***NOTE:* *Please only include non-confidential information.***

# Analysis of the most appropriate risk management option (RMOA)

**Substance Name: triclocarban**

**EC Number: 202-924-1**

**CAS Number: 101-20-2**

**Authority: FR**

**Date: 12/09/2017**

## Cover Note

In the framework of the French National Strategy on Endocrine Disruptors in 2016, the French Competent Authority requested ANSES to evaluate the toxicological profile of 1-(4-chlorophenyl)-3-(3, 4-dichlorophenyl)urea and verify whether risk management measures should be necessary for this substance

**Comments and additional relevant information are invited on this RMOA by DD Month YYYY.**

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# 1 IDENTITY OF THE SUBSTANCE

## 1.1 Other identifiers of the substance

**Table: Other Substance identifiers**

| | |
|---|---|
| **EC name (public):** | triclocarban |
| **IUPAC name (public):** | 1-(4-chlorophenyl)-3-(3, 4-dichlorophenyl)urea |
| **Index number in Annex VI of the CLP Regulation:** | |
| **Molecular formula:** | $C_{13}H_9Cl_3N_2O$ |
| **Molecular weight or molecular weight range:** | 315.5824 |
| **Synonyms:** | *1-(3',4'-Dichlorophenyl)-3-(4'-chlorophenyl)urea*<br>*1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)urea*<br>*3, 4,4`-Trichlorocarbanilide*<br>*3,4,4'-Trichlorocarbanilide*<br>*3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)urea*<br>*Triclocarban*<br>*Triclocarbon* |

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

**Structural formula:**

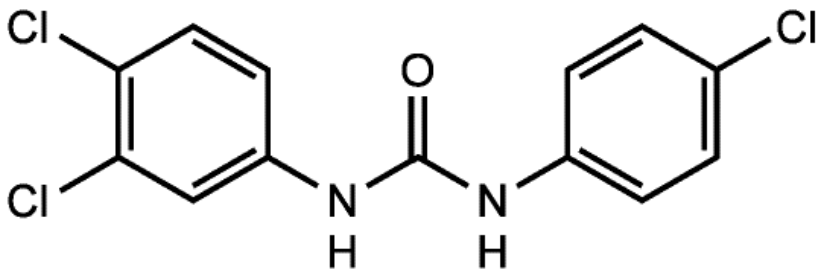
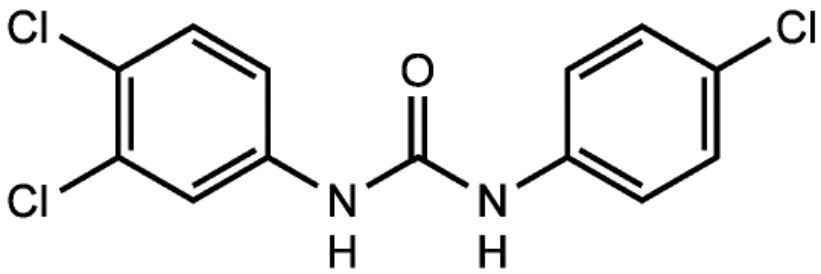


**Table:** Choose the appropriate title from this dropdown men

| | |
|---|---|
| **EC number:** | 202-924-1 |
| **EC name (public):** | triclocarban |
| **CAS number:** | 101-20-2 |
| **CAS name (public):** | |
| **IUPAC name (public):** | 1-(4-chlorophenyl)-3-(3, 4-dichlorophenyl)urea |
| **Index number in Annex VI of the CLP Regulation:** | |
| **Molecular formula:** | C13H9Cl3N2O |
| **Molecular weight or molecular weight range:** | 315.5824 |
| **Synonyms:** | *1-(3',4'-Dichlorophenyl)-3-(4'-chlorophenyl)urea*<br>*1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)urea*<br>*3, 4,4`-Trichlorocarbanilide*<br>*3,4,4'-Trichlorocarbanilide*<br>*3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)urea*<br>*Triclocarban*<br>*Triclocarbon* |

**Structural formula:**




## 1.2 Similar substances/grouping possibilities

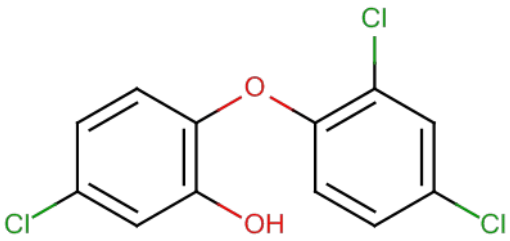
Triclocarban has a dichlorinated phenyl ring connected by an urea bond to a monochlorinated phenyl ring, and shares limited structural similarity to triclosan, i.e. both substances have two chlorinated phenyl rings. But while the phenyl rings in triclocarban are connected by an urea bond, in triclosan 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 haloganted phenol derivative.

Considerning these informations, it can be conclude that the comparison of triclosan with triclosan by read across is not relevant.

**Table: Structurally similar substance (1)**

| | |
|---|---|
| **EC number:** | 222-182-2 |
| **EC name (public):** | Triclosan |
| **CAS number:** | 3380-34-5 |
| **CAS name (public):** | |
| **IUPAC name (public):** | |
| **Index number in Annex VI of the CLP Regulation:** | - |
| **Molecular formula:** | $C_{12}H_7Cl_3O_2$ |
| **Molecular weight or molecular weight range:** | 289.542 g/mol |
| **Synonyms:** | *2,4,4'-trichloro-2'-hydroxy-diphenyl-ether*<br>*5-chloro-2-(2,4-dichlorophenoxy)phenol*<br>*phenol, 5-chloro-2-(2,4-dichlorophenoxy)-* |

**Structural formula:**




## 2 OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

**Table: Completed or ongoing processes**

| | |
|---|---|
| RMOA | ☐ Risk Management Option Analysis (RMOA) other than this RMOA |

| | | |
|---|---|---|
| REACH Processes | Evaluation | ☐ Compliance check, Final decision |
| | | ☐ Testing proposal |
| | | ☐ CoRAP and Substance Evaluation |
| | Authorisation | ☐ Candidate List |
| | | ☐ Annex XIV |
| | Restri-ction | ☐ Annex XVII[1] |
| Harmonised C&L | | ☐ Annex VI (CLP) (see section 3.1) |
| Processes under other EU legislation | | ☐ Plant Protection Products Regulation |
| | | ☒ Biocidal Product Regulation |
| Previous legislation | | ☐ Dangerous substances Directive |
| | | ☐ Existing Substances Regulation |
| (UNEP) Stockholm convention | | ☐ Assessment |
| | | ☐ In relevant Annex |
| Other rocesses/ EU legislation | | ☐ Other (provide further details below) |

1 Please specify the relevant entry.

2 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 (notified under document number C(2008) 5894) (2008/809/EC) - http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32008D0809&rid=12

# 3 HAZARD INFORMATION (INCLUDING CLASSIFICATION)

## 3.1 Classification

### 3.1.1 Harmonised Classification in Annex VI of the CLP

There is no harmonized classification.

### 3.1.2 Self classification

Classification & Labelling notified by industry to ECHA:

| Hazard Class and Category Code(s) | Hazard Statement Code(s) | Number of Notifiers |
|---|---|---|
| Aquatic Acute 1 | H400 | 88 |
| Aquatic Chronic 1 | H410 | |
| Aquatic Acute 1 | H400 | 50 |
| Aquatic Chronic 1 | H410 | |
| Skin Irrit. 2 | H315 | 8 |
| Eye Irrit. 2 | H319 | |
| Aquatic Acute 1 | H400 | |
| Aquatic Chronic 1 | H410 | |
| Aquatic Chronic 1 | H410 | 6 |
| Aquatic Acute 1 | H400 | 1 |
| Aquatic Chronic 1 | H410 | |

### 3.1.3 Proposal for Harmonised Classification in Annex VI of the CLP

### 3.1.4 CLP Notification Status

**Table: CLP Notifications**

| | CLP Notifications[3] |
|---|---|
| Number of aggregated notifications | 6 |
| Total number of notifiers | 126 |

## 3.2 Additional hazard information

*Human and environmental hazards properties presented are based on available data from the chemical safety report (CSR, 10-03-2016) of triclocarban and additional literature.*

**Human health**

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

Triclocarban (TCC) was moderately absorbed by the oral route and, depending on the vehicle and exposure conditions, poorly to moderately absorbed by dermal application (Hiles, 1978) depending on the vehicle used that greatly influences absorption of TCC.
Biotransformation appears to be rapid but did not appear to involve splitting of the basis structure. In fact, in none of the species examined, the C-N bond in TCC was cleaved as the result of metabolism.
In all species investigated, TCC was widely metabolized to compounds that were more water soluble. Thus, metabolites are more readily excreted than parental compounds. Concerning metabolites, hydroxylation followed by conjugation were observed. Principal metabolites common to all species were the sulphate and glucuronid conjugate of 2', 3' and 6-Hydroxy-triclocarban (Hiles, 1978).
**Based on the metabolisation and on the elimination of TCC, the bio-accumulation of triclocarban is likely to be low**.

- Oral acute toxicity

-Non human information

TCC in distilled water was administrated orally by gavage to wistar albino female rats at a single dose of 2000 mg/kg bw. (n=3 animals per doses). Wistar albino rats treated with the test compound TCC did not induce any mortality throughout the period of observation of 14 days. TCC did not induce any clinical signs of toxicity at the tested dose level of 2000 mg/kg bw throughout the period of observation. All the animals treated with TCC at the dose level of 2000 mg/kg bw showed normal gain in body weight as compared to control group.
**Thus, these results indicate that the test compound TCC is nontoxic to Wistar albino rats acutely exposed to the dose level of 2000 mg/kg body weight (Substainability Support Services, 2013a) available in CSR (chemical safety report) dated from 2016-03-10.**

3 C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database

-Human information
No relevant information available.

- Dermal acute toxicity

-Non human information

TCC in distilled water was evaluated for acute dermal toxicity in 5 males and 5 females wistar rats following OECD guideline 402 (Substainability Support Services, 2013b). Duration of exposure was 14 days and the dose of TCC was 2000 mg/kg bw. The test substance was applied uniformly over an exposed area of skin. The animals were then housed individually in cages with a collar around the neck in order to avoid the ingestion of the test compound. Results show that the test compound TCC did not induce any mortality throughout the observation period of 14 days. Furthermore, the test compound TCC did not elicit any clinical signs of intoxication throughout the period of observation. All the animals treated with TCC at the dose level of 2000 mg/kg bw show normal gain in body weight as observed on day $7^{th}$ and $14^{th}$ compared to day 0.
**These results indicate that the test compound TCC is nontoxic to Wistar albino rats at the tested dose level of 2000 mg/kg body weight by dermal route (CSR, 2016-03-10).**

- Inhalation acute toxicity

TCC has very low vapor pressure and high melting point, so the potential for the generation of inhabable vapors of TCC is low. Also, the normal conditions of use of this substances indicate in the CSR will not result in aerosols, particles or droplets of an inhalable size. Thus, there is no study evaluating the acute inhalation toxicity (CSR, 2016-03-10).

- Other routes

Intraperitoneal route was evaluated for acute toxicity of TCC. Information about the study was found on ECHA website by the $31^{st}$ march 2017 with no further detail. The TCC was administred to mice. No more informations are available about species, number, vehicle, time of observation. The results show that the intraperitoneal median lethal dose (LD50) of TCC in mouse was found to be 2100 mg/kg of body weight. This value indicates that TCC shall not be considered acutely toxic by the intraperitoneal route.

- Eyes Irritation and corrositivity.

The data reported here are those found on the ECHA website by the $31^{st}$ of March 2017 with no further detail. The study was conducted in compliance with GLP and according to OECD guideline 405. A single dose of 0.1 ml equaling approximately 42 mg TCC was placed in the conjunctival sac of one eye of each of three New Zealand white albino rabbits After 24h, treated eyes were rinsed with saline solution and evaluated at 1, 24, 48 and 72h and 7, 14 and 21 days for irritation according to the Draize eye irritation grading scale. No effects in cornea, iris, conjunctivae or aqueous humour were observed in any rabbit at any time. Under the conditions of the test, **TCC was therefore considered to be not irritating to the rabbit eye.**

- Skin Irritation and corrositivity.

-Non human information

The data reported here are those found on the ECHA website by the $31^{st}$ of March 2017 with no further detail. The study, which followed guildeline OECD 404,

evaluated the irritating potential of TCC on shaven back skin of rabbit. There is no information on the number of rabbits included in study. TCC was applied pure without any vehicle. The result obtained from the present study concludes that TCC is not irritating on intact skin of New Zealand white rabbit under the test condition. The skin irritation index of test compound TCC was calculated as 0.0.

Another study is available in rabbit which investigated irritation potential of TCC (Fujito, 1974). There is no information on the number of animals used. Vehicle used was acetone. TCC applied on white male albino rabbits for 24 and 48 h by open patch test was found not to be a skin irritant on 0.5, 1.0, and 3.0 percent of solutions. **Therefore, it can be concluded that TCC is not irritating to the skin of rabbits.**

The data reported here are those found on ECHA website by the 31st of March 2017 with no further detail. Finely, ground powder as a 25% suspension in corn oil was applied to the clipped intact skin of albino rabbits and removed after 24 hours. The application was covered with plastic strips to retard evaporation and avoid contamination. Observations were made over a period of several days for irritation. The data were scored according to Draize, Woodard and Calvary (1944). TCC was classified as non-irritating in rabbits when applied as a finely ground powder as a 25% suspension in corn oil.

TCC applied on guinea pig for 24 and 48 h by open patch test was realized in another study (Fujito, 1974). There is no information on the number of animals used. Vehicle used was acetone. TCC was found not to be a skin irritant in 0,5, 1,0 and 3,0 percent of solutions. **Therefore, it can be concluded that TCC is not irritating to the skin of guinea pigs.**

-Human information

TCC applied on white male humans 21 days by patch test found to be not skin irritant on 1%, 3%, 6 % and 9% in solutions. Vehicle was white petrolatum. One of the 10 subjects showed the positive response at 3% TCC. This subject did not react to higher (6% and 9%) TCC concentration. Severity of the observed reaction did not exceed a grade of 1. The significance of the effect noted at 3% TCC is questionable as it was not dose related. Based on these results, 9% TCC is considered to be of minimal irritancy potential in a 21 days cumulative irritation study. **Therefore, it can be concluded that TCC is not irritating to the skin of human (Howard, 1978).**

- Sensitisation

-Non human information

No relevant information available.

-Human information

TCC was applied on 200 humans aged between 21 and 50 years old using the Draize procedure (Marzulli and Maibach, 1973), ie an occlusive patch removed after 48-72 h was applied on the upper lateral portion of the arm. Concentration of TCC was 1,5 % and vehicle used was aqueous soap suspension. Results show that there is no sensitization in any of the 200 subjects. **Thus, TCC is considered as not sensitizing to the skin of human**.

Another study investigated the sensitizing potential of TCC using the Draize procedure (Maybach *et al*, 1978). A single concentration (9%) of TCC was applied to the upper lateral portion of the arm of each subject and covered with an

occlusive patch. Test material was applied to the same skin site three times weekly and remained in place either 48 h (during the week) or 72 h (weekends). The distribution of this group was 68% Caucasian, 18% African, 13% Mexican, and 1% American Indian and there are 185 subjects included in the study. There was not positive reaction in any of the subjects.
**Therefore, it can be concluded that TCC is not sensitization to the skin of human**.

Sensitizing potential of TCC was evaluated in 200 humans according to Draize procedure. Based on the results, it can be concluded that TCC is **not sensitizing to the human skin**.

- Repeated dose toxicity

-Non human information

Female and male Sprague-Dawley rats were treated orally with 500 mg/kg and 1000 mg/kg of TCC 5 days per week during 30 days (Monsanto report, 2011). Each dose and control group contained 10 rats per sex and the subchronic oral gavage was performed with 25% aqueous solution of TCC. 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. **No macroscopic adverse effect in male and female rats over an exposure period of 30 days was found for 1000 mg/Kg body weight of TCC.**

TCC was administered to three groups of 35 Sprague-Dawley rats in their diet at concentrations equivalent to 25, 75 and 250 mg/kg bw/d for 8 weeks(Monsanto report, 1985 ; SCCS, 2005). No control group was included in the study. 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 TCC. No necropsy was performed at the end of the study. There were no signs of toxicity or treatment related mortalities throughout the study. Mean bodyweight and food consumption were lower in the highest dose group. No compound-related pathological or histopathological findings were noted.

A two year study is described in the carcinogenicity part page 15 of the present document with 80 female and male Sprague-Dawley rats treated orally with 0, 25, 75 and 250 mg/kg bw/d **Statistically significant changes were seen in certain organ weights compared to controls with no microspopic finding nor increase in tumour incidence at any site. See below for further details.**

- Mutagenicity

*- In vitro*

TCC was assayed in *Salmonella typhimurium* for genetic toxicity (Zeiger *et al*, 1987). Four strains of *Salmonella typhimurium* were used in this study : TA 100, TA 1535, TA 1537, TA 98. In the Ames test, TCC did **not exhibit genotoxicity** in *S. typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 with and without S9 metabolic activation.

In two other *in vitro* studies using an Ames test, TCC was tested in *S. typhimurium* strains TA 98 and TA 100. TCC did **not exhibit genotoxicity** in *S. typhimurium* strains TA 98 and TA 100 with and without S9 metabolic activation.

An *in vitro* mammalian chromosome aberration test was performed using Chinese hamster ovary with different doses of TCC (31,3, 62,5, 125, 250, 500, 1000, 1500, 2000 µg/ml). Positive control substances were mitomycine C and cyclophosphamide. The genetic toxicity in Chinese Hamster Ovary (CHO) with TCC was found to be **negative**.

No evidence of genotoxicity was found in Ames test using *S. typhimurium* strains TA 100, TA 1535, TA 1537 and TA 98 with or without metabolic activation for TCC (Zeiger *et al*, 1987; 2012c). No evidence of genotoxicity was found in *in vitro* mammalian chromosome aberration test was performed using Chinese hamster ovary.

- *In vivo*

No studies are available for in vivo genotoxicity of TCC.

- Carcinogenicity

- *In vitro*

Carcinogenesis potential *in vitro* of TCC was assayed using MCF10A, a normal breast epithelial cell line or MCF-7, a breast cancer cell line with overexpression of estrogen receptor (Sood *et al*, 2013).

To investigate the ability of TCC to induce breast cell carcinogenesis, MCF10A cells were exposed repeatidly to TCC at various concentrations for 10 and 20 cycles. Results show that 200 nM concentration of TCC induces Erk-Nox pathway activation, Nox-dependant ROS elevation and Nox-independent ROS elevation. **TCC was also able to induce increased cell proliferation and DNA damage.** All this parameters play essential roles in initiation of carcinogenesis. MCF10A cells acquired also higher degrees of the constitutive endpoints of ability to an independence from cell adhesion to matrix (anchorage independent growth). Same experiences as describe above were perfomed in MCF7 cells. Results show similar effects on MCF7 cells than on MCF10A cells. It can also be concluded that TCC-induced transient endpoints (Erk-NOx pathway activation, ROS elevation, increased cell proliferation, DNA damage) were not specific to MCF10A cells or ER status.

To assess carcinogenic potency of TCC exposed breast cells, MCF10A was compared to the tumorigenic P-20 cell line, which resulted from cumulative exposure of MCF10A cells to 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) for 20 cycles, and a highly-tumorigenic, oncogenic Ras-expressing MCF10A-Ras cell line as controls. Results show that the constitutive endpoints of reduced dependence on growth factors, anchorage-independent growth, increased cell proliferation, ROS elevation, and Mek–Erk–Nox pathway activation were increasingly induced in 200nM TCC 10 and 20 cycles MCF10A cells in an exposure-dependent manner. However, none of these constitutive endpoints was induced to a comparable level to those acquired by tumorigenic P-20 and MCF10A-Ras cells.

**In conclusion, in this study, the model system revealed, that cumulative exposures of human breast cells to TCC at physiologically-achievable, nanomolar concentrations induced processes linked to carcinogenesis from a non-cancerous stage to premalignant stages in a dose- and**

**exposure-dependent manner, suggesting the ability of TCC, as a co-carcinogen, to induce breast cell pre-malignancy.**

- *In vivo*

The data reported here are those found on the ECHA website by the 31st of March 2017 with no further detail. In a two year chronic feeding study, 20 Sprague-Dawleys rats (20 animals / dose / sex) were exposed to TCC in diet for 2 years at a dosage of 0, 25, 75 or 250 mg/kg bw.day. Clinical signs (urine analysis, Haematology), body weight, water consumption, and food consumption were monitored throughout the study, while ophthalmoscopic examination and clinical chemistry examinations were conducted at regular intervals. At study termination, all animals were subject to complete necropsy and pathological examination. No differences were observed between any of the groups with regard to ophthalmic observations or food consumption. There were no treatment-related clinical signs or mortality throughout the study. No differences were observed between any of the groups with regard to food consumption, ophthalmic observations or urine analysis. Mean body weight of males at 250 mg/kg bw/d and females at 75 and 250 mg/kg bw/d were slightly reduced compared to controls during most of the study. Anemia was seen in males at 75 and 250 mg/kg bw/day and females at 250 mg/kg bw/day, and blood chemistry analysis showed a slight increase in alkaline phosphatase, blood urea nitrogen, glucose and total bilirubin at various time points for the high dose males. Statistically significant weight changes were seen and included increased liver weights in both sexes at 75 and 250 mg/kg bw/day, increased spleen weights at 75 (males) and 250 mg/kg/day (males and females), and increased testes and heart weights in males at 250 mg/kg bw/day. No microscopic changes were noted in any of the organs to account for the increased organ weights, therefore the changes may not have been biologically significant.

**In conclusion, no evidence of dose-related increase in tumor incidence was observed at any site.** Based on these results, the NOAEL and LOAEL for the study were considered to be 25 and 75 mg/kg bw/day when male and female Sprague-Dawley rats were exposed to TCC.

- Toxicity for reproduction

The data reported here are those found on ECHA website by the 31st of March 2017 with no further detail. A three generation study was performed to assay the effects of Triclocarban on fertility in rat by oral exposure at 250, 500, 1000, 3000 ppm. No treatment-related effect was found on mortality or physical in-life evaluations. Body weight and food consumption were not adversely affected by treatment throughout the study. Mating indices and male fertility were not adversely affected by treatment for all generations. In conclusion, signs of systemic toxicity, such as a changes in absolute and relative organ levels were observed in the parental generations at dose levels above 500 ppm equalling a daily exposure of about 50 mg/kg bw.The NOAEL for reproductive and developmental toxicity were determined to be 3000 ppm (280 mg/kg/d) for the parental generation, 1000 ppm (95 mg/kg/d) for the F1 generation and 3000 ppm (300 mg/kg/d) for the F2 generation.

In a reproductive toxicity study, the effect of TCC was evaluated in male Sprague-Dawley rats for 10 days (Duleba *et al*, 2011). The seven-week-old male Sprague-Dawley rats received either a normal diet or a diet supplemented with TCC (0.25% in diet). No mortalities were observed in the males. Animals treated with **TCC showed significantly increased body weight compared to control animals. Liver, seminal vesicles, ventral prostate, LABC (levator anti-bulbocavernosus muscle), and glans penis are significantly larger in the**

**TCC group compared to control group in absolute and relative weight. There were no visible abnormalities of any of the accessory sex glands, penis or testes, in treated animals and no histologically distinguishable difference between specimens from the control and treated animals.** Therefore, LOEL was considered to be 0.25% per day i.e. 250 mg/kg /day when male Sprague-Dawley rats were exposed to TCC by feed for 10 days.

The study of Kennedy *et al* (2015) aims has to discriminate the effects of one exposure during gestation and/or the lactation period in the objective to identify a period of critical exposure and to identify neuroendocrine effects on the development of pups, with a particular focus on the milk secretion.
Gestant females (SD rat) were exposed to a diet enriched or not in TCC in 0.2 % or 0.5 % (w/w). The exposure of mothers began 5 days after the conception (GD5) and continued until the weaning (PND21). Offsprings were observed daily till the end by the puberty (PND54).

This *in vivo* study explored the effects of TCC exposure (incorporated into the diet) on mothers and their progeny.

**Such experimental conditions of exposures induced no sign of severe toxicity on the mother except at the highest dose (0.5 %) where the weight gain was lower and coupled with a thyroid disruption during the gestation (decrease of T3 from 0.63 ng/mL ±0.05 (control) *vs* 0.44 ng/mL ±0.03 (highest dose of 0.5 %) which highlight an anti-thyroid potential and thus a possible PE effects.**

This study also highlights a postnatal toxicity of the TCC at the strongest dose (0.5 %), with a survival of neonates less than a week. There is no effect on the fertilization, the embryonic setting-up the ano-genital distance, the vaginal opening, and no deformations or no embryonic malformations. On the other hand the presence of stomach ulcers, leading to the death of pups is associated with the rates of TCC in plasma and in the maternal milk.

- **Endocrine disruptors characteristics**

**Assays evaluating effects of TCC on thyroid synthesis**

*In vitro* biological activities of TCC were assayed in cellular lineage of thyroid of rat FRTL-5 and microsome of thyroid of healthy rats (Wu *et al* (2016). The author estimates the effect of the TCC, (and other molecules) on various functional markers of the biosynthesis of the thyroidian hormones (TH): The active transport of the iodine within thyreocytes and activity thyroperoxydase leads to the organification of iodine and the iodification of residues tyrosine of the thyroglobuline and produce the precursors of the HT, MIT (Monodiiodotyrosine) and DIT (Diiodotyrosine).
In parallel, the author assessed the expression of the genes coding for key proteins of the TH biosynthesis: SLC5A coding for the carrier of the sodium/iodine symporter (NIS), the genes coding for the thyroperoxydase and the thyroglobuline (protein rich in residues tyrosine 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.

**This study shows an effect of TCC on the activity of the NIS on a thyroid cellular lineage with a good level of evidence and suggests that TCC could alter the biosynthesis of the TH by modulating the contribution in iodine in thyreocytes in certain conditions.**

**Assays evaluating the effects of TCC on Aryl hydrocarbon receptor**

*In vitro* biological activities of TCC were assessed in cell-based and nuclear receptor responsive bioassays. Activity of TCC to receptor for aryl hydrocarbon (AhR), estrogen (ER) and androgen (AR) was assessed by measuring luciferase activity induced by test compounds compared with solvent control (DMSO) or TCDD as positive control. Results show that TCC does not exhibit induction of AhR dependent luciferase reporter gene expression (Ahn *et al*, 2008).
Activity of the recombinant ER responsive cells (BG1-ERE cells) was evaluated by measuring luciferase activity induced by estradiol and compared results from TCC with solvent controls or positive controls. Coincubation of E2 and TCC resulted in enhanced estradiol dependent induction of luciferase gene expression, with significant increases observed at 1–10 nM estradiols.

The results show that **TCC enhanced testosterone dependent induction of luciferase gene expression in T47D-ARE cells, but only at the highest concentration (10 µM) of testosterone**.

**Assays evaluating the effects of TCC on androgen receptor**

*In vitro* androgenic activity of TCC was assayed in MDA-kb2 cell line (Christen *et al*, 2010). To assess the androgenic activity of TCC, MDA-kb2 cells were treated with different concentration of TCC for 24 h, followed by the luciferase assay. Results show that TCC has no androgenic activity at all concentrations tested in the study (0,05-5 µM).

Co-treatment of MDA-kb2 cells with Dihydrotestosterone (DHT) and TCC were **performed with luciferase assay.** The results show that DHT response was potentiated up to 130% with 0.01–5 µM of TCC. At 1 nM TCC, no enhancement of the DHT response was detectable.
MDA-kb2 cells were co-treated with 0.5 nM DHT, 10 µM flutamide and different concentrations of TCC to exclude the possibility that the contribution of glucocorticoïde hormone activity on increasing the activity of DHT. The results show that induction of the DHT response was completely inhibited by flutamide demonstrating that **TCC activate luciferase expression through the androgen receptor, and not through the glucocorticoid receptor. TCC did not compete with the AR binding but amplified the AR-mediated activity. TCC has no agonistic activity alone but enhance the DHT-induced activity.**

*In vitro* TCC effects were evaluated in a cell-based human androgen receptor (AR)-mediated bioassay system (Duleba *et al*, 2011).
Testosterone and DHT treatments induced luciferase activity in LNCaP cells transfected with probasin or simple ARE promoters. Cotreatment of androgen with TCC (1.0 nmol/L) further increased luciferase activity in LNCaP cells compared to androgen treatment alone ($P < 0,01$).

Similarly, in C4–2B cells, TCC further potentiated androgen-induced luciferase activity compared to androgen treatment alone, although the amplification was less substantial than that observed in LNCaP cells, which have higher expression of AR ($P < 0,05$).

**In both cell lines, the amplification enhanced by TCC was significantly suppressed by the strong AR binding inhibitor, bicalutamide. The enhancement of signal by TCC was suppressed by biclutamide, indicating the potentiation effect of TCC is AR-dependant.**

In a study of Yueh *et al* in 2012, ability of TCC to activate xenobiotics sensor was performed using luciferase basd reporter assays in a CV-1 cells. Of the 11 xenobiotic nuclear receptors (XenoRs) screened with TCC at the concentration of

10 mM, ERα and CAR were activated by TCC. CAR was moderately activated by TCC with 1.75-fold induction of luciferase activity. **TCC also promotes ERα activity with similar potency as estradiol.**
Then, to identify the potential role of ERα in gene induction for CYP1B1 and CYP2B6 by exposure to TCC, transient transfections with a luciferase vector containing the 2kb fragment upstream from the CYP2B6 start site (pGL3–2B6) with or without cotransfection of ERα were performed. The reporter activity of pGL3–2B6 increased with TCC treatment in a dose-dependent manner to a level compatible to that of estradiol treatment in the presence of ERα. Similar to the CYP2B6 response to TCC, CYP1B1 promoter activity was significantly induced by TCC in a dose-dependent fashion.

In vivo studies with humanized UGT1 mice (hUCG1*28) were alrealdy performed with 10 day old female mice treated intraperitoneally every 24 hours for 3 weeks with Triclocarban (20 mg/kg) or corn oil to assess expression of hepatic UGT1A genes. Following the treatment, ovary tissues were used to prepare total RNA. Quantification of CYP1B1 was performed with real time RT-PCR. **Triclocarban treatment to female hUGT1*28 mice resulted in the induction of CYP1B1.**

Finally, transfection experiments with siRNAs targeting ERα were performed to interrupt ERα gene expression in MCF7 cells. Gene expression levels for CYP1B1, CYP2B6, and ERα were analyzed by real time PCR following siRNA transfections and Triclocarban treatment. SiRNA knockdown of ERα with 58% effectiveness significantly reduced CYP1B1 and CYP2B6 gene expression in MCF7 cells, further assuring the involvement of ERα in Triclocarban-mediated gene induction.

A CAR ligand binding assay was performed with the expression vector containing the Gal4 DNA binding domain fused with the ligand binding domain of human or murine CAR transfected in CV-1 cells. Results show that **Triclocarban (10 µM) is a CAR activator but not an agonist ligand for either mouse or human CAR.**
Triclocarban induction of Xenobiotic metabolism by CAR was assessed by treating hUGT1*28 mice with 16 mg/kg of Triclocarban by the intraperitoneal route and evaluation UGT1A gene expression patterns in liver after 48 hours. In comparaison to DMSO treated mice, UGT1A1, UGT1A3, UGT1A4, UGT 1A6, UGT1A9 gene products were each induced in hUGT1*28 mice following Triclocarban administration. Real time RT-PCR analysis shows that only CYP2B10 was induced, indicating that CAR activation by Triclocarban was leading to the induction of the UGT1A genes. To examine if CAR was underlying the induction pattern, hUGT1*28/ Car-/- mice were treated with Triclocarban and expression of the UGT1A genes was examined. In comparison to hUGT1*28 mice, there was no induction of the UGT1A genes in hUGT1*28/Car-/- mice. This correlated with a nearly complete absence of Cyp2B10 gene induction following Triclocarban treatment in hUGT1*28/Car-/- mice. To access the expression of Cyp2B10 protein in microsomal preparation from treated mouse livers, Western blot analysis was performed with antibody against the CYP2B6 protein. Consistent with the transcript levels, Cyp2B10 protein expression was induced by Triclocarban treatment, and the induction was blocked in Car-/- livers.

Another *in vitro* activity of TCC was assayed in a human breast carcinoma MDA-kb2 cell line utilized for reporter gene assays, followed by luciferase assay (Kolsek *et al*, 2015).
Co-treatment of cells with TCC and Hydrocortisone (HC) revealed the same effect, since TCC increased the Hydrocortisone (HC) induced transcriptional activity to a similar degree as for the Dihydrotestosterone (DHT) induced activity.

**In conclusion, the potential Luciferase-based gene reporter assay, used in screening test of potential endocrine disruptor chemicals, show that**

**TCC can enhance testosterone dependent induction of luciferase gene expression and have weak estrogenic activities in various cell lines.**
**TCC can induce significant up regulation of pS2[4] and down regulation of ERα meaning that Triclocarban have estrogenic properties. TCC has the capacity of interfering with the receptor AhR, and to modify interelations AhR-RE in the regulation of the genic expression. TCC may also modulate the expression of the enzymes of xenobiotic and steroid metabolism via activation of the CAR receptor, with induction of CYP2B10 and UGT1A, and / or activation of AhR with induction of CYP1A1 and CYPIB1.**

- Neurotoxicity

No relevant information avalaible.

- Immunotoxicity

No relevant information available.

**In conclusion, TCC could alter the biosynthesis of the thyroidian hormones by modulating the contribution in iodine in thyreocytes in certain conditions. TCC has the capacity of interfering with the receptor AhR and can enhance testosterone dependent induction of luciferase gene expression and have weak estrogenic activities. These results suggests that TCC has an endocrine disruptor character with an important level of evidence because there is solid information on the ED effects such as Androgen or thyroid or steroidogenesis *in vitro* and some results on carcinogenicity on mammary gland *in vitro* potentially mediated by an ED mode of action. Nevertheless, due to the lack of information and in the absence of known adverse effects, it is not possible to conclude on the properties PE of this substance.**

## 3.2.1 Environment

Environmental hazards properties presented are based on available data from the chemical safety report (CSR) of triclocarban.

### 3.2.1.1 E-fate and behaviour

Triclocarban is a solid with a low water solubility (<0.01 mg/L). The estimated half-life of the substance indicates that the substance is moderately hydrolysable.
Data on ready biodegradability shows that the substance is not readily biodegradable. Moreover, triclocarban could be considered fulfilling the P/vP criteria based on predicted and experimental degradation half-life in water, sediment or soil compartment.
Triclocarban has strong adsorption properties in soil and low migration potential to the ground water due the high Koc value comprised between 4057 to 64037.

Several data on bioaccumulation potential are presented in the CSR. The highest value of BCF, issued from prediction, is 800, far below the Annex XIII criteria of 2000. Based on this data, triclocarban is not expected to bioaccumulate in the food chain. Nevertheless, these data are not assessed in detailed (see section 3.2.1.4).

4 The pS2 gene is an estrogen-responsive gene.

### 3.2.1.2 Ecotoxicity

According to the data provided by the registrant in the CSR, triclocarban is considered as toxic for aquatic organisms. The LC50 value of the substance in fish (*Oryzias latipes*) in a 96h study on the basis of mortality effect was found to be 0.085 mg/L and the lowest NOEC value was reported at 0.005 mg/L for *Pimephales promelas*.

Regarding aquatic invertebrates, the available data based on publication showed LC50 ranged between 0.01 mg/l to 0.032 mg/L and NOEC between 0.019 mg/L to 0.029 mg/L.

Toxicity results on algae species confirm the toxicity of triclocarban to aquatic organism with EC50 of 0.017 mg/L and NOEC below 0.01 mg/L.

Based on these information, triclocarban fulfils the T criteria.

### 3.2.1.3 Additionnal data on ED-properties of the substance

No data on the ED-properties and their potential related-adverse effects on the environment has been provided by the registrants. The paragraphs below summarizes some available data on ED-properties of triclocarban on aquatic species found by FR-CA in the opened literature. 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[5]).

The five 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; RI=3)[6] explore the utility of a mixture test design with 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* the water to substances with diverse mechanisms of action, including triclocarban (TCC), 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 TCC associated with or without TB were (expressed in nominal concentration):

- Control;
- 500 $\mu g.L^{-1}$ of TB;
- 5 $\mu g.L^{-1}$ of TCC;
- 10 $\mu g.L^{-1}$ of TCC;
- 500 $\mu g.L^{-1}$ of TB with 5 $\mu g.L^{-1}$ of TCC;
- 500 $\mu g.L^{-1}$ of TB with 10 $\mu g.L^{-1}$ of TCC.

5 OECD (2012) – Guidance Document on standardized test guidelines for evaluating chemicals for endocrine disruption. Series on Testing and Assessment No. 150. ENV/JM/MONO(2012)22. 24/08/2012. 524 p.

6 Ankley et al. (2010) - Use of chemical mixtures to differentiate mechanisms of endocrine action in a small fish model. Aquatic Toxicology 99 (2010) 389–396.

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

- VTG concentration in plasma in ♂ and ♀.
- 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 TCC, either alone or in combination with TB, did not induce VTG in ♂. Exposure to TB depressed VTG in ♀; treatment with TCC alone or in conjunction with the androgen did not affect VTG status. Exposure to TCC, alone or with TB, did not affect tubercle status in ♂. Exposure to TCC alone did not affect tubercle status in ♀; nevertheless the highest concentration of TCC (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 TCC 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 $\mu g.L^{-1}$ of TCC exhibited signs of overt toxicity, particularly in ♂. Three fishes (two ♂) died in the treatment TCC (10 $\mu g.L^{-1}$) and the treatment TB (500 $\mu g.L^{-1}$) + TCC (10 $\mu g.L^{-1}$), and several more ♂ ceased feeding and were relatively inactive. Therefore the toxicity observed in fish exposed to 10 $\mu g.L^{-1}$ implies that toxic effect could explained the observed and/or not observed effects of TCC, *e.g.* absence of induction of tubercles in ♀ exposed to TCC alone.

**In conclusion, this study indicated a potential androgenic activity of TCC on fish based on equivocal results due to potential other toxic effects at the highest tested concentration of TCC with and without TB.**

Chung *et al.* (2011; RI=4)[7] assessed the effects of Bisphenol A (BPA) and Triclocarban (TCC) on brain specific gene expression of aromatase AroB in early zebrafish embryos. This gene contains estrogen response elements and is estrogen responsive. TCC stimulated AroB expression only slightly when tested alone, strongly enhanced the overexpression of AroB induced by an exogenous estrogen (17β estradiol) but suppressed the expression induced by BPA. 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; RI=3)[8] assessed the effect of TCC on ♂ and ♀ adults fathead minnow fish (*Pimephales promelas)* in controlled conditions. Fish were exposed to 0.8 $\mu g.L^{-1}$ of TCC (measured concentration). After 48h of exposure, the following endpoints were measured:

- VTG gene expression in liver;
- Several genes expression in testes, known for being related to 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;

7 Chung *et al.* (2011) - Effects of bisphenol A and triclocarban on brain-specific expression of aromatase in early zebrafish embryos. PNAS (2011), vol. 108, no. 43, p. 17732–17737.

8 Zenobio *et al.* (2014) – Effects of triclocarban, N,N-diethyl-meta-toluamide, and a mixture of pharmaceuticals and personal care products on fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry, Vol. 33, No. 4, pp. 910–919.

- 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 TCC, relative expression of *vtg* in livers was significantly increased in ♀ (around 2-fold change) and ♂ (around 2.5-fold change), indicating a potential estrogenic activity of TCC. In addition, TCC downregulated *ar* and *star* in testes and upregulated *lpl* in livers. Considering the non significant difference with control of GSI, HIS, 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 TCC 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 indicated a potential ED-effect of TCC through estrogenic, androgenic and steroidogenesis activity at a concentration of 0.8 µg.$L^{-1}$.**

Schultz *et al.* (2012; RI=2)[9] assessed the effect of TCC on fish in controlled conditions with the hypothesis of an estrogenic activity. ♂ and ♀ adults of fathead minnow (*Pimephales promelas)* were exposed to 560 and 1600 ng.$L^{-1}$ of TCC. 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 TCC treatment with controls, nor positive control was observed for mortality, HIS, GSI, histopathological changes in liver and gonads or secondary sexual characteristics. These results confirmed that the tested concentrations are low doses, that induce no toxic effects. VTG concentration in plasma were significantly increased only in E2-positive control in male fish compared with the negative controls. No significant difference of TCC treatment with solvent control. This result could be related 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 TCC. Considering the short-term exposure (48h) in Zenobio *et al.* (2014) and the genomic level of the endpoint, the transitional effect of TCC 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

9 Schultz *et al.* (2012) - Effects of Triclosan and Triclocarban, Two Ubiquitous Environmental Contaminants, on Anatomy, Physiology, and Behavior of the Fathead Minnow (*Pimephales promelas*). Arch Environ Contam Toxicol (2012) 63:114–124.

to attack and number of attacks) was significantly decreased for the TCC-treatment at 1600 ng.$L^{-1}$, compared to control.

**In conclusion, data on ED-effect on fish indicated a potential ED-effect of TCC on fish adult when exposed at concentration < 10 µg.$L^{-1}$. Different modes of action can be hypothesized: estrogenic, androgenic and steroidogenesis activity. It has to be noted that considering the reliability of these data, and because no ED-related adverse effects was demonstrated, more data are needed to confirm the ED-properties of TCC on fish.**

In addition, effect of TCC have also been investigated in other aquatic specie. Giudice *et al.* (2010; RI=3)[10] assessed the effect of TCC 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 TCC 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 µg.$L^{-1}$ (measured concentration). A significant positive dose response relationship between TCC concentrations and the increase of number of unshelled, shelled and total embryos revealed an effect on reproduction (NOEC = 0.47 µg.$L^{-1}$; $EC_{50}$ = 2.5 µg.$L^{-1}$). The greatest increase was showed for the unshelled embryos.

According to the authors, this significant increase of number of embryos has been previously found in experiments with exogenous estrogenic EDCs (BPA, octylphenol, nonylphenol, ethynylestradiol), and hence could be an ED-related adverse effect. The hypothesis is that TCC could amplify the binding affinity and consequently increase the transcriptional activity of naturally present estrogens to estrogen receptor. No data is currently available to confirm this mode of action. 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, this study demonstrated the chronic effect on reproduction (increased number of embryos) of New Zealand mudsail of TCC with a NOEC = 0.47 µg.$L^{-1}$. Nevertheless, no data allowed to consider this adverse effect related to an estrogenic activity of TCC.**

10 Giudice *et al.* (2010) – The antimicrobial triclocarban stimulates embryo production in the freshwater mudsnail *potamopyrgus antipodarum*. Environmental Toxicology and Chemistry, vol. 29, No.4, pp. 966-970.

### 3.2.1.4 PBT-properties of the substance

Not assessed in this document. According to ECHA, PBT properties have been reviewed by NL in a justification document where it was requested to perform a CCh on that point.

# 4 INFORMATION ON (AGGREGATED) TONNAGE AND USES[11]

## 4.1 Tonnage and registration status

**Table: Tonnage and registration status**

| **From ECHA dissemination site** | |
|---|---|
| Registrations | ☒ Full registration(s) (Art. 10)<br>☐ Intermediate registration(s) (Art. 17 and/or 18) |
| Total tonnage band for substance (excluding volume registered under Art 17 or Art 18, or directly exported) | **100 - 1 000 tonnes per year.** |

11 *Please provide here the date when the dissemination site was accessed.*

## 4.2 Overview of uses

Triclocarban is used in the following products: coating products, cosmetics and personal care products, fillers, putties, plasters, modelling clay, finger paints, inks and toners, pharmaceuticals, washing & cleaning products and air care products.

This substance is used in the following areas: formulation of mixtures and/or re-packaging.

Release to the environment of this substance is likely to occur from industrial use: formulation of mixtures, in processing aids at industrial sites and as an intermediate step in further manufacturing of another substance (use of intermediates). Other release to the environment of this substance is likely to occur from: indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), outdoor use and outdoor use in long-life materials with low release rate (e.g. metal, wooden and plastic construction and building materials).

This substance can be found in products with material based on: stone, plaster, cement, glass or ceramic (e.g. dishes, pots/pans, food storage containers, construction and isolation material).

**Table: Uses**

| | **Use(s)** |
|---|---|
| **Formulation** | Manufacture of construction chemical<br>Formulations of preparation<br>Cosmetics, personal care products |
| **Uses at industrial sites** | Automated application of water-borne adhesive<br>Industrial<br>Industrial end-use stage |
| **Consumer Uses** | Personnal care products<br>Air freshner of consumer use<br>Consumer use |
| **Article service life** | Service life of cured/installed construction chemical |

## 4.3 Additional information

# 5 JUSTIFICATION FOR THE RISK MANAGEMENT OPTION

## 5.1 Need for (further) risk management

Triclocarban is used in the following products: coating products, cosmetics and personal care products, fillers, putties, plasters, modelling clay, finger paints, inks and toners, pharmaceuticals, washing & cleaning products and air care products. In the framework on the French National Strategy on Endocrine Disruptors in 2016, the French Competent Authority requested ANSES to evaluate its toxicological and ecotoxicological profile and verify whether risk management measures should be necessary for this substance.

Based on the metabolisation and on the elimination of triclocarban (TCC), the bio-accumulation of TCC is likely to be low.

Concerning the TCC, toxicological data shows that TCC is nontoxic to Wistar albino rats acutely exposed to the dose level of 2000 mg/kg body weight by oral or dermal route. TCC was therefore considered to be not irritating to the rabbit eye and not irritating to the skin of human, rabbits and guinea pigs. TCC is considered as not sensitizing to the skin of human.

Concerning the genotoxicity, no evidence of genotoxicity was found in Ames test using S. typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 with or without metabolic activation for TCC. No evidence of genotoxicity was found in in vitro mammalian chromosome aberration test was performed using Chinese hamster ovary with different doses of TCC (31, 3, 62,5, 125, 250, 500, 1000, 1500, 2000 µg/ml).

This study shows an effect of TCC on the activity of the NIS (Sodium-iodine symporter) on a thyroid cellular lineage with a good level of evidence and suggests that TCC could alter the biosynthesis of the TH by modulating the contribution in iodine in thyreocytes in certain conditions.

Regarding the activation of the receptors ER, AR, AhR, CAR, TCC activates luciferase expression through the androgen receptor, and not through the glucocorticoid receptor. TCC did not compete with the AR binding but amplified the AR-mediated activity. TCC has no agonistic activity alone but enhance the DHT-induced activity. TCC also promotes ERα activity with similar potency as estradiol. TCC is a CAR activator but not an agonist ligand for either mouse or human CAR. TCC can induce significant up regulation of pS2 and down regulation of ERα meaning that Triclocarban have estrogenic properties. TCC has the capacity of interfering with the receptor AhR, and to modify interelations AhR-RE in the regulation of the genic expression. TCC has the capacity of interfering with the receptor AhR and can enhance testosterone dependent induction of luciferase gene expression and have weak estrogenic activities.

Regarding the modulation of the expression of the enzymes of xenobiotic and steroid metabolism, TCC may induce activation of the CAR receptor, with induction of CYP2B10 and UGT1A, and / or activation of AhR with induction of CYP1A1 and CYPIB1.

Regarding the activation of thyroidian hormones, TCC could alter the biosynthesis of the thyroidian hormones by modulating the contribution in iodine in thyreocytes in certain conditions.

Environmental and ecotoxicological data show that TCC could be considered fulfilling the P/vP criteria based on predicted and experimental degradation half-life in water, sediment or soil compartment, as well as the T criteria with the lowest NOEC value reported at 0.005 mg/L for fish (*Pimephales promelas).* The highest value of BCF, issued from prediction, is 800, leading to the conclusion that TCC is not expected to bioaccumulate in the food chain.

Regarding The ED potential in the environmental organisms, one study indicates that TCC could have a potential androgenic activity on fish as it enhances AR-mediated response to a well-know androgen (Trenbolone). Other studies suggest that TCC has both estrogenic and androgenic activity 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. Finally, it is demonstrated a chronic effect on reproduction (increased number of embryos) of New Zealand mudsail after TCC treatment. This significant increase of number of embryos has been previously found in experiments with exogenous estrogenic ED compounds (BPA, octylphenol, nonylphenol, ethynylestradiol).

However, concerning the environmental ED potential, supplementary data with recognized guidelines would allow to confirm or invalidate the proposed assumptions.

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 no enough data to identify potential ED effects. The result suggests that TCC 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.

**Table: SVHC Roadmap 2020 criteria**

| | Yes | No |
|---|---|---|
| a) Art 57 criteria fulfilled? | Non-conclusive data | |
| b) Registrations in accordance with Article 10? | x | |
| c) Registrations include uses within scope of authorisation? | x | |
| d) Known uses <u>not</u> already regulated by specific EU legislation that provides a pressure for substitution? | x | |

## 5.2 Conclusions on the most appropriate (combination of) risk management options

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.

## 5.3 References

- *Chemical safety report 2016*