclocarban intake at a dose of 4.7 µg/kg/day by lactating mice from PND0 to PND10 (with a focus on three endpoints in the adult offspring):

1- increase in body weight by 9% in females and 6% in males,

2- decrease in brain weight by 10 – 12 % in both sexes,

3- increase in triglycerides hepatic level by 290 % and increase of several adipose tissues weight (average: 30% in the examined tissues) in female. This is associated with a decrease in the liver of the expression of PPAR alpha a master nuclear receptor

regulating beta-oxidation of fatty acids and of two PPARalpha target genes (i.e., CPT1A, CPT2)

Concerning long term endocrine changes, adult females exposed to triclocarban during neonatal life *via* maternal milk showed a decrease by half of the mRNA levels of *Lep* and *Adipoq* in adipose tissue. The adipokines leptin and adiponectin are both involved in the regulation of energetic metabolism and the ratio of leptin to adiponectin is used as a marker of insulin sensitivity.

Leptin is involved in satiety mechanisms and its plasma levels correlate with fat mass expansion while high levels of adinopectin are associated with insulin sensitivity.

While the decrease in the weight of brain is not understood and the authors do not give any explanation, a mode of action could be suggested to link the body weight increase with the changes observed both in the liver and the adipose tissues which both express specific receptors for leptin and adiponectin.

- A decrease in *Lep* mRNA causes reduced synthesis and secretion of leptin by adipocytes resulting in an increase of appetite which leads to an increase of body weight
- A decrease in adiponectin signalling will favor a decrease in ß-oxidation of fatty acids and thus an increase in lipid content in the liver resulting in decreased insulin sensitivity.

- In the developmental study of Costa *et al*., (2019), gestational and lactational exposure was assessed by gavaging female Wistar rats with 0.3, 1.5 or 3 mg triclocarban/kg/d. No effect was found in F0 dams, but in F1 adult females, triclocarban induced a decrease of estradiol and progesterone levels measured at the proestrous stage.

The eMSCA conclude that no consequence of this endocrine disorder (decrease of estradiol and progesterone levels in F1 females) was observed on the weight of uterus or on estrous cyclicity. The increase of pre-implantation loss in a dose dependant manner was observed in pregnant F1 females on lactational day 11, but hormonal levels were not evaluated in these females.

Dermal route

- The Registrant also provided a reproduction and/or teratogenicity study performed in rat and rabbit models through dermal application of triclocarban suspensions in the scapular region (Monsanto, 1966, not cited in the registration dossier (2019) [22]).

-Experiment 1: Fertility and reproductive performance were investigated in rodents only. Albinos rats (PND30; 30 males / 60 females per group) were dosed with 0, 40 and 200 mg triclocarban /kg bw/d. Males (10/groups) were daily treated from the 40th day of age until the end of mating. Females (20/groups) were dosed two weeks prior to mating (86 days of age) up to the end of experiment. Control group was treated in a similar manner with corn oil. At 100 days of age, females were caged in pairs and mated with one male from the same group. No death was observed in treated groups during the study.

Dermal exposure to triclocarban had no effect on both, body weight gain and food intake. Male and female fertility mating indices, gestation time, parturition incidence, as well as the number of implantation sites, percentage of corpora lutea, the number of fetuses and the number of resorption sites were not affected. Also, the number of delivered pups, number of viable pups at birth and at the beginning of lactation (PND1 & PND4) and progeny body weights at various point of lactation, were similar in control and treated groups. All delivered pups were without external/internal abnormalities at birth and at weaning.

[22] See 7.2 on Procedure. The data has been provided directly but not via the registration dossier.

-Experiment 2: The teratogenicity of triclocarban has been investigated in both female rats and rabbits. Albino female rats (20 females/ groups) and Dutch Belted rabbits (68 females/doses/groups) were dermally exposed to 40 mg triclocarban /kg bw/d and 200 mg triclocarban /kg bw/d from the sixth gestational days (GD6) to the fifteenth gestational day (GD15) or to eighteenth gestational day (GD18), respectively.

No significant differences between triclocarban treated and control females body weight at any time during gestation were found in both, rodent and rabbit models. No death or abnormal general behavior was attributable to triclocarban. All female rats were free of gross uterine abnormalities at GD20 (rats) or GD28 (rabbits). All fetuses appeared normal and the number of fetal resorptions was similar between the control and the 40 mg/kg bw/d dosed group. In rats only, 55 % of females dosed with 200 mg triclocarban /kg bw/d exhibited one or more resorption sites and a slight decrease in the mean number of viable pups at the end of the study was observed. In rabbits at GD28, viability index were not statistically significant compared to control. In both experiments, body weights from treated fetuses were comparable to control, and without apparent external or internal abnormalities.

Taken together, studies on rats and rabbits showed that fertility, reproductive performance, teratology, and peri-natal and post-natal performance of treated animals were unaffected by repeated dermal exposure to triclocarban at doses of 40 and 200 mg/kg bw/d. However, a slight decrease in the number implantation site/corpus luteum was observedfrom 92.5% in vehicle vs 81 and 85 % in treated-rabbits.

The eMSCA concludes that this study shows some alterations in the fertility of the couple (reduction in the number of viable fetuses at birth and increase in the number of resorption sites). Since both sexes of the couple were treated, it is not possible to know whether triclocarban affected male or female or both reproductive functions.

7.9.10. Others effects

- Neurotoxicity

The studies of Kajta *et al*., (2019, 2020) used primary cultures of neocortical neurons from mice at 15-17 days of gestation with triclocarban at 0.1, 1, 10 and 100 µM for 6 hours or 24 hours .

In Kajta et al. (2019), it was shown that treatment of primary cultures of neocortical neurons with triclocarban for 6 hours or 24 hours induced apoptosis, as evidenced by reduced mitochondrial membrane potential, decreased antiapoptotic(BCL2)/proapoptotic (BAX) ratio, and increased apoptotic fragmentation of cell nuclei. These effects were associated with reactive oxygen species (ROS) formation, increased lactate dehydrogenase (LDH) release and caspase-3 activity. The increased LDH release and caspase activity were reduced or blocked by antagonists or siRNA for CAR (constitutive androstane receptor) and AhR (aryl hydrocarbon receptor). Triclocarban exerted a biphasic effect on the expression of these receptors, which were confirmed to be present in neurons. Triclocarban also induced epigenetic modifications illustrated by changes in histone desacetylase, sirtuin and DNA methyltransferase activities, as well as a reduction in global DNA hypomethylation correlated with the hypomethylation of the CAR and BAX genes, and reduced protein sumoylation.

A second study (Kajta *et al*., 2020) shows that in addition to apoptosis induction and modifications in LDH release and caspase activity found in the first study, exposure to triclocarban triggered also modifications in the autophagosome by changing the expression levels of several related genes. Furthermore, ERalpha and GPER1 signalling pathways were also modified since triclocarban reduced their expression levels at 6 hours and 24 hours post-treatments, probably through an increased methylation state level. Both estrogen receptors were involved in triclocarban-induced changes in LDH release and caspase-3 activity as shown by the use of selective antagonists, suggesting that triclocarban reduced the neuroprotective effects of these receptors. siRNAS for ERalpha and GPER1 did not block

the triclocarban-induced effects on the autophagosome, suggesting that this effect was independent from that affecting estrogen receptor signalling.

In the two studies, the lowest effective concentration appeared to be 10 µM.

These *in vitro* analyses detailed the mechanisms of disrupted neuronal integrity following short and long-term exposure of primary cultures of cortical cells to triclocarban. The experiments are well designed and performed; the use of primary cultures of cortical cells is relevant in terms of potential impact of triclocarban at developmental stages of the nervous system, although these data need to be confirmed *in vivo*. Triclocarban seems to exert at least partly its effects through the modulation of the signaling pathways involving AhR and CAR, in one hand, and estrogen receptors, in the other hand. Interestingly, recent studies using Ahr knockout mice show that this receptor is not only a xenobiotic receptor, but it also exerts several physiological functions including neural functions (locomotor activity, myelin sheat organization around the axons; Shackleford et al., PNAS 2018). Unfortunately, no analyses were performed in order to investigate whether the signalling pathways involving AhR/CAR and ERalpha/GPER1 are connected with respect to the observed modifications in LDH release and caspase-3 activity. The authors suggested an interaction between AhR and ERalpha on the basis of previous studies in other cell types. Nevertheless, on the basis of the second study, there is evidence of a mode of endocrine disruption action at least in part for the regulation of LDH release and caspase-3 activity.

- Immunotoxicity

No relevant information available.

- Cardiotoxicity

Chaudary *et al*., 2018, and Xie *et al*., (2018) (not included in the registration dossier) investigated the impact of triclocarban on cardiac functions *in vitro* and *in vivo*, respectively. Deleterious effects were observed on cardiomyocytes. Functional analysis showed that 10µM of triclocarban significantly decreases the metabolic activity and causes arrhythmic beating. In absence of serum, triclocarban abolishes beating at 10µM and still causes arrhithmia beating at 3µM. The study shows that cardiomyocytes exposed to triclocarban exhibit cardiac dysfunctions.

Moreover, *in vivo,* triclocarban exposure inhibits fatty acid synthesis and beta-oxidation through reduced expression of peroxisome proliferator-activated receptor alpha and its target genes. Tricarboxylic acid cycle is as well impacted which would lead to insufficient energy supply to the heart leading to heart toxicity.

These studies do not show evidence of a mode of action linked to endocrine disruption for the alteration of cardiac function.

7.9.11. Hazard assessment of physico-chemical properties

Not assessed as part of the Substance Evaluation.

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

Not assessed as part of the Substance Evaluation.

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

The Substance Evaluation targeted reprotoxicity and endocrine disruption. Endocrine disruption is discussed in section 7.10 below together with environmental data.

Regarding the effects of triclocarban on reproductive function:

Several studies relate to the possible reprotoxic effects of triclocarban in rats at [25-1000] mg/kg bw/d range doses, ie therapeutic doses. The results agree on a dose-dependent reprotoxic effect in males. These studies clearly show that doses equal to or higher than 75 mg/kg/d mainly affect spermatogenesis. However, it is not possible to agree with the statement that doses lower than 75 mg/kg/d (two-year carcinogenicity study) or at 300 mg/kg/d (one year oral toxicity study) or in three generation reproduction study) can be safe doses. In fact, the protocols used detected only at massive level spermatogenesis deterioration. Furthermore, it is not possible to define a mode of action based on these data because they are limited to organ and incomplete histological observations. Data on the female are fragmented and irregular depending on the studies, whether it concerns the development of the reproductive organs, the mammary gland, or fertility data: no monitoring of the menstrual cycles, few data on ovaries and mammary gland histology, little data on the parameters during the gestation.

Taken together, the studies on rodents show reprotoxic effects of triclocarban in males at the therapeutic dose (750 ppm ≈ 75 mg/kg/d or 3000 ppm ≈ 300 mg/kg/d), and suggest possible effects during the gestation maintenance, but do not specify any effects on the development and function of the females sexual organs.

- Chen et al., (2008) suggest that triclocarban potentialise the effect of testosterone both sex organs, and may exacerbate androgenic effects.
- Kennedy *et al.*, (2014) did not show effects on fertility, embryonic implantation, anogenital distance, vaginal opening, and no embryonic malformations when dams were exposed to 0.2 and 0.5% of triclocarban in their diet. However, during lactation, there is presence of stomach ulcers in pups, leading to their death (13% survival rate beyond weaning at 0.2% and no survivors at 0.5% of triclocarban). This was associated with high triclocarban levels in plasma and in maternal milk (X4 vs blood levels), showing acute toxicity of triclocarban *via* lactation.
- Enright *et al*. (2017) showed the passage of triclocarban into the placenta and in the milk when dams were exposed at very low dose (4,7 µg/kg/d) through drinking water. Triclocarban was recovered in many tissues and organs, and in some of them, after lactation exposure in a greater extent in female than in male (heart, brains, adrenals and gonads).
- Costa *et al*. (2019) found no effect in F0 dams exposed by gavage to 0.3, 1.5 or 3 mg /kg/d of triclocarban during the gestational and lactional period, but observed a decrease of estradiol and progesterone levels in F1 adult females. They also observed an increase of pre-implantation loss in a dose dependant manner in F1 females on lactational PND 11.
- Some ecotoxicological studies also investigated the reproductive effects of environmental doses of triclocarban in ecotoxicological experimental models (see section 7.10.1 for the full studies description). Villeneuve *et al.* (2017) showed a significant decrease of cumulative fecundity in female (less than 50% spawned eggs compared to the control) after exposure to triclocarban, linked to an increased number of preovulatory atretic follicles in females. Wang *et al.* (2016) reported that triclocarban slightly retarded the development of oocytes in zebrafish females; and reduced spermatogenesis in males. Giudice *et al.* (2010) demonstrated the chronic effect of triclocarban on reproduction (increased number of embryos) of New Zealand mudsail.

Considering the numerous observations of adverse effects on reproductive function in males (testicular alteration and fertility) and females (number of pre-implantation and/or implantation sites) due to triclocarban, and on the offspring via lactation (gastric ulcerations and high triclocarban levels in plasma and in maternal milk), this substance is considered to have a potential for reproductive toxicity. Thus, a C&L process is expected

to provide a harmonised classification and classify triclocarban as Reprotoxic (fertility and adverse effects *via* lactation).

7.10. Assessment of endocrine disrupting (ED) properties

Triclocarban was added to the community rolling action plan (CoRAP) for substance evaluation as suspected reprotoxic, a potential endocrine disruptor and due to its wide dispersive use and microbial resistance. Both human health relevant and wildlife relevant endocrine disruption were evaluated.

7.10.1. Endocrine disruption – Environment

No data on the ED-properties and their potential related-adverse effects on the environment has been provided by the Registrant(s). Relevant available data on ED-properties of triclocarban on aquatic species found in the open literature (Scopus, until August 2019) are summarized below. Those data have been assessed in a weight of evidence approach, according to the OECD Conceptual Framework for evaluating chemicals for endocrine disruption (OECD, 2012).

The available studies presented below should be considered at level 3 of the OECD Conceptual Framework (OECD, 2012) - *i.e. in vivo* assays providing data about selected endocrine mechanism(s) / pathways(s).

- Ankley *et al.* (2010) explored a mixture test design using fathead minnow fish (*Pimephales promelas*) for detecting different classes of EDCs (agonists of the estrogen and androgen receptors, inhibitors of steroid synthesis, and antagonists of the androgen receptors). Adults of both sexes were exposed during 21 days *via* water to substances with diverse mechanisms of action, including triclocarban, in absence or presence of 17β-trenbolone (TB), a potent androgen receptor agonist which masculinizes female fathead minnows. The applied protocol was equivalent to the OECD TG 229.

The replicated tested conditions with triclocarban associated with or without TB were:

- Negative control;
- 0.5 $\mu g.L^{-1}$ of TB;
- 5 $\mu g.L^{-1}$ of triclocarban;
- 10 $\mu g.L^{-1}$ of triclocarban;
- 0.5 $\mu g.L^{-1}$ of TB with 5 $\mu g.L^{-1}$ of triclocarban;
- 0.5 $\mu g.L^{-1}$ of TB with 10 $\mu g.L^{-1}$ of triclocarban.

At the end of the exposure period, the following endpoints were measured:

- Vitellogenin (VTG) concentration in plasma in male and female.
- Number of nuptial tubercles, that is in accordance with the OECD TG 229 as the main indicator of exogenous androgenic exposure when measured on female *P. promelas*.

Exposure to triclocarban, either alone or in combination with TB, did not induce VTG in male. Exposure to TB depressed VTG in female; treatment with triclocarban alone or with the androgen did not affect VTG status. Exposure to triclocarban, alone or with TB, did not affect tubercle status in male. Exposure to triclocarban alone did not affect tubercle status in female; nevertheless the highest concentration of triclocarban (10 $\mu g.L^{-1}$) significantly enhanced the induction of tubercles by TB. These results in fish were in accordance with recent studies reporting that triclocarban enhanced AR-mediated responses to androgens in mammalian cell cultures *via* the androgen receptor (Ahn *et al.*, 2008; Christen *et al.*, 2010; Duleba *et al.*, 2011).

It is worth noting that fish exposed to 10 µg.L^{-1} of triclocarban exhibited signs of overt toxicity, particularly in male. Three fishes (two male) died during the treatment with triclocarban (10 µg.L^{-1}) and with TB (0.5 µg.L^{-1}) + triclocarban (10 µg.L^{-1}), and several more male ceased feeding and were relatively inactive.

In conclusion, the study Ankley *et al.* (2010) indicated a potential androgen-enhancing activity of triclocarban on fish, based on equivocal results due to potential other toxic effects at the highest tested concentration of triclocarban with and without TB.

- Villeneuve *et al.* (2017) tried to confirm the results from Ankley *et al.* (2010) at lower doses and with additonal parameters. The study examined the effects of triclocarban alone and in combination with 17β-trenbolone (TB), a potent androgen, on fish reproduction function. Adult fathead minnow fishes (*Pimephales promelas*) were continuously exposed to either 1 or 5 µg.L^{-1} triclocarban, to 0.5 µg.L^{-1} TB or a mixture of 5 µg/L triclocarban and 0.5 µg.L^{-1} TB for 22 days, and a variety of endocrine related endpoints were examined. No mortality, abnormal behaviour, change in body weight and gonadosomatic index (GSI) were recorded in the triclocarban treated groups. Exposure to 1 µg.L^{-1} triclocarban had no effect on reproduction. Nevertheless, cumulative fecundity was significantly reduced in fathead minnows exposed to 5 µg.L^{-1} triclocarban, with a significant decrease in the number of spawned eggs (less than 50% compared to control; i.e. 3.6, 4.2, 1.8 mean spawns and 78, 74 and 36 eggs per spawns for the control, low and high triclocarban treatments respectively), linked to an increase number of preovulatory atretic follicles in females (9.5% of follicles for treated fish compared to 0.4% for controls). No effect of triclocarban exposure on secondary sex characteristics, plasma VTG, Estradiol (E2) and Testosterone (T), gene expression of *11βhsd, 3βhsd, 17 βhsd, ar, lhr, cyp 19a1a, vtgr* and *star* in testis and/or ovaries was observed. Only an increase in T production in testis and a decrease expression of *fshr* in ovaries with triclocarban at 5 µg.L^{-1} were noted. However, in terms of impact of triclocarban co-exposure on the anticipated responses to AR agonism, no significant augmentation effect was demonstrated.

The most relevant point to be noted in the study Villeneuve *et al.* (2017) is the significant effect of triclocarban at the dose rate of 5 µg.L^{-1} on the reproduction, with a significant decrease in the number of spawned eggs (less than 50% compared to control), linked to an increased number of preovulatory atretic follicles in females.

- Chung *et al.* (2011) assessed the effects of bisphenol A (BPA) and triclocarban on brain specific gene expression of aromatase *AroB* in early zebrafish embryos. This gene contains estrogen response elements and is therefore estrogen responsive. Triclocarban at 0,25 µM slightly stimulated *AroB* expression when tested alone, strongly enhanced overexpression of *AroB* induced by an exogenous estrogen (17β estradiol) but suppressed the expression induced by BPA. Authors didn't propose any signalling path, nor mechanism of action for triclocarban.Nevertheless, considering that no statistical analysis was performed by the authors, the results of this study should be considered with caution.

- Zenobio *et al.* (2014) assessed the effect of triclocarban on male and female adults fathead minnow fish (*Pimephales promelas)* in controlled conditions. Fish were exposed to 0.8 µg.L^{-1} of triclocarban (measured concentration). After 48h of exposure, the following endpoints were measured:
    - VTG gene expression in liver;
    - Expression levels of several genes in testes, known for being their involvement in steroidogenesis:
        - hepatic lipoprotein lipase (lpl) gene expression in testes;
        - androgen receptor (ar) gene expression in testes;
        - steroidogenic acute regulatory protein (star) gene expression in testes;
    - Gonado-Somatic Index (GSI);

- Hepato-Somatic Index (HSI);
- Condition factor;
- Mortality.

Compared to control conditions, when fish were exposed to 0.8 µg.L^{-1} of triclocarban, relative expression of *vtg* in livers was significantly increased in female (around 2-fold change) and male (around 2.5-fold change), indicating a potential estrogenic activity of triclocarban. In addition, triclocarban downregulated *ar* and *star* in testes and upregulated *lpl* in livers. Considering the non significant difference with control of GSI, HSI, condition factor, and mortality, the observed effect on gene expression could be considered as not related to systemic effects. According to the authors, these results suggest that triclocarban has both estrogenic and androgenic activity through regulation of *vtg* and *ar* and might impact steroidogenesis through decline in cholesterol levels and inhibition of *star* expression.

In conclusion, the study of Zenobio *et al.* (2014) indicated a potential ED-effect of triclocarban through estrogenic, androgenic and steroidogenesis activity at a concentration of 0.8 µg.L^{-1}.

- Schultz *et al.* (2012) assessed the effect of triclocarban on fishes in controlled conditions with the hypothesis of an estrogenic activity. Male and female adults of fathead minnow (*Pimephales promelas)* were exposed to 0,56 and 1,60 µg.L^{-1} of triclocarban. A treatment of 17β-E2 at 30 ng.L^{-1} was used as a positive control. After 21 days of exposure, the following endpoint were measured:

  - VTG concentration in plasma;
  - Secondary sexual characteristics (tubercles; dorsal pad and color intensity);
  - HSI;
  - GSI;
  - Histopathological changes in liver and gonads;
  - Male ability to defend a nest;
  - Mortality.

No significant difference of triclocarban treatment with controls, nor positive control was observed for mortality, HSI, GSI, histopathological changes in liver and gonads or secondary sexual characteristics. These results confirmed that low doses of triclocarban induced no toxic effects. VTG concentration in plasma were significantly increased only in E2-positive control in male fishes compared with the negative controls. No significant difference was observed between groups treated with triclocarban and solvent control. This result could be compared to the data from Zenobio *et al.* (2014), mentioned above, that demonstrated in fathead minnow fish adults an upregulation of VTG genes in liver after 48h of exposure to 0.8 µg.L^{-1} of triclocarban. Considering the short-term exposure (48h) in Zenobio *et al.* (2014) and the genomic level of the endpoint, the transitional effect of triclocarban in VTG gene expression is questioned by the VTG results of Schultz *et al.* (2012). Concerning the male ability to defend a nest, the total aggression index (the product of time to attack and number of attacks) was significantly decreased for the triclocarban-treatment at 1600 ng.L^{-1}, compared to control.

In conclusion, the study of Schultz *et al*. (2012) indicated no change in VTG plasma concentration in fathead minnow with triclocarban treatment up to 1.6 µg/L. The major point raised in this study is about the the male ability to defend a nest. The total aggression index (the product of time to attack and number of attacks) was significantly decreased for the triclocarban-treatment at 1.6 µg/L, compared to control.

- The study of Shi *et al*. (2019) analysed the effect of triclocarban on *Danio rerio* embryos according to OECD 236 guideline (fish embryo toxicity test) at 0.3, 30 and 300 µg/L. The

embryos were exposed from 4 to 120 hpf (hour post fertilisation). No effect compared to control was observed for the lowest concentration of 0.3 µg/L. Significant increase in malformations was recorded at 300 µg/L only with 23.3% of malformed embryos at 120 hour post-fertilisation (hpf) (yolk sac edema, pericardial edema and notochord bending). A significant delay in hatching was observed at 30 and 300 µg/L (78.3% and 64.2% at 72 hpf compared to 89.2% in control). Nevertheless, all the surviving embryos were hatched out from the chorion after 96 hpf and entered the larval stage at 120 hpf. The heart rates were significantly decreased at 120 hpf for the embryos exposed to 30 and 300 µg/L. Some endocrine genes expressions were measured: at 120 *hpf*, *Vtg1*, *Esr2b* and *Cyp19b* were upregulated at 30 and 300 µg/L; and Esr1 was also upregulated but only at 300 µg/L.

Nevetheless, at the two highest tested concentrations of 30 and 300 µg/L, significant mortality was also observed (20 and 49% at 120 hpf).

In the study of Shi *et al*. (2019), some effects on *Danio rerio* embryos were recorded (malformations, delay in hatching, decreased heart rates, upregulation of endocrine gene expression such as Vtg1, Esr1, Esr2b and Cyp19b). However, as significant mortality was also recorded at the effect doses, it is not possible to link the adverse effects and potential endocrine mechanisms.

- The study of Wang *et al.* (2016) suffers several deficiencies (poor reporting of effects, no statistical analysis, high differences between nominal and mesured concentrations...) and its reliability is difficult to assess. Nevertheless interesting results can be reported even if they must be considered with care. Exposure of *Danio rerio* to triclocarban alone (2-5 µg/L nominal; 140 – 310 ng/L measured) during 21 days seemed to slightly retard development of oocytes and to slightly reduce spermatogenesis. Moreover, triclocarban in mixture with inorganic mercury modulated the expression of different genes, included cyp 19a and cyp17 involved in steroid synthesis in testes and ovaries.

- In addition, effect of triclocarban has also been investigated in other aquatic species. Giudice *et al.* (2010) assessed the effect of triclocarban on freshwater mudsail *Potamopyrgus antipodarum* according to a protocol equivalent to the OECD TG 242 (*Potamopyrgus antipodarum* Reproduction Test). The aim of this study was to assess the potential effects of prolonged exposure to triclocarban on reproduction and survival of the freshwater mudsnail. Organisms were exposed during 4 weeks to a range of 6 concentrations from 0.05 to 10.5 $\mu g.L^{-1}$ (measured concentration). A significant positive dose response relationship between triclocarban concentrations and the increase in number of unshelled, shelled and total embryos revealed an effect on reproduction (NOEC = 0.47 $\mu g.L^{-1}$; EC_{50} = 2.5 $\mu g.L^{-1}$). The greatest increase was shown for the unshelled embryos.

  The authors indicate that this significant increase of number of embryos has been previously found in experiments with exogenous estrogenic EDCs (BPA, octylphenol, nonylphenol, ethynylestradiol). Nevertheless according to the OECD TG 242, the reproduction test is not suitable to demonstrate an endocrine mediated mode of action solely on the basis of a decreased or increased embryo number.

  In conclusion, Giudice *et al.* (2010) demonstrated a chronic effect of triclocarban on reproduction (increased number in embryos) of New Zealand mudsail, with a NOEC = 0.47 $\mu g.L^{-1}$. Nevertheless, no data allowed to consider this adverse effect related to an estrogenic activity of triclocarban.

- Rochester *et al.* (2017) summarises more general effects of triclocarban on reproduction and development of different species as follows:

- A chronic exposure of 28 days to 0.3 µg/L triclocarban altered the number of mud snail (*Potamopyrgus antipodarum*) embryos in the brood pouch in a nonmonotonic fashion.
- For an exposure lasting until 10 days at 100 µg/L, triclocarban retarded population growth and reduced cumulative offspring and lifespan of Brachionus koreanus. Triclocarban also altered the expression of xenobiotic metabolizing genes.

- In a study lasting 80 hours, triclocarban increased mortality rates in embryos of zebrafish and sea urchin at exposures greater than 350 µg/L (600 to 10 000 µg/L) but did not have effects on other developmental parameters. The NOEC for zebrafish was 100 µg/L. For sea urchin, triclocarban decreased larval length and increased abnormalities at 0.64 and 1.6 µg/L respectively.
- An exposure at 18 µg/L triclocarban during 24 hours caused mortality in *Artemia salina*. DNA damage and apoptosis of *Artemia salina* nauplii coelomocytes were apparent as 12 and 24 hours after exposure.

- Concerning the thyroid pathway, the study Dong *et al.* (2018) analysed some thyroid protein level and gene expression in link with developmental effects. Nevertheless, this study has been deemed of low reliability due to some deficiencies, as no analytical measure was conducted and results were not consistent with other studies (high NOEC mortality). Zebrafish embryos exposed from 6 to 120 hpf showed a NOEC for mortality of 133.3 µg/L. Delay in yolk absorption and swim bladder development were noted at 133.3 and 147.5 µg/L, as well as a decrease in heart rate at 147.5 µg/L. No effect on somites, pericardial edema or axial spinal curvature were observed for sub-lethal concentrations. An increase in T4 level was measured as well as an upregulation of *NIS* and *Deio1* and a downregulation of *TPO* at 133.3 µg/L, but no effect on T3 level or expression of *TSHβ, TSHR, TRHR2, TRα, and β, TRH, TG* and *deio2* was observed.

- The study of Zhou *et al.* (2019) put forward the hypothesis that the developmental abnormalities and hepatotoxicity observed in zebrafish embryos would be linked to mechanisms of endoplasmic reticulum stress and unfolded protein response (UPR) (range of tested concentrations from 2.5 to 10 µg/L). According to Wei *et al.* (2018), the abnormal development in early stage of zebrafish could be the consequence of an oxidative stress induction and enzymatic or non-enzymatic antioxidant disruptions mediated by triclocarban (range of tested concentrations in zebrafish embryos from 1.25 to 20 µg/L).

- The study of Barros *et al*. (2017) explored the effets of triclocarban on crustacans *Gammarus locusta* (tested concentrations: 0.1 to 2.5 µg/L). Significant alterations were observed in all biochemical markers of oxidative stress (catalase (CAT), glutathione-S-transferase (GST) and lipid peroxidation (LPO)) and neurotransmission (acetylcholinesterase (AChE)). While AChE showed a dose-response curve (with a significant increased activity at a triclocarban concentration of 2.5 µg/L), oxidative stress markers did not follow a dose-response curve, with a significant increase at 0.1 and/or 0.5 µg/L and a decreased activity at the highest concentration (2.5 µg/L). The same effect was observed in the females' behavioural response (increase activity), whereas males' behaviour was not affected by triclocarban exposure.

Conclusions of the eMSCA:

Regarding the ED potential in environmental organisms, one study indicates that triclocarban could have a potential androgenic activity on fish as it enhances AR-mediated response to a well-know androgen (Trenbolone). Nevertheless systemic toxicity was also recorded at the dose rate leading to this activity. Other studies suggest that triclocarban has both, estrogenic and androgenic activities, through regulation of *vtg* and *ar* gene expression and might impact steroidogenesis through decline in cholesterol levels and inhibition of *star* gene expression in fish. Effect of triclocarban on fish reproduction is also reported, with a significant decrease on the number of spawned eggs linked to an increase number of preovulatory atretic follicles in females. Exposure of fish to triclocarban seemed to slightly delay the development of oocytes and to slightly reduce spermatogenesis. Finally, triclocarban could also have a chronic effect on reproduction of mudsails (increased number of embryos).

However, the studies available on environmental species (including recent data) are too scarce and suffer too many deficiencies to draw clear conclusions on the environmental ED properties of triclocarban.

7.10.2. Endocrine disruption - Human health

The data presented in section 7.9 are supplemented with the data presented below to address the ED concern. No data on the ED-properties and their potential related-adverse effects on human health has been provided by the Registrant(s). Relevant available data on ED-properties of triclocarban on human health found in the open literature (until January 2020) are summarized below. Those data have been assessed in a weight of evidence approach, according to the OECD Conceptual Framework for evaluating chemicals for endocrine disruption (OECD, 2012).

7.10.2.1. Triclocarban and male reproductive function

Three ED MoA can be stated to explain the male reprotoxicity of triclocarban.

1) Does triclocarban act *via* an estrogenic pathway?

It is well known that an excess in estrogens concentration or action in the testis negatively impacts spermatogenesis (Delbes et al., 2005, Leavy et al., 2017). Most of the *in vitro* studies recently carried out to assess the endocrine effects of triclocarban are mainly comparative studies comparing these effects to triclosan, for which the estrogenic effects have been established. They use transfected cell lines coupled to reporter genes and tools referenced in screening tests (MCF7 vs MDA-MB-231; CV1, MDA-kb2). The effects on gene expression were characterized using conventional techniques (RT-QPR, Immunoblots, siRNA, etc.). It was clearly shown that triclocarban binds to ERalpha, increases the transcription of ERE–luciferase reporter, ERalpha-dependent genes such as *Ps2, Cyp1a1, Cyp1b1, Cyp2b6* and stimulates MCF7 proliferation (Ahn *et al*., 2008; Yueh *et al*., 2012, Tarnow *et al.,* 2013, Huang *et al*., 2014, Rochester et al. 2017).

However, the hypothesis that triclocarban inhibits testicular spermatogenesis *via* its estrogenic potency must be considered with caution in particular when considering that no effect were observed on reliable bomarkers of estrogenic effects *in vivo* in females and for the following reasons:.
i)triclocarban binds to and activates CAR which inhibits ER-mediated signalling pathway (Min *et al., 2002,* Yueh *et al*., 2012). ii) triclocarban inhibits the aromatase and decreases the estradiol production (Li *et al.,* 2017).

2) Does triclocarban act *via* an androgenic pathway?

Triclocarban is a powerful AR agonist (Ahn *et al*., 2008; Blake et al., 2010, Kolsek *et al*, 2015) and it potentiates testosterone effects in the Hershberger test through an unclear mechanism (Chen *et al*., 2008; Ahn *et al*., 2008; Christen *et al*., 2010).

It is well-known that an increase in androgenic effect can be deleterious for spermatogenesis when it results in a decrease in LH secretion leading to a large decrease in the testicular testosterone production. However, *in vivo* experiments showed that LH and testosterone plasma levels were not changed after a triclocarban exposure (Duleba *et al*., 2011, Kennedy et al., 2014).

Thus, the mediation of male reprotoxic effect of triclocarban *via* the androgenic property is doubtful.

3) Does triclocarban act *via* an effect on steroidogenesis?

Although triclocarban was shown to act on the synthesis of pregnenolone and progesterone, as well as sex hormones (Tonoli *et al*., 2015), these observations are not sufficient to state a solid hypothesis of an ED MoA of the reprotoxic effect of triclocarban. Furthermore, triclocarban inhibits the aromatase and decreases the estradiol production (Li *et al.,* 2017).

In conclusion, as the male reproductive function is controlled by a precise balance in multiple endocrine processes and triclocarban has the potency to interact with several of these processes, triclocarban displays endocrine disruption properties.

However, available data, and specifically the *in vivo* data, are presently too limited to make a statement or a clear-cut sequence of events from an initial triclocarban-induced endocrine change to the alteration of male reproductive function.

7.10.2.2. Triclocarban and female reproductive function

Data on the female are fragmented and irregular depending on the different studies, whether it concerns the development of the reproductive organs and the mammary gland, or fertility data: no monitoring of the oestrous cycles, few data on ovaries and mammary gland histology, little data on the parameters of gestation. However, an effect on the implementation of gestation cannot be ruled out with regard to pre-implantation losses. Nevertheless, because of non SPF (Specific Pathogen Free) conditions, the two-year carcinogenicity study of triclocarban in rat model is marred by bacterial infections which have led to high mortality in adulthood and to the prolonged use of antibiotics, and these studies have to be considered with caution.

In conclusion, the studies in rodent suggest possible triclocarban effects on gestation maintenance, but do not specify any effects on the development and function of the female sexual organs. The results suggest that triclocarban can induce preimplantatory losses in two different species. In one study in rats, this appears to be related to adverse effects on fertility and viability of offspring. Given the fact that no endocrine data is provided, no reliable histology evaluation of the ovaries, it is difficult to conclude on a possible endocrine mode of action of triclocarban. Preimplantatory losses can indeed be related to other mechanisms such as oocyte cytotoxicity, or non-endocrine-related epigenetic reprogramming.

7.10.2.3. Triclocarban and thyroid function

Human data:

- Ley *et al.,* (Reprod Toxicol, 2017, 143-149) reported the thyroid function of 78 pregnant women from the Canadian STORCK cohort, exposed to triclosan during pregnancy (after 16.9 weeks) and to triclosan and triclocarban after delivery. They did not observe any modification of thyroid hormones; no dosage of triclocarban was performed in this study.

- Aker *et al*., (Environ Health, 2019, 28) performed complementary analysis on 602 pregnant women from the Puerto Rico PROTECT cohort. They showed, on repeated measures (first and second trimesters of pregnancy), that each interquartile increase in triclocarban maternal levels was associated with a significant 10% decrease of maternal TSH levels and with a significant 3% increase of maternal T3 levels. However, these women are particularly exposed to triclocarban as their blood and urine levels are 37 times higher than those observed in American women from the NHANES for example. Furthermore, no fetal or neonatal endpoints were considered in this study.

- Aker *et al.,* (Environ Int, 2018, 341-349) reported the thyroid function of 439 pregnant women from the LIFECODES cohort (n = 1600 pregnant women). Less than 15% of women exhibited detectable urine triclocarban levels (first, second and third trimesters); LOD = 0,1 µg/L). In these women, Aker *et al*., reported a 5.71% decrease of maternal T3 levels which was no significant when corrected with gestational age. Furthermore, no fetal or neonatal endpoints were considered in this study.

In conclusion, these three studies provide no relevant data concerning the potency of triclocarban to determine thyroid adverse effect during pregnancy.

In vitro and non Human data:

- *In vitro* biological activities of triclocarban were assayed in rat FRTL-5 thyroid cells and microsome of thyroid from healthy rats (Wu *et al*., 2016). The authors estimated the effect of triclocarban, (and other molecules) on various functional markers of the biosynthesis of

the thyroid hormones (TH). The active transport of the iodine within thyreocytes and activity of thyroperoxydase were required for the organification of iodine, the iodification of the tyrosine residue of the thyroglobuline and production of the precursors of the HT, MIT (Monodiiodotyrosine) and DIT (Diiodotyrosine).

In parallel, the authors assessed the expression of genes coding for key proteins of the TH biosynthesis: *slca5* encoding the iodine carrier the sodium/iodine symporter (NIS), the genes coding for the thyroperoxydase and the thyroglobuline (protein rich in tyrosine residues, establishing colloid and serving as reserve of substratum for the production of MIT and DIT) as well as the expression of factors of transcriptions under thyroid control Pax8, Foxe1, and Nkx2-1.

A dose-dependent non-competitive inhibition of NIS-mediated iodine incorporation by FRTL5 cells was evidenced both during short-term (1h) coincubation with triclocarban and NaI and incubation with NaI after 24-48 h exposure and cessation of the exposure for concentration as low as 0.3 µM. Triclocarban also inhibited TPO microsomal activity but with a very low potency (IC50 >300µM). For concentrations below 3 µM, triclocarban had an effect neither on the expression of *tpo, Slc5a5* (NIS) and *tg* (thyroglobulin) genes encoding three critical proteins for TH biosynthesis nor on the three thyroid-target gene expression .

This study shows an effect of triclocarban on the activity of the NIS on a thyroid cellular lineage with a good level of evidence and suggests that triclocarban could alter the biosynthesis of the TH by inhibiting iodine uptake and TPO activity in thyreocytes under certain conditions. However, effects on thyroid function have almost never be investigated in vivo. Thus it is difficult to conclude on the consequences on the thyroid function of such biochemical alterations in a whole thyroid gland.

7.10.2.4. Effects of triclocarban on metabolic function

- The study of Enright *et al*., 2017, as described by the authors, aimed to determine firstly whether an environmentally relevant concentration of triclocarban is transferred from mother mice to offspring through the placenta and *via* maternal milk and secondly, / to investigate whether neonatal exposure had long term metabolic effects and to study some related endocrine changes.

Dams were exposed through drinking water from offspring birth to PND10 and associations between exposure to triclocarban and alterations in lipid metabolism were investigated in male and female offspring.

- Hepatic triglycerides levels

At PND56, a three-fold increase in hepatic triglycerides level was found in triclocarban-exposed female offspring (94 and 24 mg/dL in triclocarban and control groups respectively) whereas no change was observed in males (n = 4-5 from 4-5 different litters).

- Genes expression

The expression of genes was evaluated by RT-qPCR in liver and adipose tissue (origin not specified) from both sexes at PND42 (n = 5 from 5 different litters).

In liver, levels of mRNA for Peroxisome proliferator-activated receptor alpha (PPAR alpha),carnitine acyltransferase 1A (CPT1A) and carnitine acyltransferase 2 (CPT2), which are all involved in ß-oxidation of fatty acids, were decreased by half in triclocarban female group compared with controls. On the opposite, the expression of these genes was unchanged after triclocarban treatment in male offspring.

In the adipose tissue, the expressions of *Lep* and *Adipoq* encoding leptin and adiponectin, respectively were decreased by half in triclocarban group compared with controls in females but were unchanged in males.

- The aim of the study by Li *et al*., 2018 (not included in the registration dossier) was to perform a systemic evaluation of hepatic endogenous metabolism alterations after triclocarban exposure, and to determine the underlying metabolic pathway. The authors

studied the correlation between key genes and metabolic pathways involved in liver metabolism alterations induced by triclocarban.

Triclocarban was administered to male C57BL/6 mice (5–6 weeks old) intragastrically at the doses of 0, 3, 10, 30, and 90 mg/kg body weight (diluted in 10% polyethylene glycol 400 in water) once a day for 35 days.

The authors did not mention whether the same part of the liver was used for histology biochemistry or metabolomics, which is very important especially for the metabolomics approach as the liver is a heterogeneous tissue.

The eMSCA noted effects of triclocarban exposure on the mouse liver visceral coefficient and pathology.The body weight, liver weight, and organ coefficient of treated mice decreased significantly when the dose of triclocarban reached or exceeded 10 mg/kg, compared to control group. Changes were also observed for blood biochemical indicators, such as an increase of alkaline phosphatase (ALT), choline esterase (CHE), and total protein (TP) concentrations, and a decrease of glucose (GLU) concentrations (only at 10 mg/kg).

Histological observations showed that, unlike the control group, treated liver cells were edematous when the dose reached or exceeded 10 mg/kg, with significant edema occurring in liver cells around the central vein of the liver in mice exposed to the highest dose (90 mg/kg).

Metabolomic data analysis, completed by multivariate statistical analysis, showed that control groups can be differentiated from the exposed groups for both matrices (plasma and liver).

These results indicate that triclocarban affects the metabolic secretion of endogenous substances in mouse liver and plasma. The identification showed that among those metabolites, fatty acids and lipid substances were the most affected.

The results for plasma and liver pathway analysis showed that triclocarban affected 3 endogenous substance-related metabolic pathways in plasma and 8 endogenous substance-related metabolic pathways in liver.

Triclocarban affected endogenous substance related metabolic pathway such as the linoleic acid metabolism, the valine, leucine, and isoleucine biosynthesis, and the glycerophospholipid metabolism in the plasma. In the liver, the same pathways were identified, as well as the arachidonic acid metabolism, the nicotinate and nicotinamide metabolism, glycerophospholipid metabolism, the alanine, aspartate, and glutamate metabolism, and the inositol phosphate metabolism.

Further *in silico* analysis identified pathways modulated by triclocarban, namely liver fatty acid synthesis and metabolism, glycolysis, and gluconeogenesis.

<u>Effects of triclocarban on the related enzyme activity in fatty acid metabolism in mouse liver</u>

Compared with the control group, the triclocarban-exposed groups had reduced Fatty Acid Synthetase (FAS) and acetyl CoA Carboxylase (ACC) activity which are associated with liver fatty acid synthesis. PPARγ regulates intrahepatic fatty acid production and PPARγ mRNA levels were also decreased; however, the CD36 mRNA level was increased in the triclocarban exposed groups compared to the control group. CD36 is highly expressed in tissues with high fatty acid oxidative metabolism, such as the liver, where it promotes absorption of fatty acids.

Likewise, compared with the control group, the triclocarban-exposed groups had higher oleic acid and linoleic acid proportions in the plasma but lower percentages in the liver. Another study reported that specific CD36 overexpression in muscle tissue reduced the proportions of free fatty acids (FFAs), triglyceride (TG), and cholesterol in the blood (Ibrahimi *et al*., 1999). In this paper, no significant difference in TG or cholesterol content between the control and exposed groups, suggesting that other factors might be responsible for the decreased proportion of FFAs in the liver. ACS and CPT1 enzyme activity increased with increasing triclocarban dose, and the PPARα mRNA level was also increased

at all the triclocarban concentrations tested, with higher concentrations at the intermediate doses (10 and 30 mg/kg). The main function of PPARα in the liver is to regulate fatty acid oxidative metabolism and energy consumption (Pyper *et al*., 2010). PPARα also regulates the activities of long-chain ACS and carnitine aminotransferase 1, of which ACS can activate many fatty acids to produce acyl-coenzyme A. CPT1 is necessary for acyl carnitine to enter mitochondria (Reddy and Hashimoto, 2001) and is a key enzyme for fatty acid β-oxidation, which regulates fatty acid oxidation (Kim *et al*., 2000). The authors concluded that after 35 days of exposure, triclocarban promotes the β-oxidation of fatty acids in mouse liver, which is consistent with the results of the liver metabolic pathway analysis. These data are associated with a decrease in fatty acid content in the liver and may explain the reduced liver weight.

Effects of triclocarban on glucose metabolism-related enzymes in mouse liver

In the liver, the triclocarban treatment groups had decreased pyruvate dehydrogenase activity in the liver compared with the control group, indicating that triclocarban inhibits the normal glycolysis in the liver. Additionally, G6PC mRNA levels were increased with the higher triclocarban doses (>3mg/kg bw).

Results also suggest that triclocarban drives gluconeogenesis in the liver in close coordination with liver glycolysis, and the results in this paper confirmed that inhibition of the major regulatory glycolytic enzymes enhanced the potency of gluconeogenic enzymes.

In the paper, the authors showed that the proportion of citric acid in the liver increased with the triclocarban dose. Additionally, citric acid inhibited the activity of phosphofructokinase. This indicates that triclocarban inhibits normal glycolysis in mouse liver and promote gluconeogenesis after 35 days of triclocarban exposure.

Effects of triclocarban on energy metabolism in mouse liver

The results indicated that triclocarban promoted the β-oxidation of fatty acids and gluconeogenesis providing acetyl coenzyme A and energy, but inhibited aerobic glycolysis in mitochondria.

After 35 days of exposure to triclocarban, fatty acid oxidation and glycosylation in the liver were up-regulated, glycolysis was down-regulated, and the proportion of sugars in the liver was higher in mice exposed to triclocarban.

The authors concluded by suggesting some hypothises. Abnormal liver glucose metabolism is the main pathological feature of metabolic syndrome, which includes type 2 diabetes, obesity, and non-alcoholic fatty liver, but liver insulin resistance may be the main pathogenic link between these diseases. Indeed, the most obvious pathophysiological feature was abnormal gluconeogenesis, which leads to an increase in hepatic glucose output.

The eMSCA concludes that the results of this paper suggest that triclocarban within the relatively high concentrations tested, i.e. 3, 10, 30, and 90 mg/kg body weight, may cause liver insulin resistance in mice, although the blood glucose level of mice in the exposed group was lower, suggesting that the efficiency of glucose utilization in other exposed tissues may be greater, but this remains to be confirmed through longer-term exposure experiments. It is important to note that concentrations above 10 mg/kg bw may be hepatotoxic and should be considered with caution.

- The eMSCA evaluated the paper of *Dong et al*., (2019) (not included in the registration dossier) which investigates the impact of triclocarban on lipid homeostasis in male rats. The rationale was based on evidences that triclocarban has been shown to interact with the aryl hydrocarbon receptor (AhR), ER, AR and constitutive androstane receptor as well as the thyroid system in human breast cancer cells (Tarnow et al., 2013) and in receptor-based bioassay screens (Ahn et al., 2008). The authors demonstrated that gavage of 4-week male Sprague-Dawley rats with triclocarban at doses of 50 μg, 20 mg and 100 mg/kg administered every other day for 56 days (n = 8 rats/group) impacted lipid homeostasis mostly at the lowest dose and triggered toxicity mostly at the two highest doses. Hence

on the one hand, low-dose triclocarban induced dyslipidemia manifested by enhanced plasma TG and LDL and liver accumulation of lipids resulting from de novo lipogenesis (DNL) and gut microbiota fermentation. Modes of action may involve activation of AhR as demonstrated with TCDF in mice (Zhang et al., 2015). On the other hand, high doses of triclocarban were toxic even though ASAT and ALAT remained in the control range values. Significant histopathological alterations were evident at the two highest doses with the 100mg dose more toxic than the 20 mg dose (according to the authors). Plasma creatinine was increased for the 3 doses, with the highest increase for the highest triclocarban dose, which is indicative of renal dysfunction. Expression levels of AhR, Cyp1a1 and Cyp1b1 and of inflammatory cytokines (IL-1β and IL-6) were significantly augmented. Based on the enhancement of the expression of genes involved in DNL, the authors concluded on lipid accumulation in the liver.

Dealing with endocrine disrupting effect of triclocarban on metabolism, attention must be paid to ectopic accumulation of lipids in liver known as steatosis and which constitutes one adverse effect of triclocarban.

Fatty liver is a very common sickness in industrial countries with a 30% prevalence. It is the first step in a pathological process which may conduct to fibrosis, cirrhosis and cancer.

A clear distinction must be made between high doses of triclocarban (some mg/kg/day) which elicit toxic effects (Chaudary *et al*., 2018; Li *et al*., 2018; Dong *et al*., 2019) and low doses (some µg/kg/day) which promotes liver steatosis (Enright *et al*., 2017; Dong *et al*., 2019).

Exposure to triclocarban during neonatal life by giving less than 5µg/kg/day to the lactating mother provoked a three-fold increase of liver triglycerides levels with a drop in the expression of fatty acid beta-oxidation markers in females at adulthood (Enright *et al*., 2017). This was associated with a diminution by half of *Lep* and *Adipoq* gene expression. Importantly, leptin in addition to reducing food intake protects metabolic organs from lipid accumulation including the liver which express leptin receptors. Therefore, decreased expression of leptin is a plausible cause of hepatic steatosis. Besides, adiponectin enhances insulin sensitivity in target organs. Thus, decreased expression of adiponectin indicates enhanced resistance to insulin, which characterizes a steatotic liver. However, in physiological conditions, a decrease of leptin signalling causes an increased food intake and gain weight normalizing leptin levels and hepatic lipid metabolism. Furthermore, leptin is positively expressed with fat mass in contrary to adiponectin and the ratio of leptin to adiponectin is an indicator of resistance to insulin. In response to triclocarban, at least at the mRNA level both leptin and adiponectin decrease which indicates that adipocytes may be dysfunctional causing ectopic accumulation of lipids in the liver.

Accumulation of hepatic lipids was also observed in adult male mice after an exposure to 50 µg/kg of triclocarban every two days for 8 weeks (Dong *et al*., 2019). This was associated with a AhR agonist activity that is also described in *in vitro* studies. It is known that AhR promotes accumulation of lipids in the liver (Duval *et al*., 2017) as it was observed for TCDF in mice (Zhang *et al*., 2015). Thus, the AhR agonist effect of triclocarban is a plausible cause of of hepatic steatosis in male rats exposed postnatally.

The major discrepancies between the studies of Enright 2017 and Dong 2019 resides in the periods of exposure to trichlocarban and the sex-dependency of the adverse effects. It is known that adipocytes are differentiated early in development (end of fetal life and neonatal period of life), it could be hypothesized that triclocarban exposure could disrupt the adipocyte differentiation program in females while in post-weaning animals, the liver would be a major target at least in males.

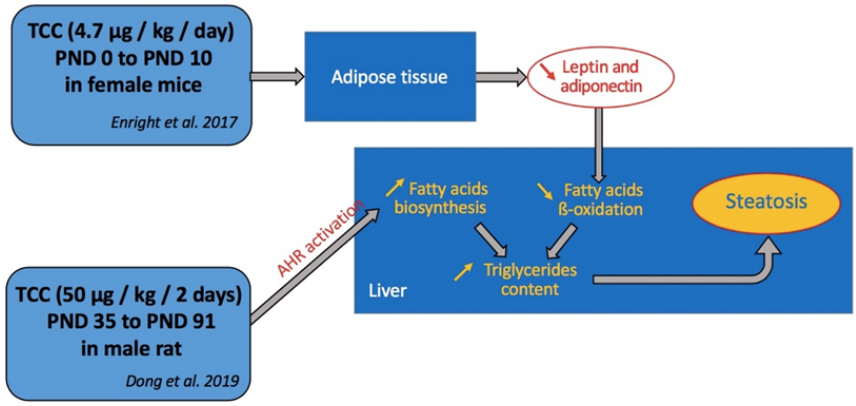


Figure 1. Scheme of the endocrine linkages between hepatic steatosis and triclocarban exposure

Legend to figure: Enright et al (2017) observed that low doses of triclocarban induced a decrease by half in the mRNA levels encoding leptin and adiponectin in the adipose tissue. It is known that decrease in plasma leptin or adiponectin is associated with a decrease in the ß-oxidation of fatty acids. Effectively, Enright et al observed a triclocarban-induced decrease in the expression of key enzymes involved in this metabolic pathway which conducted to a 4-fold increase in hepatic triglycerides content (steatosis). Dong et al observed that low doses of triclocarban activate aryl hydrocarbon receptor (AHR) and its transcriptional targets such as enzymes involved in the biosynthesis of fatty acids. This resulted in an increase in the content of fatty acids in the liver promoting steatosis.

The eMSCA concluded that high doses of triclocarban (> 20 mg/kg bw) would be hepatotoxic triggering inflammation but that non-hepatotoxic doses in the range of the concentrations used in the papers of Li *et al.* (3 and 10 mg/kg bw) and Dong *et al.* (50 µg/kg bw) alters hepatic metabolism and can induce hepatic steatosis *via* ED MoA, possibly through a mechanism involving AhR activation and enzymes involved in the biosynthesis of fatty acids. Worthy of note, hepatic steatosis coupled to inflammation leads to steatohepatitis which in turn can lead to more severe diseases such as fibrosis and cancer. However, more data especially coming from *in vivo* experiments using non hepatotoxic and low doses of triclocarban through longer-term exposure experiments would have to be published to confirm the endocrine disrupting modes of action of triclocarban.

7.10.3. Conclusion on endocrine disrupting properties (combined / separate)

Using environmental species, some studies reported adverse effects of triclocarban on reproduction and development. In particular, in fish, it was shown that triclocarban drastically decreased the number of spawned eggs and increased the number of preovulatory atretic follicles. Interestingly, these alterations were associated with a decrease in *fshr* expression in ovaries. In fish, triclocarban exhibited both estrogenic and androgenic activities through regulation of *vtg* and *ar* gene expression and might impact steroidogenesis through decline in cholesterol levels and inhibition of *star* gene expression. Triclocarban could have also a potential androgenic activity on fish as it enhanced AR-mediated response to a well-know androgen (trenbolone), but a systemic toxicity was associated with this effect. Although studies available on environmental species alert on the reprotoxicity of triclocarban, the eMSCA concludes that they are too scarce and incomplete to claim an ED mode of action of triclocarban.

Using experimental rodent studies, low doses of triclocarban were reported to induce a hepatic steatosis. It is plausible that this effect resulted from an ED mode of action involving changes in the expression levels of leptin and adiponectin and/or AhR activation. *In vitro* studies support also an interaction of triclocarban with AhR, which is known to be involved in metabolic disorders (metabolic syndrome, hepatic metabolism and liver steatosis).

Other *in vitro* studies pointed out that triclocarban activates AR and ER, potentializes both androgenic and estrogenic pathways and impacts steroidogenesis pathways particularly by reducing *Cyp19A1* expression. This could underlie the reprotoxic effects reported by the registrant in males for triclocarban and the possible effects on gestation maintenance. However, no *in vivo* experimental data linking these mechanisms and reproductive alterations are available.

Other triclocarban-induced alterations have been described in *in vitro* models. In thyreocytes, triclocarban could alter the biosynthesis of thyroid hormones by inhibiting the the two limiting steps of TH biosynthesis:iodine uptake and organification. Studies using mammary cell lines showed triclocarban-induced effects, which were potentially mediated through an ED mode of action.

Overall, although *in vivo* data are needed to confirm the ED mode of action of triclocarban, in particular for the hepatic and reproductive effects, the available observations allow making the assumption that this compound can exhibit an ED mode of action in multiple systems.

7.11. PBT and VPVB assessment

Not assessed as part of the Substance Evaluation.

7.12. Exposure assessment

Not assessed as part of the Substance Evaluation.

7.13. Risk characterisation

Not assessed as part of the Substance Evaluation.

7.14. References

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7.15. Abbreviations

ACC: acetyl CoA carboxylase
ACS: acetyl-CoA synthetase
AChE: acetylcholinesterase
ALP: alkaline phosphatase
ar: androgen receptor
AST: aspartate aminotransferase
BPA: bisphenol A
CAT: catalase
CHE: cholinesterase
CS: citrate synthetase
CTP1: carnitine palmitoyltransferase I
Deio: deiodinase
E2: estradiol
Esr: estrogen receptor
FAS: fatty acid synthetase
FFAs: free fatty acids
GLU: glucose
GS: glutathione-S-transferase
GSI: gonadosomatic index
hpf: Hour post fertilisation
HRMS: high-resolution mass spectrometric
HSI: Hepato-Somatic Index
IL: Interleukin
lpl: hepatic lipoprotein lipase
LDH: lactate dehydrogenase
LPO: lipid peroxidation
NIS: sodium/iodide symporter
PDH: pyruvate dehydrogenase
PLS-DA: partial least squares-discriminant analysis
star: steroidogenic acute regulatory protein
T: testosterone
TB: 17β-trenbolone
Tbc: to be confirmed
TCA cycle: tricarboxylic acid cycle
TG: thyroglobulin
TG: triglyceride
TP: total protein
TPO: thyroperoxidase
TR: thyroid receptors
TRH: thyrotropin releasing hormone
TSH: thyroid-stimulating hormone
TSHR: thyroid-stimulating hormone receptor
UPR: unfolded protein response
VIP: variable important in the projection
VTG: vitellogenin