Federale Overheidsdienst
VOLKSGEZONDHEID,
VEILIGHEID VAN DE VOEDSELKETEN
EN LEEFMILIEU

DG Environment - Risk Management of Chemical Substances

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

1,2,4-triazole

EC No 206-022-9

CAS No 288-88-0

Evaluating Member State(s): BE CA

Dated: 25 March 2021

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2015

Before concluding the substance evaluation a Decision to request further information was issued on: 31 July 2017

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

1,2,4-triazole was originally selected for substance evaluation in order to clarify the following concerns:

- Reprotoxicity
- other hazard based concern (neurotoxicity, carcinogenicity, endocrine disruption)
- wide dispersive use
- exposure of environment
- high aggregated tonnage
- other exposure/risk based concern (consumer exposure)

During the evaluation no other concerns were identified.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

None.

3. CONCLUSION OF SUBSTANCE EVALUATION

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

Table 1

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

*The RAC opinion was adopted on 15 March 2019 based on BE CA proposal for a more stringent classification for reprotoxicity (see section 4.1.1).

In addition to the proposal for classification and labelling for reprotoxicity, in the course of the substance evaluation, the eMSCA concluded that the concern for endocrine disruption had to be clarified. Consequently, the Registrants were requested to perform a Steroidogenesis Assay (OECD TG 456) (final decision sent to the Registrants on 31/07/2017).

In the course of the substance evaluation follow-up procedure the eMSCA concluded that the concern for endocrine disruption had to be further clarified. Hence, a follow-up draft decion was submitted to ECHA and sent to the Registrants. Due to a change in the

registration scope from full registration (>1000 t/a) to transported and on-site isolated intermediate (1-10 t/a) under strictly controlled conditions, rather limited exposure of workers and environment to 1,2,4-triazole is expected[2].

Based on the Registrants declarations regarding fulfilment of Art. 17(3) and Art. 18(4) of REACH Regulation the eMSCA considered that in these circumstances no further testing should be required and decided to close the substance evaluation procedure.

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

BE CA submitted a proposal for a more stringent reproductive toxicity classification (Repr. 1B H360FD instead of Repr. 2 H361d) on 15 March 2018. ECHA's Committee of Risk Assessment (RAC) agreed with the proposal of the BE CA and consequently the opinion of RAC has been adopted on 15 March 2019 (https://echa.europa.eu/documents/10162/5e989545-ffd0-1c47-54b9-deced9006a94).

This new classification however has not yet been included in Annex VI of the CLP Regulation.

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

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

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

5.2. Other actions

If the registration scope change from intermediate to full registration, the Substance should be put on the Community rolling Action Plan (CoRAP) and re-evaluated again as the concern for endocrine disruption remains unanswered.

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

Not applicable.

[2] While at present only the Intermediate registrations are actively manufacturing / importing the substance, there exists one cease manufacture Full registration which could potentially resume manufacture / import at any time. This is the reason why the disseminated information still appears as "full registration".

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

1,2,4-triazole was originally selected for substance evaluation in order to clarify the following concerns:

- Reprotoxicity
- other hazard based concern (neurotoxicity, carcinogenicity, endocrine disruption)
- wide dispersive use
- exposure of environment
- high aggregated tonnage
- other exposure/risk based concern (consumer exposure)

During the evaluation no other concerns were identified.

Table 2: Evaluated endpoints

EVALUATED ENDPOINTS Endpoint evaluated	Outcome/conclusion
Reprotoxicity	More stringent classification for reproductive toxicity (Repr. 1B H360FD) was agreed by the RAC on 15 March 2019.
Other hazard: Neurotoxicity	Concern not further evaluated due to more stringent classification for reproductive toxicity (see above).
Other hazard: Carcinogenicity	No data available.
Other hazard: Endocrine Disruption	The concern has not been clarified but at present there is no follow up foreseen due to no or limited exposure. The intrinsic property should be however further inverstigated if/when the scope of the registration dossier changes and exposure is again possible or if/when new hazard information becomes available.
Wide dispersive use, exposure of environment, high aggergated tonnage, other exposure/risk based concern (consumer exposure)	Concern not substantiated. No further action due to a dossier update (change from full registration (>1000 t/a) to transported and on-site isolated intermediate (1-10 t/a) under strictly controlled conditions.)

7.2. Procedure

Substance Evaluation:

The evaluation of 1,2,4-triazole started on 17 March 2015 and focused on the human health endpoints. However, the endpoints related to environment were also examined in the course of this evaluation. An exposure assessment was also performed.

The registration dossier of 1,2,4-triazole (date 22/07/2015) was used as a basis for the evaluation. The Registrants provided full study reports for the endpoints of concern. The information from the registration dossier was then verified with data from publicly available literature (i.e. scientific publications (see reference list)). For some endpoints data were generated with EpiSuite v4.1.

The possible outcome of this evaluation was consulted during the open session of the Endocrine Disruptor Expert Group meeting held at ECHA (21-22 October 2015) where a representative of the Registrants was also present. Comments provided during this discussion were taken into account in drafting of the decision.

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-54 meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation. The Final Decision was sent to the Regitrants on 31 July 2017.

Classification and labelling:

In order to address the concern for reproductive toxicity and development, BE CA submitted to ECHA a proposal for a more stringent reproductive toxicity classification (Repr. 1B H360FD instead of Repr. 2 H361d) on 15 March 2018. ECHA's Committee of Risk Assessment (RAC) agreed with the proposal of the BE CA and consequently the opinion of RAC has been adopted on 15 March 2019. This new classification however has not yet been included in Annex VI of the CLP Regulation.

Follow-up substance evaluation:

The Registrants performed the requested steroidogenesis assay (OECD TG 456) within the deadline set and updated their dossier on 7 February 2018.

The eMSCA evaluated the new data and considered that further testing was needed in order to address the concern for endocrine disruption for human health and environment. The proposed testing strategy was also consulted with the Endocrine Disruptor Expert Group (meeting held at ECHA (9-10 October 2018). Comments provided during this discussion were taken into account in drafting of the follow-up decision.

The evaluating MSCA submitted a draft decision under Article 46(3) of the REACH Regulation. on 4 February 2019.

On 27 March 2019 ECHA notified the Registrants' of the draft decision and invited them to provide comments.

On 20 June 2019 the eMSCA has been informed that the Registrants's submitted an update of the registration dossier in which the status of dossier was changed from full registration (>1000 t/a) to transported and on-site isolated intermediate (1-10 t/a) under strictly controlled conditions.

Due to this dossier update and based on the Registrants declarations regarding fulfilment of Art. 17(3) and Art. 18(4) of REACH Regulation the eMSCA concluded that at this moment there is no or limited exposure to human and environment due to strictly controlled conditions applied. Hence there is currently no need to continue with further testing requests to clarify concerns related to endocrine disruption. However, the endocrine disruption potential of 1,2,4-triazole, an intrinsic property of a substance, remains unanswered and should be further investigated once there is a change in the scope of the dossier or if new information regarding this endpoint becomes available.

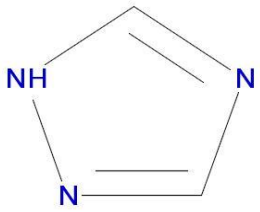
7.3. Identity of the substance

Table 3: Substance identity

SUBSTANCE IDENTITY	
Public name:	1,2,4-triazole
EC number:	206-022-9
CAS number:	288-88-0
Index number in Annex VI of the CLP Regulation:	613-111-00-X
Molecular formula:	C2H3N3
Molecular weight range:	69.0653
Synonyms:	1,2,4-Triazol 1,2,4-triazool 1,2,4-triazole 1H-1,2,4-Triazole

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

Structural formula:



7.4. Physico-chemical properties

Table 4: Summary of physico-chemical properties

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Solid *at 20°C and 1013 hPa*
Vapour pressure	*0.22 Pa at 20°C (OECD TG 104 (effusion method: vapour pressure balance))*
Water solubility	*730 g/L at 25°C (OECD TG 105 (flask method))*
Partition coefficient n-octanol/water (Log Kow)	*25° C, pH 5: log Pow = -0.62* *25°C, pH 7: log Pow = -0.71* *25° C, pH 9: log Pow = -0.68*

	(OECD TG 107 (Shake flask method))
Flammability	*not highly flammable (EU A.10)*
Explosive properties	*It can be expected that the substance is non-explosive.*
Oxidising properties	*Not expected to have oxidising properties, since the substance does not contain oxygen, fluorine or chlorine.*
Granulometry	*No particles smaller than 100 µm determined (ISO 13317-2 (Fixed Pipette Method))*
Stability in organic solvents and identity of relevant degradation products	*Data waived.*
Dissociation constant	*pKa = 10 at 22°C (OECD TG 112 (Dissociation constant; photometric method))*

7.5. Manufacture and uses

7.5.1. Quantities

Table 5: Quantities

AGGREGATED TONNAGE (PER YEAR)				
☒ 1 – 10 t[3]	☐ 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[2]

7.5.2. Overview of uses

Information from ECHA dissemination website (http://echa.europa.eu/en/information-on-chemicals/registered-substances).

The information in Table 6 represents the information currently available on the ECHA's dissemination website. This is however not consistent with the information provided in the dossier update (20 June 2019): the registrants declare that they have only intermediate uses.

Table 6 : Overview of uses

USES	
Manufacture	Manufacture and use as interemediate: - chemical production or refinery in closed process with likehood of exposure or process with equivalent containment conditions - Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions

	- Chemical production where opportunity for exposure arises - Transfer of substance or mixture (charging and discharging) at dedicated facilities - Use as laboratory reagent
Formulation	Substance: - Chemical production where opportunity for exposure arises - Use in artificial fertilizers Distribution: - Transfer of substance or mixture (charging and discharging) at non-dedicated facilities - Transfer of substance or mixture into small containers (dedicated filling line, including weighing) Formulation at SKW Piesteritz GmbH: - Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions - Transfer of substance or mixture (charging and discharging) at dedicated facilities -Transfer of substance or mixture into small containers (dedicated filling line, including weighing)
Uses at industrial sites	Intermediate - Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions - Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions - Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions - Transfer of substance or mixture (charging and discharging) at dedicated facilities Substance - chemical production where opportunity for exposure arises
Uses by professional workers	Handling and application of liquid and solid fertilizers by farmers - transfer of a substance or preparation into small containers (dedicated filling line, including weighing) Handling of nitrification inhibitors, handling of liquid and solid fertilizers with nitrification inhibitors by retailers - transfer of substance or preparation (charging/discharging) at dedicated facilities.
Uses advised against	Formulation into mixture

Use as semiconductors was removed from the registration dossier in the update of 22 July 2015.

On 20 June 2019 the scope of the registration was changed from full registration to intermediate use under strictly controlled conditions.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 7: Overview of harmonised classification

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
613-111-00-X	1,2,4-triazole	206-022-9	288-88-0	Acute Tox. 4*	H302		
				Eye Irrit. 2	H319		
				Repr. 2	H361d		

On 15 March 2019 the RAC adopted an opinion on more stringent classification for 1,2,4-triazole:

Repr. 1B, H360FD

Acute Tox. 4, H302

Eye Irrit. 2, H319

Oral ATE = 1320 mg/kg bw

https://www.echa.europa.eu/documents/10162/5e989545-ffd0-1c47-54b9-deced9006a94

This new classification however has not yet been included in Annex VI of the CLP Regulation.

7.6.2. Self-classification

In the registration(s):

Acute Tox. 4 H302

Eye Irrit. 2 H319

Repr. 2 H361d

Above mentioned notifications as listed in the registration dossier are in line with the harmonized 1,2,4-triazole classification according to annex VI of CLP. However it has to be noted that on 15 March 2019, RAC adopted an opinion regarding more stringent reproductive toxicity classification (Repr. 1B H360FD). This new classification has not yet been included in annex VI of the CLP Regulation.

The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory (ECHA's Classification and Labelling Inventory was last consulted by the eMSCA on 20 March 2020):

Aquatic Chronic 3 H412 (1 notification).

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

7.7.1.1.1. Hydrolysis

Table 8: Studies on hydrolysis

Method	Results	Remarks	Reference
equivalent or similar to OECD TG 111 (Hydrolysis as a Function of pH)	Recovery (in %): pH 5: >= 84 — < 88 at 25 °C after 30 d pH 7: > 85.5 — < 88.2 at 25 °C after 30 d pH 9: > 86.9 — < 87.7 at 25 °C after 30 d Transformation products: not measured	2 (reliable with restrictions) Non-GLP	Registration dossier (study report, 1983)

More then 84% of the substance could be recovered after 30 days in the hydrolysis test at different pH levels. It is assumed that the substance does not hydrolyse.

7.7.1.2. Biodegradation

7.7.1.2.1. Screening test : Biodegradtion in water

Table 9: Screening tests for biodegradation in water

Method	Results	Remarks	Reference
Test type: ready biodegradability aerobic activated sludge, domestic, adapted OECD TG 301 A (old version) (Ready Biodegradabiltiy: Modified AFNOR Test) EU Method C.4-A (Determination of the "Ready" Biodegradability - Dissolved Organic Carbon (DOC) Die-Away Test)	under test conditions no biodegradation observed % Degradation of test substance: 0 after 0 d (DOC removal) 16 after 24 d (DOC removal)	1 (reliable without restriction) GLP	Registration dossier (study report, 1995)

Method	Results	Remarks	Reference
Test type: inherent biodegradability: Modified Zahn-Wellens Test OECD TG 302B	under test conditions no biodegradation observed % Degradation of test substance: 1 after 28 d (DOC removal)	2 (reliable with restrictions) No data on GLP	US EPA (2009) Registration dossier (study report)

Two ready biodegradation tests showed no significant degradation. In a DOC Die-Away test 16% degradation was seen after 24 days. In a Zahn-Wellens test 1% degradation was seen. It can be concluded that 1,2,4 triazole is not readily biodegradable.

7.7.1.2.2. Simulation test: Biodegradation in soil

Table 10: Simulation tests for biodegradation in soil

Method	Results	Remarks	Reference
Aerobic degradation 3 soil types : sandy loam(Laacher Hof AXXa) loamy (BBA 2.2)sand silt loam (Laacher Hof AIII) SETAC, Procedures for assessing the environmental fate and ecotoxicity of pesticides, part 1, 1.1 Dutch Board for the Authorisation of Agrochemicals (CTB). G. 1.1 UA EPA Environmental fate §162-1, Aerobic soil metabolism studies BBA Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln im Boden -Abbau, Umwandlung und Metabolismus	DT50 = 7.98d (mean) 2.34 d(FOMC)/6.32 d (non-linear first order) 9.34 d(FOMC)/9.91 d (non-linear first order) 12.27 d(first order) Degradation products were measured Applied conc about 0.06 mg/kg dry soil: equivalent to an application rate of triazole releasing fungicides of 750 g a.i./ha, reaching the soil for 50%, incorporation in 5 cm of soil and assuming a soil bulk density of 1500 kg/m3, a maximum metabolite formation of 50% and a molar mass ratio of triazole to parent of 0.25.	2 (reliable with restrictions) GLP	Notox, 2000

In an aerobic soil degradation study an average half life of 8 days (best fit kinetics) or 9.5d (non-linear first order once-compartment kinetics) was determined for 1,2,4-triazole.

Triazolyl acetic acid was identified as major degradation product (max. 7% and 3% AR resp.). Two other minor degradation products were identified of which hydroxytriazole (1,2-dihydro- l,2,4-triazolone) with a maximum of 2.6% AR. CO2-formation (ultimate degradation) after 120 d was ≤33 % AR. Non-extractable residues (NER) formation accounted for ≤ 65% AR at the end of the study.

7.7.1.2.3. Field study

Table 11: Field study summary

Method	Results	Remarks	Reference
Test type: field trial Aerobic 4 Soil types: Silt loam (#1) Silt loam (#1) Silty clay loam (#2) Silty clay (#2) Sandy loam (#3) Sandy loam (#3) Loam (#4) Clay loam (#4) EC-Directive 91/414/EEC Annex I Part 7 and Annex II Part 9 SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides BBA guideline, part IV, 4-1, Fate and Behavior of Plant Protection Compounds in Soil ECPA Guidance Document on Field Soil Dissipation Studies	Half-life (DT50): 7.8 d (#1) 21.2 d (#2) 6.8 d (#3) 28.1 d (#4) Transformation products: not measured	1 (reliable without restriction) GLP	Registration dossier (study report, 2004)

From the results presented above it can be concluded that 1,2,4-triazole degrades under the investigated conditions (in northern and southern Europe) with no observation of a significant vertical movement. Half-life were determined between 6.8 to 28.1d.

7.7.1.3. Conclusion

1,2,4-triazole is hydrolytically stable and not readily degradable. The degradation half-life in soil was determined between 6.8 - 28.1 days.

7.7.2. Environmental distribution

7.7.2.1. Adsorption/desorption

Table 12: Studies on adsorption/desorption

Method	Results	Remarks	Reference
Study type: adsorption/desorption (soil) batch equilibrium method equivalent or similar to OECD TG 106 (Adsorption - Desorption Using a Batch Equilibrium Method)	Adsorption coefficient: Koc: 120 at 25 °C (1.2 % Org. Carbon) (Organic matter) Koc: 43 at 25 °C (3 % Org. Carbon) (Organic matter) Koc: 202 at 25 °C (0.2% Org. Carbon) (Organic matter) Koc: 104 at 25 °C (1.2% Org. Carbon) (Organic matter) Koc: 89 at 25 °C (1.4% Org. Carbon) (Organic matter) Transformation products: no	1 (reliable without restriction) GLP	Registration dossier (study report, 1988)
Study type: adsorption/desorption (soil) batch equilibrium method equivalent or similar to OECD TG 106 (Adsorption - Desorption Using a Batch Equilibrium Method)	Adsorption coefficient: Freundlich adsorption constant k: 0.19 at 20 °C (% Org. C: 1.4) (Organic matter) Freundlich adsorption constant k: 0.22 at 20 °C (% Org. C: 1) (Organic matter) Freundlich adsorption constant k: 0.52 at 20 °C (% Org. C: 2.6) (Organic matter) Freundlich adsorption constant k: 1.32 at 20 °C (% Org. C: 9.3) (Organic matter) Freundlich adsorption constant k: 3.35 at 20 °C (% Org. C: 43.1) (Organic matter)	2 (reliable with restrictions) Non-GLP	Registration dossier (study report, 1983)

The koc values for 1,2,4-triazole varied between 43 to 202 in different soil types. Based on these results it can be expected that the substance is slightly mobile to immobile in soil according to Mensink *et al.* (1995).
The substance is very soluble in water (700 g/L at 20°C) and has a estimated log Kow value < 1. In addition 1,2,4-triazole has a low vapour pressure (0.22 Pa). Therefore it could be assumed that when only the aquatic compartment is considered, the substance will mainly stay in water.
However, the BE CA notes that when the substance is applied directly to soil, it has a high potential to bind to soil and to form non-extractable residues.

7.7.2.2. Distribution

According to the Level III Fugacity model (Epiweb v. 4.1), using a water solubility of 730g/l, a log Kow of -0.71 (pH 7) and the highest logKow of 202, the substance will mainly distribute to soil under equal emissions (eMSCA, 2017)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	5.19	2.57e+003	1000
Water	24	360	1000
Soil	70.6	720	1000
Sediment	0.231	3.24e+003	0

Persistence Time: 522 hr

7.7.3. Bioaccumulation

No experimental data on bioaccumulation are available.

Experimental Log Kow:

25° C, pH 5: log Pow = -0.62
25°C, pH 7: log Pow = -0.71
25° C, pH 9: log Pow = -0.68

BE CA agrees that based of the experimental log Kow it can be assumed that the substance has a low bioaccumulation potential.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

7.8.1.1.1. Short-term toxicity to fish

Table 13: Short-term effects on fish

Method	Results	Remarks	Reference
Danio rerio freshwater semi-static OECD TG 203 (Fish, Acute Toxicity Test) EU Method C.1 (Acute Toxicity for Fish)	96hLC50: > 150 mg/L test mat. (nominal) based on: mortality 96hLC50: > 97 mg/L test mat. (meas. (arithm. mean)) based on: mortality	1 (reliable without restriction) GLP **read-across with sodium 1,2,4-triazol-1-ide** Substance was not stable during the test	Registration dossier (study report, 2001)
Salmo gairdneri (new name: Oncorhynchus mykiss) freshwater static OECD TG 203 (Fish, Acute Toxicity Test)	96hLC50: 760 mg/L test mat. (nominal) based on: mortality	2 (reliable with restrictions) Non-GLP Test substance dissolved in the tanks wihthout preparation of a stock solution Substance concentration not maintained during the test	Registration dossier (study report, 1983)

An acute toxicity test (OECD TG 203) with sodium 1,2,4 -triazol-1-ide (the sodium salt of the substance) was performed. No effects were seen with Danio rerio up to 150 mg/L (nominal concentration). However, the substance was not stable during the test.
In another non-GLP acute toxity test (OECD TG 203) with Oncorhynchus mykiss an LC50 of 760mg/L (nominal) could be determined. However, the test concentration was not maintained during the test.

7.8.1.1.2. Long term toxicity to fish

Table 14: Long-term effects on fish

Method	Results	Remarks	Reference
Oncorhynchus mykiss freshwater juvenile fish: growth semi-static OECD TG 215 (Fish, Juvenile Growth Test)	28d NOEC: > 100 mg/L test mat. (nominal) based on: mortality	1 (reliable without restriction) GLP	Registration dossier (study report, 2001)

In a juvenile fish test (OECD TG 215) with Oncorhynchus mykiss, no effects were seen up to a concentration of 100 mg/L nominal. The substance was quite stable (97-99% of nominal concentration).

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertabrates

Table 15: Short-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater static EU Method C.2 (Acute Toxicity for Daphnia) OECD TG 202 (Daphnia sp. Acute Immobilisation Test)	48hEC50 (48 h): > 494.7 mg/L test mat. (meas. (initial)) based on: mobility	1 (reliable without restriction) GLP	Registration dossier (study report, 1996)
Daphnia magna freshwater static OECD TG 202 (Daphnia sp. Acute Immobilisation Test)	24hEC50: 900 mg/L test mat. (nominal) based on: mobility (calculated)	2 (reliable with restrictions) Non-GLP Substance concentration not maintained during the test Exposure duration 24h instead of 48h	Registration dossier (study report, 1982)

Two Daphnia magna short term toxicity tests (OECD TG 202) are available.
In the first test no effects were seen after 48 hours up to a concentration of 494.7 mg/L. In the second test a EC50 value of 900 mg/L was determined after 24 hours of testing instead of 48h. However test concentration was not maintained during this study.

7.8.1.2.2. . Long-term toxicity to aquatic invertabrates

No data available.

7.8.1.3. Algae and aquatic plants

Table 16: Effects on algae and aquatic plants

Method	Results	Remarks	Reference
Pseudokirchnerella subcapitata (reported as Raphidocelis subcapitata) (algae) freshwater static EU Method C.3 (Algal Inhibition test) OECD TG 201 (Alga, Growth Inhibition Test)	72hEC50: 14 mg/L test mat. (meas. (initial)) based on: biomass (95% CL = 8.1 - 24 mg/L) 72hEC50: 45 mg/L test mat. (meas. (initial)) based on: growth rate (95% CL = 31 - 74 mg/L) 72hNOEC: 3.49 mg/L test mat. (meas. (initial)) based on: biomass 72hNOEC: 3.49 mg/L test mat. (meas. (initial)) based on: growth rate 72hEC10: 4.5 mg/L test mat. (meas. (initial)) based on: biomass (95% CL = 1.9 - 7.7 mg/L) 72hEC10: 8.7 mg/L test mat. (meas. (initial)) based on: growth rate (95% CL = 6.1 - 12 mg/L)	1 (reliable without restriction) GLP	Registration dossier (study report, 1998)
Scenedesmus subspicatus (new name: Desmodesmus subspicatus) (algae) freshwater static Equivalent or similar to OECD TG 201 (Alga, Growth Inhibition Test)	5dEC50: 6.3 mg/L test mat. (nominal) based on: growth rate (calculated) 5dEC50: 6.5 mg/L test mat. (nominal) based on: growth rate (graphically determined)	2 (reliable with restrictions) Non-GLP Cell concentration measured at 120h instead of 24, 48 and 72h	Registration dossier (study report, 1982)

In an algae growth inhibition test (OECD TG 201) with *Pseudokirchnerella subcapitata* an EC50 (72h) of 14 mg/L and a NOEC of 3.49 mg/L were determined.

In an algae growth inhibition test (OECD TG 201) with *Desmodesmus subspicatus* an EC50 (5 days) of 6.3 mg/L was determined.

7.8.1.4. Sediment organisms

No data available.

7.8.1.5. Other aquatic organisms

No data available.

7.8.2. Terrestrial compartment

7.8.2.1. Toxicity to soil macro-organisms

The results are summarised in the following table:

Table 17: Effects on soil macro-organisms

Method	Results	Remarks	Reference
Eisenia fetida (annelids) short-term toxicity (laboratory study) Substrate: artificial soil OECD TG 207 (Earthworm, Acute Toxicity Tests)	14dLC50: > 1000 mg/kg soil dw test mat. (nominal) based on: mortality	1 (reliable without restriction) GLP	Registration dossier (study report, 1986)
Eisenia fetida (annelids) *Long-term toxicity (laboratory study)* *Substrate: artificial soil* *ISO 11268-2 (Effects of Pollutants on Earthworms. 2. Determination of Effects on Reproduction)*	28dNOEC: >70.81 µg/kg soil dw test mat. (nominal) based on: reproduction	2 (reliable with restrictions)	Registration dossier (study report, 2000)

No effects were seen in both short-term and long-term earthworm toxicity tests (OECD TG 207 & ISO 11268-2) up to a concentration of 1000 mg/kg and 0.0708 mg/kg respectively (Eisenia fetida).

7.8.3. Microbiological activity in sewage treatment systems

Table 18: Effects on micro-organisms

Method	Results	Remarks	Reference
activated sludge freshwater static	3hEC50: > 1000 mg/L test mat. (nominal) based on: respiration rate	1 (reliable without restriction) GLP	Registration dossier (study report, 2011)

Method	Results	Remarks	Reference
OECD TG 209 (Activated Sludge, Respiration Inhibition Test)			

No effects were seen in microorganisms up to 1000 mg/L in an activated sludge respiration inhibition test (OECD TG 209).

7.8.4. PNEC derivation and other hazard conclusions

Not evaluated.

7.8.5. Conclusions for classification and labelling

Based on the available data, the BE CA agrees with the registrant not to classify the substance for environment:

Acute Toxicity:

All L(E)C50s >1 mg/l => no classification warranted for Acute Aquatic Toxicity

Chronic Toxicity:

Only NOECs available for 2 trophic levels : fish and algae.

Based on the NOECs : all > 1 mg/l no classification is warranted.

Based on surrogate approach (acute toxicity of other trophic level) : 48hLC50 Daphnia (> 494.7 mg/L) > 100 mg/l no classification is warranted.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

7.9.1.1. Basic toxicokinetics

Table 19: Summary of the basic toxicokinetics data

Method	Results	Remarks	Reference
Rats (SD), 2/sex/group Oral : gavage Single oral dose 0.08 , 9.8 , 173.1 mg/rat (2/sex/dose)	Main elimination route: urinary excretion with average recoveries of 92.0%, 86.2%, 89.8% for the low, mid and high dosed animals. Recovery of the radioactivity in feces averaged 8.1, 15.2 and 7.9 % for the low, mid and high dosed animals. No significant differences were observed in excretion patterns	1 (reliable without restriction)	Registration dossier (study report, 1986)

Method	Results	Remarks	Reference
According to EPA OPPTS 870.7485 (Metabolism and Pharmacokinetics)	between different sexes of rats of the same dose level. Excretion of the radioactivity during the first 48 hours after administration accounted for 97.7% for low, 97.6% for mid and 84.8% for high dose animals. The average total recoveries of administered 14C-Triazole was excellent for low (101.0%), mid (102.8%) and high (100.2%) dosed animals.		
Rat (SD), male Oral: unspecified Single dose: 10 mg/kg bw 10males/dose Equivalent or similar to OECD TG 417 (Toxicokinetics)	≥90% of the radioactive dose is eliminated in the urine in form of unchanged 1,2,4-triazole within the first 8 hours after oral treatment. Three additional metabolites each less than 3% were observed (identification not performed)	3 (not reliable)	Registration dossier (study report (1980)
Rat (SD), male Oral: intravenous and intraduodenal IV : 0.1-100 mg/kg Oral : 1 mg/kg Intraduodenal :1 mg/kg Equivalent or similar to OECD TG 417 (Toxicokinetics)	Distribution in tissues: in all tissues (all tissue concentration were similar then no tissue preference) Nearly complete renal elimination of radioactivity was found. About 3-4% of the applied dose was excreted with the feces. The plasma half-life was about 12 hours and was independent of the dose applied 15-20% of the applied dose was subjected to enterohepatic circulation.	3 (not reliable)	Registration dossier (study report (1978)

Registrant claims triazole shows no potential for bioaccumulation. The eMSCA supports this conclusion.

7.9.1.2. Dermal absorption

Table 20: Summary of dermal absorption results

Method	Results	Remarks	Reference
In vitro Approx. 5mg/cm² According to OECD TG 428 (Skin Absorption: *in vitro* method)	Penetration into the viable skin layers and the receptor fluid out of the test item dilution with 132 ± 71.0 µg/cm² (2.73 ± 1.45% of applied dose).	1 (reliable without restriction)	Registration dossier (study report, 2011)

The eMSCA agrees with the registrants that 1,2,4-triazole can penetrate the skin.

7.9.2. Acute toxicity and Corrosion/Irritation

7.9.2.1. Acute toxicity by oral route :

Table 21 : Summary of the acute oral toxicity studies

Method	Results	Remarks	Reference
In rats (Wistar) (5/sex/dose) Gavage : 1000, 1500 and 2000 mg/kg bw OECD TG 401 and 423	Mortality : 1000mg/kg bw : no mortality In males : at 1500 mg/kg bw, 2 rats died after 24h and 2 after 2d and at 2000 mg/kg bw, 4 died after 24h and 1 after 2d. In females : at 1500 mg/kg bw, 3 rats died after 24h and 2 after 3d and at 2000 mg/kg bw, 4 died after 24h and 1 after 2d. Clinical signs : ≥ 1000 mg/kg : sedated, ventral recumbency, dyspnea Body weight gain of the surviving animals not affected LD50 : 1320.39 mg/kg	1 (reliable without restriction)	Registration dossier (study report, 1989)
In rats (Crl:CD BR) (3males/dose) By gavage : 500 and 5000 mg/kg bw OECD TG 423 (deviations : deviant starting doses of 500 and 5000 mg/kg, and study conducted in males instead of the more sensitive	Mortality : at 500 mg/kg bw no death and at 5000 mg/kg bw all rats died within 10min Clinical signs and bw: no effects Necropsy : 5000 mg/kg : reddened duodenum and reddened glandular portion of stomach 500 mg/kg : no visible lesions LD50 : >500 and <5000 mg/kg	2 (reliable with restrictions)	Registration dossier (study report, 1992)

females)			
In rats (Wistar) By gavage (15 rats/sex/dose) : 100 (only ♀), 250, 500, 1000 (30♂ and 15♀), 1250, 1500, 1750, 1850 (only ♂), 2000 (15♂ and 30♀) and 2500 (14♂ and 15♀) mg/kg bw OECD TG 423 (deviations : source, purity, temperature, light cycle, humidity not provided)	Mortality : no information Clinical signs : reduction in general well-being, sedation, breathing disorders Necropsy : no major changes LD50 (females) : 1648 mg/kg LD50 (males) : 1650 mg/kg	2 (reliable with restrictions)	Registration dossier (study report, 1976)

The acute oral toxicity of 1,2,4-triazole was evaluated in an acute oral gavage study in rats (Registration dossier (study report, 1989)). The oral LD50 was 1320.39 mg/kg bw. This result is supported by other oral LD50 values which were of 1648 and 1650 mg/kg bw respectively in females and in males in the registration dossier (study report, 1976) and between 500 and 5000 mg/kg bw in the registration dossier (study report, 1992).

1,2,4-triazole has a harmonised classification as **Acute Toxicity Category 4* ; H302 (Harmfull if swallowed)**.

A proposal for Harmonised Classification and Labelling was introduced by the BE CA on 15 March 2018. In March 2019, RAC (Risk Assessment Committee) decided to classify 1,2,4-triazole has Acute Toxicity Category 4; H302 (Harmful if swallowed) and assign the acute toxicity estimate of 1320 mg/kg bw. More details can be found in the CLH report and the Annex I of the CLH report : https://www.echa.europa.eu/web/guest/harmonised-classification-and-labelling-previous-consultations/-/substance-rev/19733/term?_viewsubstances_WAR_echarevsubstanceportlet_SEARCH_CRITERIA_EC_NUMBER=206-022-9&_viewsubstances_WAR_echarevsubstanceportlet_DISS=true.
Based on the available information, the eMSCA concludes that there is no further concern for acute oral toxicity and thus no additional testing is needed.

7.9.2.2. Acute toxicity by inhalation :

Table 22 : Summary of the acute inhalation toxicity studies

Method	Results	Remarks	Reference
Mouse (NMRI) (10 males/doses) Dust Duration of exposure : 6 h Dose : unspecified OECD TG 403 (deviations : doses, source, purity, temperature, light cycle, humidity	Mortality : none Clinical signs : none	3 (not reliable)	Registration dossier (study report, 1976)

and quantitative results not provided			

Based on the results of the granulometry study (no particles smaller than 78 µm), and the available information (Registration dossier (study report, 1976)), the eMSCA concludes that there is no concern for acute inhalation toxicity and thus no further testing is needed.

7.9.2.3. Acute toxicity by dermal route :

Table 23 : Summary of the acute dermal toxicity studies

Method	Results	Remarks	Reference
In rats (Wistar) (10/sex/doses ; except at 1000 mg/kg bw only 5/sex, at 2000 and 4000 mg/kg bw females only) Occlusive : 1000, 2000 (only ♀), 2500, 3500, 4000 (only ♀), 5000 mg/kg bw Duration of exposure : 24 h OECD TG 402 (deviations : source, purity, temperature, light cycle, humidity not provided)	Mortality : no information Clinical signs : reduction in general well-being, sedation, breathing disorders Necropsy : no major changes LD50 (♂) : 4200 mg/kg bw LD50 (♀) : 3129 mg/kg bw	1 (reliable without restrictions)	Registration dossier (study report, 1976)
In rats (Wistar) (5/sex/dose) Semiocclusive : 2000 mg/kg bw Duration of exposure : 24 h OECD TG 402	Mortality : no animal died Clinical signs : scales, with recovery after 11 observation days Body weight gain : not affected Necropsy : lungs : discoloration, dark red (2 animals) LD50 : > 2000 mg/kg	2 (reliable with restrictions)	Registration dossier (study report, 1989)
In rabbit (New Zealand Xhite) (2males/dose) Occlusive : 0.2, 2.0 and 5.0 g/kg bw Duration of exposure :	Mortality : ≥2.0 g : all animals died by d4 Clinical signs : ≥2.0 g : abdominal breathing, ataxia, clear nasal discharge, moribundity, tremors, ..	2 (reliable with restrictions)	Registration dossier (study report, 1992)

24 h OECD TG 402 (deviations : only 2 animals per dose and no females)	Body weight : no effect Necropsy : ≥2.0 g : numerous gross findings LD50 >0.2 - < 2 g/kg		

According to the registrants' assessment 1,2,4-triazole is not acutely toxic via the dermal route.
The eMSCA concludes that based on the available information, there is no concern for acute dermal toxicity and thus no further testing is needed.

7.9.2.4. Skin irritation :

Table 24 : Summary of the skin irritation studies

Method	Results	Remarks	Reference
In 2 rabbits (NZW) Occlusive : 500 mg of the test substance Duration of exposure : 24 h OECD TG 404 (deviations : chemical characteristics, temperature, light cycle and humidity not provided, no grading scale of irritation, incorrect number of animals)	Erythema : mean : score of 0/4 Edema : mean : score of 0/4 Not irritating	2 (reliable with restriction)	Registration dossier (study report, 1976)
In 2 male rabbits (NZW) Occlusive : 0.5 g of the test substance Duration of exposure : 24 h OECD TG 404	Primary irritation score (PIS) : mean : 0.25/4 (intact skin) and 0.38/4 (shaved skin) Slightly irritating	1 (reliable without restrictions) Supporting study Experimental result	Registration dossier (study report, 1992)

The registrants concluded that 1,2,4-triazole is not skin irritating.
The eMSCA assessed that based on the available information there is no concern for skin irritation and thus no further testing is needed.

7.9.2.5. Eye irritation :

Table 25 : Summary of the eye irritation studies

Methods	Results	Remarks	Reference
In 2 rabbits (NZW)	Mean score (24-48h) :	2 (reliable with	Registration dossier

Single administration 50mg of the test substance OECD TG 405 (deviations : source, purity, chemical characteristics, temperature, light cycle and humidity not provided, grading scale not reported)	Corneal opacity : 0.5/4 Iritis : 0.5/2 Conjunctival redness : 2.5/4 Chemosis : 1/4 In one animal, slight redness and swelling of the conjunctivae was still present 5 d after application whereas the other animal was normal. The animals were normal 7 d after application Irritating	restrictions)	(study report, 1976)
In 2 males rabbits 0.1g of the test substance Equivalent to OECD TG 405	Mean score (72 h): Corneal opacity : 32.5/80 Iritis : 0.8/10 Conjunctival redness : 2/3 Chemosis : 1.8/4 All the parameters were fully reversible Irritating	1 (reliable without restriction)	Registration dossier (study report, 1992)

1,2,4-triazole has a harmonised classification as Eye Irrit. 2 H319 : **Causes serious eye irritation** based on the results of the 2 rabbit studies.
The eMSCA supports this classification and concludes that there is no further concern for eye irritation. No additional testing is therefore needed.

7.9.3. Sensitisation

7.9.3.1. Skin :

Table 26 : Summary of the skin sensitisation studies

Methods	Results	Remarks	Reference
Guinea pig maximisation test (GPMT) Guinea pig (Dunkin-Hartley) 5 animals/sex/dose for control group and 10 animals/sex/dose for treated group	Number of animals with positive reaction : For control group : 1/10, 0/10, 0/10, 0/10 respectively at 1 h, 6 h, 24 h and 48 h after challenge For treated group : 1/20, 1/20, 1/20, 0/19 respectively at 1 h, 6 h, 24	1 (reliable without restriction)	Registration dossier (study report, 1985)

Induction : ID : 0.1 ml. 25 % solution Challenge : epicutaneous : 0.5 ml. 50 % solution OECD TG 406	h and 48 h after challenge Not sensitising		
Mouse local lymph node assay (LLNA) Female mouse (CBA/CaOlaHsd) 5animals/dose Concentration : 0, 10, 25 or 34.6 % (equivalent to 0, 15, 37.5 or 51.9 mg) + positive control Vehicle : dimethyl sulphoxide OECD TG 429	Disintegrations per minute (DPM) : 5753, 6801, 10766, 9713 respectively at 0, 10, 25 and 34.6 % (for positive control : 45145) Stimulation index : 1, 1.2, 1.9, 1.7 respectively at 0, 10, 25 and 34.6 % (for positive control : 7.8) Not sensitising	1 (reliable without restriction)	Registration dossier (study report, 2005)
GPMT Guinea pig (Dunkin-Hartley) 10males/dose Concentration : 10 % Induction : ID : 0.1 ml Challenge : epicutaneous : 0.5 g OECD TG 406	Number of animals with positive reaction : 1st reading : 48 h : 0/10 2nd reading : 72 h : 0/10 Not sensitising	1 (reliable without restriction)	Registration dossier (study report, 1998)

Based on the results from the available studies, the eMSCA concludes that there is no concern for skin sensitisation and thus no further testing is needed.

7.9.4. Repeated dose toxicity

Table 27 : Summary of the repeated dose toxicity study

Studies	Reliability	LOAEL	NOAEL
Subchronic study in rats (Wistar), 90 days (Registration dossier (study report, 1979)) Doses : 0, 100, 500 and 2500	1	2500 ppm Males : 212.3 mg/kg bw/d Females : 266.69	500 ppm Males : 37.85 mg/kg bw/d Females : 54.20

ppm OECD TG 408		mg/kg bw/d	mg/kg bw/d
Chronic study in rats, 12 months (Anonymous, 2010) OECD TG 452 Not reported in the registration dossier	1	Males : 58 mg/kg bw/d Females : 71 mg/kg bw/d	Males : 21 mg/kg bw/d Females : 26 mg/kg bw/d
Combined subchronic toxicity and neurotoxicity screening study in rats, 14 weeks (Wahle & Sheets, 2004. cited in JMPR, 2008) OECD TG 408 and 424 Not reported in the registration dossier	3	Males : 183 mg/kg bw/d Females : 234 mg/kg bw/d	Males : 33 mg/kg bw/d Females : 41 mg/kg bw/d
Subacute study in mice, 28 days (Wahle, 2004. Cited in JMPR, 2008) Not reported in the registration dossier		Males : 356 mg/kg bw/d Females : not identified	Males : 90 mg/kg bw/d Females : 479 mg/kg bw/d
Subchronic study in mice, 90days (Wahle, 2004. Cited in JMPR, 2008) Not reported in the registration dossier		Males : 487 mg/kg bw/d Females : 1346 mg/kg bw/d	Males : 161 mg/kg bw/d Females : 663 mg/kg bw/d
Subacute study in rats, 30days (Anonymous, 2006) Not reported the registration dossier		8 mg/kg bw/d	8 mg/kg bw/d

Studies in rats :

A subchronic study (Registration dossier (study report, 1979)), following OECD TG 408 (Repeated dose 90-day oral toxicity in rodents), was conducted. 15 rats/sex/group were treated with 0, 100, 500 and 2500 ppm corresponding respectively to 0, 7.79, 37.85, 212.30 mg/kg bw/d in males and 0, 10.23, 54.20 and 266.69 mg/kg bw/d in females. The mortality and the food consumption were regarded but no modifications were observed. In the highest dose group, 2 males and 2 females exhibited slight convulsions. The body weight and body weight gain parameters in the low and mid-dose group were in the same range as in the control group. In a higher-dose group a significant lower body weight was observed: in the males for the entire study period and in the females in the majority of the observation dates (at the end of the study : in males, 301 g at the highest dose vs 330 g in control and in females, 191 g at the highest dose vs 182 g in control).

The haematology evaluation, made one month after start of study with 5 rats/sex/group, revealed only in males a dose related decrease of haemoglobin and haematocrit (the differences were significant ($p<0.05$) at 2500 ppm and also at 500 ppm for haematocrit). In addition the Mean corpuscular hemoglobin (MCH) and corpuscular volume MCV value were significantly lower at 2500 ppm. At the end of the study, the analysis indicated a statistically significant decrease of haemoglobin, haematocrit, MCH and MCV at 2500 ppm in males. The blood cells counts of the treated animals did not differ significantly from the controls.

Table 28 : Haematology evaluation in males (one month after start of the study and at the the end of the study)

	1 month after start of the study				End of the study			
Ppm	HGB (g/l)	HCT (l/l)	MCH (pg/e)	MCV (fl)	HGB	HCT	MCH	MCV
0	151	0.481	20.3	65	162	0.517	18.3	59
100	147	0.465	20.4	64	161	0.510	18.3	58
500	144	0.450*	20.2	63	155	0.488	18.2	57
2500	142*	0.449*	19.1*	61**	151*	0.475*	17.5*	55*

* : $p<0.05$, ** : $p<0.01$

The clinical chemistry did not reveal any dose related effects (cholesterol, ALT, ..). One and 3 months after start of study protein-bound-iodine (PBI) was determined and there were no significant differences between control and treated group observed.

At the highest dose group, the thymus, heart, spleen and testicule weights were significantly lower in males, whereas the lungs were found to be significantly lower in both sexes.

Table 29 : Summary of the absolute organ weight evaluation (in mg)

	Males				Females			
	0	100	500	2500	0	100	500	2500
BW (in g)	335	342	344	306**	195	195	187	184*
Thyroid	16	16	17	16	13	14	16	15
Thymus	339	323	339	287*	268	233	240	238
Heart	963	938	962	876**	628	631	605	589
Lung	1217	1190	1230	1106*	883	887	847	804**
Liver	11765	12106	12285	11693	6712	7088	6952	9775
Spleen	603	597	578	534*	426	424	421	382
Testis/ovary	3418	3308	3247	3215*	68	69	70	72

* : $p<0.05$; ** : $p<0.01$

The necropsy did not reveal any macroscopic modification whereas 2 males of the highest dose group showed signs of moderately defined centrolobular fat infiltration in the liver parenchyma cells.

According to the results, the NOAEL was of 500 ppm (corresponding to 37.85 mg/kg bw/d in males and 54.20 mg/kg bw/d in females) and the LOAEL was 2500 ppm (corresponding to 212.30 mg/kg bw/d in males and 266.69 mg/kg bw/d in females).

A chronic study was conducted in rats (Anonymous, 2010), following OECD TG 452 (one-year repeated oral toxicity study). The test substance 1,2,4-triazole was administered by feed during 12 months at 30 rats/sex/group . The dietary levels were 0, 125, 375, 1000 and 2000 ppm corresponding to 0, 6.9, 21, 58 and 113 mg/kg bw/d in males and 0, 8.3, 26, 71 and 136 mg/kg bw/d in females. A treatment-related lower body weights and lower body weight gains were observed in both sexes at the 2 highest dose groups (bwg difference of 8-19 % at 1000 ppm and 8-20 % at 2000 ppm). Mortality, clinical signs, food consumption, hematology, clinical chemistry, urinalysis, organ weight, gross pathology, estrous cycle staging, sperm analysis parameters were unaffected. The histopathology reveals test-substance–related morphologic changes in brain of both sexes in the highest dose group (decreased population of Purkinje cells within the cerebellar vermis). The micropathology examination of reproductive organs revealed no treatment-related effects. According to the results, the NOAEL was of 21 mg/kg bw/d in male and 26 mg/kg bw/d in female and the LOAEL was 58 mg/kg bw/d in male and 71 mg/kg bw/d in female.

A combined subchronic toxicity and neurotoxicity screening study in rats was conducted with the test-substance 1,2,4-triazole following OECD TG 408 (repeated dose 90-day oral toxicity in rodents) and 424 (neurotoxicity study in rodents) (Wahle, 2004). 20 rats/sex/group received during 14 weeks 0, 250, 500, 3000 or 1000/4000 ppm (1000 ppm for 4 weeks and 4000 ppm thereafter), corresponding to 0, 16, 33, 183 and 210 mg/kg bw/d in males and 0, 19, 41, 234 and 275 mg/kg bw/d in females. At the end of the study, body weight in the 2 higher dose group were lower than control (reductions compared to control of 7 %/6 % at 3000 ppm and 8 %/5 % at 1000/4000 ppm, respectively in males/females). A significant decrease of TSH value was noted in males at 500, 3000 and 1000/4000 ppm group, in females there was a decrease in all dose group however these decreases were only significant at 250 and 1000/4000 ppm. At the 2 highest doses, muscle fasciculations, tremor, gait incoordination, urine stain, red nasal and lacrymal stain, decreased activity in the open field were noted. At these 2 doses,a decreased absolute brain weight was observed and the micropathology examination showed a degeneration/necrosis in the cerebellum and an extensive loss of Purkinje cells, variable with matter degeneration and gliosis. There was also an increase incidence and severity of individual nerve fiber degeneration in the peripheral nerves (sciatic, tibial, sural) at these 2 doses (axon degeneration (fragmentation or lysis) often with macrophage response).

The mortality, food consumption, hematology and urinalysis parameters were unaffected.

Table 30 : Summary of the principal changes in the combined subchronic toxicity and neurotoxicity screening study (Anonymous, 2004)

	Males					Females				
ppm	0	250	500	1000 /4000	3000	0	250	500	1000 /4000	3000
TSH (ng/ml)	6.35	5.19	4.68 *	4.58*	4.14 *	7.48	4.36 *	6.43	4.47*	5.27
T4 (µg/dl)	4.61	4.54	4.37	4.02	3.99	2.49	2.89	2.89	2.71	3.22

T3 (ng/ml)	0.68	0.70	0.69	0.58	0.66	0.85	0.78	0.75	0.69	0.84
Cerebellum : NAD (by animals)	9/10		8/10	2/10	0/10	10/10		10/10		
Cerebellum : Degeneration/necrosis				10/10 *	9/10 *				10/10 *	10/10*

* : p<0.05

Based on the results, the NOAEL was 33 mg/kg bw/d in male and 41 mg/kg bw/d in female and the LOAEL was 183mg/kg bw/d in male and 234 mg/kg bw/d in female.

A subacute study (30days) was conducted in rats (Anonymous, 2006). The test substance 1,2,4-triazole was administrated by oral route in rats. The dietary levels were 0, 8, 57, 400 mg/kg/bw/d.

Decrease in adrenal weight was seen at 8 mg/kg bw/d. Slight hematology changes were observed at 57mg/kg bw/d and clinical signs (staggering, tremors, hunched) and decreased body weight at 400mg/kg bw/d.

Based on the results, the NOAEL was <8mg/kg bw/d and the LOAEL was 8 mg/kg bw/d.

Studies in mice :

A subacute study (28 days) was conducted in mice (Wahle, 2004). The test substance 1,2,4-triazole was administrated by feed at 15 mice/sex/dose. The dietary levels were 0, 50, 250, 500 and 2000 ppm.

No treatment-related effects were seen on survival, clinical signs, body weight, food consumption, haematological or clinical chemistry parameters, organ weights or on glossy observable lesions.

Males in the control and in the highest dose did not show loss of Purkinje cells in any of the cerebellar brain sections.

Some testicular or epididymal effects were observed in the highest dose group (see table 33).

Table 31: Overview testicular or epididymal effects

	0 ppm	50 ppm	250 ppm	500 ppm	2500 ppm
Testicular degeneration	3	ND	ND	ND	5
Apoptic bodies (testes)	2 (1)[a]	4 (1)	1 (1)	3 (1)	5 (1)
Spermatid degerenation/depletion/asynchrony (testes)	1 (1)	1 (1)	1 (1)	0	5 (1.4)
Focal tubular atrophy (testes)	1 (1)	2 (1)	1 (2)	2 (2)	4 (1.8)
Exfoliated germ cells/debris (epididymides)	0	1 (1)	1 (3)	0	3 (2)

ND : not determined

[a] : average severity score (1 minimal to 5 severe)

Based on the results, the NOAEL was 90 mg/kg bw/d in male and 479 mg/kg bw/d in female and the LOAEL was 356 mg/kg bw/d in male and not identified in female.

A subchronic study (90days) was conducted in mice (Wahle, 2004). The test substance 1,2,4-triazole was administrated by feed at 20 mice/sex/dose. The dietary levels were 0, 500, 1000, 3000 and 6000 ppm.

No treatment-related effects were observed on mortality, haematological or clinical chemistry parameters.

Treatment related clinical signs were observed, including tremors in both sex at the highest dose group, at these 2 doses yellow staining of the ventrum in males and food spillage in females and at 6000 ppm rough coat in males.

There were some body weight and organ weight changes (see table 34) and body weight gain was also modified (3.1/3.5, 3.6/3.1, 1.7/3.0, 1.1*/2.7, -3.1*/0.9* respectively at 0, 500, 1000, 3000 and 6000 ppm in males/females).

Table 32 : Body weight and organ weight.

Doses (ppm)	0	500	1000	3000	6000
Males					
Terminal bw (g)	37.3	37.0	36.4	34.9*	31.3*
Abs. brain weight (g)	0.488	0.491	0.476	0.465*	0.445*
Rel. brain weight (%)	1.328	1.378	1.365	1.376	1.462*
Abs. testis weight	0.253	0.247	0.233	0.233	0.219*
Females					
Terminal bw (g)	29.1	28.4	28.4	28.7	26.6*
Brain weight (g)	0.485	0.489	0.483	0.475	0.451*

* : $p<0.05$

The significant modification of the brain weight was correlated with a decrease of numbers of purkinje cells in the cerebella at 6000 ppm (15 males out of 20 and 10 females out of 18 vs 0 in control group). 9 males out of 11 and 1 female out of 3 showing tremors had also Purkinje cells loss.

An increase of total cytochrome P450 activity was seen at the highest dose group in both sexes. In liver tissue, 7-ethoxycoumarin deethylase (ECOD), 7-ethoxyresorufin deethylase (EROD) and aldrin epoxide (ALD) were observed in both sexes at 3000 and 6000 ppm after 4 weeks and at 6000 ppm after 13 weeks. A slight increase of UDP-glucuronyltransferase (GLU-T) activity was also seen at 6000 ppm. These modifications were not correlated with changes in liver weight or histopathology.

At 6000 ppm absolute testes weights decreased significantly (87 % of control). The same decreasing trend, but not significant, was observed at 1000 and 3000 ppm (92 % of controls).

Histopathological changes in the testes were observed. These include increased incidence of apoptotic-like bodies (4/20, 4/20, 7/20, 11/20 and 12/20, respectively at 0, 500, 1000, 3000 and 6000 ppm), spermatid degeneration, depletion, and asynchrony (5/20 and 15/20 of the males at 3000 and 6000 ppm, respectively), and minimal or slight focal tubular atrophy (2/20, 3/20, and 10/20 of the males at 1000, 3000, and 6000 ppm, resp.).

Changes in the epididymides (increased germ cells and debris in the luminal duct (10/20) and one male with aspermia) at 6000 ppm were considered as secondary to the testicular effects. Minimally increased apoptotic-like bodies and tubular atrophy seen at 1000 ppm were considered treatment-related but not adverse. Testicular atrophy at 1000 ppm was considered to be spontaneous due to their limited tissue distribution (focal and/or unilateral) and the lack of accompanying spermatid degeneration/depletion/asynchrony.

Based on the results, the NOAEL was 161 mg/kg bw/d in male and 663 mg/kg bw/d in female and the LOAEL was 487mg/kg bw/d in male and 1346 mg/kg bw/d in female.The registrant assessed that the substance is not toxic after repeated dose exposure.

Based on the available information, the eMSCA concluded that effects observed in the reproductive and in the neurological systems are of concern. Based on the available information, BE CA concluded that effects observed in the neurological systems are of concern. However, based on the RAC opinion to classify 1,2,4-triazole as Repr. 1B H360FD and based on the recent update in the registration status (from full registration to transported and on-site isolated intermediate under strictrictly controlled conditions), further investigations where deemed unnecessary. However this should be further investigated once there is a change in the scope of the dossier or if new information regarding this endpoint becomes available.

7.9.5. Mutagenicity

Table 33: Summary of the *In vitro* studies

Methods	Results	Remarks	Reference
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) *S. typh* TA 1535, TA 1537, TA 1538, TA 98 and TA 100 (with and without met. act.) Test conc. : 5, 50, 500 and 5000 µg/plate Test 1 & 2 : 15, 50, 150, 500 and 1500 µg/plate OECD TG 471	Evaluation of results : negative Test results : negative for *S. Typh* TA 1535, TA 1537, TA 1538, TA 98 and TA 100 with and without met. act.; cytotoxicity : yes (5000 µg/plate)	2 (reliable with restrictions)	Registration dossier (study report, 1985)
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) *S. typh* TA 1535, TA 1537, TA98 and TA 100 (with and without met. act.) Test conc. : pre-experiment: 1.0, 3.3, 10.0, 33.3, 100.0, 333.3, 1000.0 and 5000.0 µg/plate Experiment I & II : 10.0, 33.3, 100.0, 333.3, 1000.0	Evaluation of results : negative Test results : negative for *S. typh* TA 1535, TA 1537, TA 98 and TA 100 (with and without met. act.) ; cytotoxicity : yes (1000.0 and 5000.0 µg/plate	2 (reliable with restrictions)	Registration dossier (study report, 1989)

and 5000.0 µg/plate OECD TG 471			
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) *S. typh* TA 1535, TA 1537, TA 98 and TA 100 (with and without met. act.) Test conc. : 100, 500, 2500, 5000 and 7500 µg/plate EPA OPPTS 870.5100 – Bacterial reverse mutation test	Evaluation of results : negative Test results : negative for *S. typh* TA 1535, TA 1537, TA 98 and TA 100 (with and without met. act.) ; cytotoxicity : no	2 (reliable with restrictions)	Registration dossier (study report, 1981)
Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay (gene mutation) Chinese hamster Ovary (CHO) (with and without met. act.) Test conc. : 0, 2.7, 5.4, 10.8, 21.6, 43.2, 86.4, 172.8, 645.5 and 691 µg/ml OECD TG 476	Evaluation of results : negative Test results : negative for Chinese hamster ovary (with and without met. act.) ; cytotoxicity : no	1 (reliable without restriction)	Registration dossier (study report, 2007)
In vitro mammalian chromosome aberration test (chromosome aberration) Lymphocytes (with and without met. act.) Test conc. : 0, 172.8, 645.5 and 691 µg/plate OECD TG 473	Evaluation of results : negative Test results : negative for lymphocytes (with and without met. act.) ; cytotoxicity : no	1 (reliable without restriction)	Registration dossier (study report, 2007)

Three Ames tests were carried out with the test substance 1,2,4-triazole. No substantial increases in revertant colony number of any of the five tested stains were observed, either in the presence or absence of metabolic activation.

A chromosomic aberrations study on rat lymphocytes showed no significant increase in the frequencies of cells with aberrations in either the presence or absence of S9 activation.

A mouse lymphoma gene mutation test in the HPRT locus revealed no increase of the frequency of TGR mutants.

Table 34: Summary of the *In vivo* study

Methods	results	Remarks	Reference
Micronucleus assay (chromosome aberration) Mouse male/female Oral : gavage : 1200 mg/kg bw OECD TG 474	Evaluation of results : negative Test results : genotoxicity : negative ; toxicity : no effects	2 (reliable with restrictions)	Registration dossier (study report, 1985)

The *in vivo* mutagenic test indicated that the test substance 1,2,4-triazole shows no evidence of mutagenic potential on bone marrow cell toxicity when administered orally. The registrant concluded that 1,2,4-triazole is not mutagenic and the eMSCA agrees with this conclusion. Therefore there is no need for further mutagenicity testing.

7.9.6. Carcinogenicity

No data available.

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

Table 35: Summary of the reproductive studies

Method	Results	Remarks	Reference
developmental study (OECD TG 414) rats/Wistar 25 females / dose oral (gavage), GD 6-15 0, 10, 30, 100 mg/kg bw/day	Maternal toxicity: no mortality and no treatment-related clinical signs were observed. Bwg was statistically significant reduced (79.8 g vs 92.9 g in control group) during pregnancy at the highest dose. Food consumption was unaffected. Developmental toxicity: at 100 mg/kg, fetal weights were statistically significant reduced and the number of runts per litter was statistically significant increased. Malformations were observed in all groups. Microphtalmia/anophtalmia were observed in 5 animals in 5 different litters (1 control, 1 at 10 mg/kg, 3 at 100 mg/kg). False posture of right hind leg was observed in 1 animal at 30 mg/kg. Dysplasia and assymmetry of body of vertebrape and vertebral arches of thoracic spine and abnormal position of one rib was observed in 1 animal at 100 mg/kg. <u>Historical values (1984)</u> 6 microphalmia (1,1,4) /1344 foetuses	GLP key study Purity : 95.3 % Aqueous 0.5 % solution.	Renhof, 1988a (cited in JMPR, 2008)

	(170 dams, 8 studies). Historical values for the same lab and the same strain was reported in the next study. Maternal NOAEL: 30 mg/kg bw/day Developmental NOAEL : 30 mg/kg bw/day		
developmental study (OECD TG 414) rats/Wistar 25 females /dose oral (gavage), GD 6-15 0, 100, 200 mg/kg bw/day	<u>Maternal toxicity:</u> no mortality and no treatment-related clinical signs were observed. Bwg was statistically significant reduced 60.4g* vs 96.9g in control group) at 200 mg/kg during pregnancy. At 100 mg/kg bw/d, the reduction of bwg was not statistically signicant. Food consumption was comparable in all groups. <u>Developmental toxicity:</u> fetal and placental weights were statistically significantly reduced in treated animals and the number of runts per litter was statistically significantly increased. Fetal weight (in g) : 3.55, 3.06*, 2.35** respectively at 0, 100 and 200 mg/kg bw/d Placental weight (in g) : 0.59, 0.52*, 0.49** The number of foetuses with skeletal deviations was statistically significantly increased at 100 mg/kg. At 200 mg/kg, the number of surviving foetuses per dam was reduced and the number of resorptions per litter was increased (53.2 % vs 3.9 % in controls). Malformations were observed in all groups. Microphtalmia was observed in 2 animals of the same litter in controls (1 litter/21) (2 foetuses/253). Undescended testicle occurred in 2/253 (0.8 %), 11/226 (4.9 %) and 6/138 (4.3 %) foetuses at 0, 100 and 200 mg/kg. The incidence per litter was respectively 2/21 (9.5 %), 7/19 (36.8 %) and 5/25 (20 %). Hydronephrosis occurred in 1/253 (0.4 %), 1/226 (0.4 %) and 7/138 (5.1 %) at 0,100 and 200 mg/kg. The incidence per litter was respectively 1/21 (4.8 %), 1/19 (5.3 %) and 6/25 (24 %). Cleft palate was observed at 200 mg/kg in 4/138 (2.9 %) fetuses. The incidence per litter was 3/25 (12 %). Long bone dysplasia was observed at 200 mg/kg in 3/138 (2.2 %) fetuses. The incidence per litter was 3/25 (12 %). False posture of hind legs was observed	GLP supporting study Purity : 94 % Aqueous 0.5% solution	Renhof, 1988b (cited in JMPR, 2008)

	in 1 animal at 200 mg/kg. Incidence per fetuses: 0.7 %, incidence per litter: 4 %. Historical controls: 1) 1984 (8 studies, 170 dams, 1344 foetuses): Microphtalmia: 6 (1, 1, 4) Malformations of vertebral column (kink): 1 Malformations of long bone: 2 (1, 1) Internal hydrocephalus: 1 2) 1985 (16 studies, 221 dams, 2277 foetuses): Microphtalmia: 3 (1, 1, 1) Anophtalmia: 1 Exericephaly:1 Malformations of vertebral column: 2 Malformations of long bone: 1 Internal hydrocephalus: 1 Hydronephrosis:1 Undescended testicle:/ Maternal NOAEL: 100 mg/kg bw/day Developmental NOAEL : no NOAEL could be derived.		
developmental study (non-guideline) rats/Wistar-derived 10 females /dose oral (gavage), GD 7-17 0, 25, 100 mg/kg bw/day	Maternal observations were restricted to bw on days 1, 7-17 and 22. Offspring observations : litter weights of live pups on postnatal days 1 and 5, and the number of live and dead pups on these days. No specific examination for malformations was conducted. Under the study conditions, no effects were observed on maternal weight gain or on fetuses.	Non GLP study Purity and vehicle not reported. Raw data were not available. Results were reported in the JMPR evaluation.	Wickramaratne, 1987 (cited in JMPR, 2008)
developmental study (OECD TG 414) rabbits/NZW 25 females / dose oral (gavage), GD 6-28 0, 5, 15, 30, 45 mg/kg bw/day	Maternal toxicity : 5 moribund animals of the highest dose were sacrificed and one rabbit delivered on day 29 before scheduled sacrifice. These animals showed decreased food consumption and body weight loss. Clinical signs in the highest group were a decreased motor activity, clear perinasal substance, ptosis, excessive salivation, hyperpnoea. Body weight gains were significantly reduced for the entire study period at the highest dose (0.37 kg vs 0.65 kg in control) however body weight did not significant differ among the groups. A significant decrease in gravid uterine weight was also observed (0.46 kg** at the highest	Purity : 94 % Aqueous 0.5% solution Raw data were not available. Results were reported in the US EPA evaluation and in the JMPR	Hoberman, 2004 (cited in JMPR, 2008)

	dose vs 0.56 kg in control). There were no total litter losses. No effects on implantations, on the number of corpora lutea or on the number of viable fetuses were observed. One single dead fetus was noted in the group at 30 mg/kg bw per day. Developmental toxicity: a significant decrease in fetal weight was observed at the maternal toxic dose (45 mg/kg bw/d) (39.46 g/litters** vs 44.3 in control). At this dose, urinary tract malformations were observed in 4/157 foetuses from 2/19 litters : low set and small kidney(s) in three fetuses from the same litter; absent left kidney and absent left ureter in one of these same three fetuses and an absent kidney in one fetus from a different litter. There were no other dose-dependent or significant differences in the litter or fetal incidences of any gross external, soft tissues or skeletal alterations. Skeletal ossification averages per foetus per litter did not differ among the groups. There were no treatment-related effects on the fetal sex ratio. Maternal NOAEL: 30 mg/kg bw/day Developmental NOAEL : 30 mg/kg bw/day	evaluation	
2-generation study (OECD TG 416) rats/Wistar Hannover 30 animals/sex/dose oral (diet) 0, 250, 500, 3000 ppm (equivalent to 0, 15.4-17.5, 30.9-36.2, 188.6-217.9 mg/kg bw in P0) (equivalent to 0, 16-18.9 and 30-37.5 mg/kg bw in F1)	Parental toxicity No mortality and no treatment-related clinical signs were observed in any group. Bw was statistically significantly reduced at 3000 ppm in P0 males (-11.4 %) and females (-11.6 %). Bwg was also statistically significantly reduced at this dose in P0 males (33.2 %). Bwg was not evaluated in P0 females since 28 of 30 females were not pregnant. In F1, terminal bw was statistically significantly lower in males at 250 (94 %) and 500 (93 %) ppm but was not affected in females. Organ weights Brain weight was statistically significantly reduced at 3000 ppm in P0 males (95.9 %) and P0 females (94.8 %). Absolute and relative ovaries weight was statistically significantly increased at 3000 ppm in P0 females. The weight of the thyroid was also reduced in both sexes. Some other organs showed also a reduction in weight but not in both sexes (spleen in female, thymus in	GLP key study Purity : 99.9-101 % Mixed in ethanol before mixing in the diet.	Young and Sheets, 2005 (cited in JMPR, 2008)

	male). Histopathology Brain : minimal to marked degeneration/necrosis was observed in 30/30 males (2 marked, 22 moderate, 4 slight, 2 minimal) and 28/30 females (8 moderate, 14 slight, 6 minimal) in P0 at the highest dose. Ovaries : statistically increased number of total corpora lutea (corpora lutea produced from multiple oestrus cycles) at 3000 ppm. Effects on fertility The fertility index was statistically significantly reduced ($p \leq 0.01$) in P0 at 3000 ppm (7.1 % vs 76.7 % in controls). Only 2/28 inseminated females were pregnant. In these 2 pregnant females, one had 2 implantation sites and 1 live foetus, the other had 1 implantation site and 1 live foetus. The number of implantation was also statistically significantly reduced at 3000 ppm (3 vs 265 in controls) A decrease in the number of corpora lutea at the middle dose in P0 and F1 has been observed (P0 : 24.9 ± 7.1, 23.0 ± 6.8, 15.6 ± 8.3, 41.3 ± 6.5 in controls, 250, 500 and 3000 ppm) (F1 : 48.9 ± 7.9, 39.3 ± 7.4 in controls and 500 ppm) . The other reproductive parameters (as gestation index, estrous cycle length, gestation length) were not modified. F0 adults/F1 pups : number of litters : 22, 25, 25 and 2 respectively at 0, 250, 500 and 3000 ppm F1 adults/ F2 pups : number of litters : 27, 26, 25 respectively at 0, 250 and 500 ppm. The mean weight of the viable fetus were modified at D21 (47.6 g* at 500 ppm vs 50.2 g in control group). Concerning male fertility, a statistically significant decrease (74 %) in epididymal sperm counts was observed at 3000 ppm in P0. A slight decrease in testicular sperm counts was observed at all doses in P0 (88 %, 89 % and 85 % at 250, 500 and 3000 ppm), the decrease was statistically significant at the lowest and highest dose. The sperm count was not modified in the P1. The % of abnormal sperm was increased in		

	the P0 at 500 and 3000 ppm (1.4 and 1.5 respectively versus 0.8 in controls) as well as the % of detached sperm (1.6 and 2.8 versus 0.5). These values were within the historical values. NOAEL parental toxicity: 500 ppm, based on lower body weight and degenerative findings in the cerebellum at 3000 ppm. NOAEL fertility : could not be determined based on the decrease in testicular sperm counts at 250 ppm. NOAEL development : 500 ppm (the highest dose allowing assessment of the developmental effects).		

Discussion:

Development :

Developmental disturbances were observed in a developmental toxicity study performed in rats (Renhof M., 1988a). This study indicated an increased incidence of cleft palates (in 3/25 litters (12% litter incidence) at 200 mg/kg bw/d). In comparison with historical control data (1986-1989) only one case of cleft palate was reported in the year 1987 (litter incidence 4.17%) and only one case in 1989 (litter incidence 7.69%). Moreover, the high rate of resorptions (53%) observed at this highest dose may have masked the number of malformations, which are known to be very rare in rats. Additionally, in males, the incidence of cryptorchism was above the historical values at the treated dose group (100 and 200 mg/kg) in this developmental toxicity study in rats (in 2/253, 11/226 and 6/138 pups respectively at 0, 100 and 200 mg/kg bw/d) and an increase of pre and post-implantation losses was observed (number of implantation loss per dam : 0.5, 0.3 and 6.3** respectively at 0, 100 and 200 mg/kg bw/d). In conjunction, the mean number of foetus per dams was significantly decreased. Finally, a significant dose-related decrease in mean foetus weight was seen at 100 and 200 mg/kg bw/d. These severe effects are not explained by the maternal toxicity as the bw change appear only at the highest dose level.

Furthermore, in the other developmental toxicity study performed in rats (Renhof M., 1988b) a significant increased incidence of runts was noted at the highest dose level (100 mg/kg bw/d). In addition, the mean foetus weight was significantly decreased at 100 mg/kg bw/d compared to the control group. However, these effects appear at the same dose level as the bw modification.

In the prenatal developmental toxicity study in rabbits (Hoberman, 2004), an important increase in the mortality rate (20%) was observed at the highest dose (45 mg/kg bw/d). However, a dose-related increase in the incidence of dead or resorbed conceptuses per litter was already observed at 30 mg/kg bw/d (3.1, 4.7, 4.8, 6.4 and 7.0 respectively at 0, 5, 15, 30 and 45 mg/kg bw/d). This examination may have masked the number of malformations.

No conclusion about developmental toxicity can be drawn from the 2-generation toxicity study (Yound A.D. and Sheets L.P., 2005) as the severe decrease in the fertility index at 3000 ppm (corresponding to less than 235 mg/kg bw/d) lead to a premature termination of this dose level. Consequently, the highest dose for the F1-generation was 500 ppm corresponding to less than 40 mg/kg bw/d. Thus, a concern cannot be excluded.Cleft palate and hydronephrosis are malformations commonly observed with some triazole-derivatives.

Triazole-derivatives are antimycotic compounds used as fungicides in agriculture but also as antimycotic in human and veterinary medicine. The common target for all triazoles in fungi is the enzyme CYP51, involved in the steroid biosynthesis and therefore in the formation of the fungal walls.

In mammals, different Cytochrome P450 enzymes are also potential targets of the triazole-derivatives. Depending of the azoles and the tissue, different specific inhibition or induction have been described.

1,2,4-Triazole itself is a metabolite of different triazole-derivatives but the proportion of this metabolite may considerably differ from one compound to another. Amounts varying from 1 % to 65 % have been found in the urine of rats exposed to different azoles.

Cleft palate is a specific malformation implying a disturbance in the process of cranial morphogenesis and the most validated hypothesis to explain this disturbance is the alteration in the endogenous level of Retinoic Acid. Excess in Retinoic Acid is responsible of a series of malformations and resulted also in increased rates of fetal resoption and stillbirths. This excess of Retinoic Acid caused by at least some triazole-derivatives would be the consequence of the inhibition of the CYP26 family. These enzymes are expressed differently during embryonic development, depending of time and tissue.

To compare the teratogenic potential of different triazole-derivatives, different alternative *in vitro* models have been used.

Menegola *et al.* (Menegola *et al.*, 2001) have used the rat whole embryo model to investigate the teratogenic activity of different azoles. In one of the studies, 1,2,4-triazole was compared to two well-known teratogenic triazoles, flusilazole and fluconazole. These two triazole-derivatives compounds showed similar teratogenic effects as concentrations as low as 3.125 to 250 µM for flusilazole and 62.5 to 500 µM for fluconazole but no teratogenic activity was detected with 1,2,4-Triazole. Concentrations as high as 5000 µM induced only slight developmental retardation and blood discoloration.

Fertility:

In the 2-generation reproductive toxicity study performed in rats (Young A.D. and Sheets L.P., 2005), almost complete infertility was observed at the top dose (fertility index: 76.7, 83.3, 86.2 and 7.1 % at 0, 250, 500 and 3000 ppm, respectively) in the P generation only, considering the highest dose was not tested in F1. Therefore, clear evidence of an adverse effect on fertility at the top dose was shown. The adverse effect occurred together with other toxic effects. At the highest dose, mild to moderate brain cerebellar degeneration/necrosis was observed in both sexes in the parental generation. Nevertheless, the cerebellum is not involved in the reproductive axis (hypothalamic-pituitary-gonadal axis). Furthermore, maternal body weight during gestation period was statistically significantly reduced at the highest dose however, this modification could be explained by the low number of pregnant females (since 28 out of 30 dams were not pregnant). The adverse effects on fertility are not considered to be secondary non-specific consequences of systemic toxicity since systemic toxicity appeared to be minimal. Further more, adverse effects on fertility are supported by other effects observed in this study : increased incidence of uterus dilatation, reduction in epididymal sperm counts and reduction of normal sperm morphology percentage can also explain the adverse effects on fertility.

Furthermore, a few studies revealed histopathological modifications in testis. In a subacute toxicity study (Wahle B.S., 2004a), an increased incidence of spermatid degeneration/depletion/asynchrony was observed without any other signs of toxicity (no effects on survival, clinical signs, bw or organ weigth). This effect was confirmed by a subchronic toxicity study (Wahle B.S., 2004b) in which a statistically significant higher incidence of spermatid degeneration/depletion/asynchrony was also noted. In this last study, an increased incidence of tremors, yellow staining and rough coat were observed in

males at the highest dose, the bw was also modified however these effects are not considered severe enough to explain the important modifications observed in testis and epididymis. In a combined 90-day repeated dose toxicity study and neurotoxicity study (Wahle B.S. and Sheets L.P., 2004), a lower uterus weight and a slight increased number of corpora lutea were observed at the 2 highest dose levels (3000 ppm and 1000/4000 ppm). Simultaneously, a bw change was noted which was significant only at 3000 ppm).

Conclusion:

Based on the available information, the eMSCA concluded that effects observed in the reproductive systems is of concern. To address the concern for the reproductive toxicity (fertility and development), the BE CA submitted to the RAC a CLH dossier proposing a more stringent classification (Repr. 1B H360FD instead of Repr. 2 H361d). RAC agreed with the proposal of the BE CA and consequently the opinion of RAC has been adopted on 15 March 2019. This new classification however has not yet been included in the Adaptation to Technical Progress. More details can be found in the CLH report and the Annex I of the CLH report : https://www.echa.europa.eu/web/guest/harmonised-classification-and-labelling-previous-consultations/-/substance-rev/19733/term?_viewsubstances_WAR_echarevsubstanceportlet_SEARCH_CRITERIA_EC_NUMBER=206-022-9&_viewsubstances_WAR_echarevsubstanceportlet_DISS=true.
Regarding the concern for neurological system, based on the classification Repr. 1B H360FD and due to no or limited exposure to 1,2,4-triazole as a consequence of the change in the registration dossier status, no added value was found to continue.further investigations where deemed unnecessary. However this should be further investigated once there is a change in the scope of the dossier or if new information regarding this endpoint becomes available.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated.

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

Not evaluated.

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

Based on the available information, the eMSCA concluded that effects observed in the reproductive and in the neurological systems are of concern. To address the concern for the reproductive toxicity (fertility and development), the BE CA submitted to the RAC a CLH dossier proposing a more stringent classification (Repr. 1B H360FD instead of Repr. 2 H361d). RAC agreed with the proposal of the BE CA and consequently the opinion of RAC has been adopted on 15 March 2019. This new classification however has not yet been included in the Adaptation to Technical Progress.

Regarding the concern for the neurological system, based on the classification Repr. 1B H360FD and due to no or limited exposure to 1,2,4-triazole as a consequence of the change in the registration dossier status, further investigations where deemed unnecessary.

Previously identified concern for carcinogenicity was however not assessed due to lack of data.

7.10. Assessment of endocrine disrupting (ED) properties

OECD CF level 1: Existing data and non-test information

Table 36: Summary of OECD CF level 1 data

Non-test data			
Estrogen activity			
Method	**Result**	**Description of Result**	**References**
OECD QSAR Toolbox	negative	Non-ER binder (Relative Binding Activity <0.00001)	e-MS, 2015
Tox21_Aromatase_Inhibition	negative	Inhibition of aromatase using MCF-7 aro ERE cells stably transfected with testosterone receptor –responsive luciferase reporter	TOX21, 2015
ToxCast High-throughput H295R steroidogenesis assay	negative	Modified OECD TG 456 Negative outcome for all of the 10 measured hormones, incl. oestradiol and testosterone	iCSS ToxCast Dashboard (2016)

Estrogen receptor binding profiler (OECD (Q)SAR toolbox)

The eMSCA has run the OECD (Q)SAR toolbox for Grouping Chemicals into Categories (hereafter called the QSAR Toolbox) and concluded that 1,2,4-triazole is a non binder to ER reseptor.

Also included in the QSAR Toolbox is the ERES profiler developed by US-EPA, which is an effects-based automated system used to predict estrogen receptor binding affinity. ERES found no alerts for categorization of 1,2,4-triazole as estrogen receptor binder.

BE CA have not consulted commercial models for the evaluation of the endocrine disruption potential.

1,2,4-triazole is classified as a non-binder (Relative Binding Activity <0.00001) in the Expert System for Estrogen Binding Affinity in the 'Report of the expert consultation to evaluate an estrogen receptor binding affinity model for hazard identification' (figure 3 in OECD series on testing and assessment, number 111).

Interactive Chemical Safety for Sustainability (iCSS) ToxCast Dashboard

The iCSS ToxCast Dashboard is an interactive tool for distribution, visualization, and use of chemical screening data from the Toxicity Forecaster (ToxCast) project and the Toxicity Testing in the 21st century (Tox21) collaboration and integrates data from various sources, including:

- ToxCast and Tox21 - High-throughput chemical screening data
- ExpoCast - Chemical exposure data and prediction models
- DSSTox - High quality chemical structures and annotations
- PhysChemDB - Physical Chemical Properties Database
- CPCat - Chemicals listed by associated categories of chemical and product use

This database contains more detailed information on the assays than EDSP21 and includes more than only information on Endocrine disruption properties. Furthermore this database is updated occasionaly.

Estimated cytotoxicity threshold for 1,2,4-triazole = 161.93µM=11mg [log (161.93)=2.209]

- **Estrogen activity** (ERpathway : incl. ERtranscriptional activity, ER cofactor recruitement and dimerization, ER binding, estrogen-dependant cell proliferation assay).
  The ToxCast ER biological model integrates 18 in vitro ToxCast/Tox 21 assay endpoints for ER-based pathway activity.
  18 assays were performed with 1,2,4-triazole of which 2 were positive

Table 37 : ToxCast 21 assays on ER pathway

Assay ID (AR biological pathway mode, Judson et al, 2015)	Assay Name	Short Assay Description	Estrogen (E) or Anti-Estrogen (Anti-E) Signal Detection	Result
A1	NVS_NR_bER	Cell-free radioligand binding assay using bovine uterus-derived ER.	E/Anti-E	Negative
A2	NVS_NR_hER	Cell-free radioligand binding assay using human ER from MCF-7 cells.	E/Anti-E	Negative
A3	NVS_NR_mERa	Cell-free radioligand binding assay using mouse ERα	E/Anti-E	Negative
A4	OT_ER_ERaERa_0480	Fluorescent protein-fragment complementation assay measuring ERα homodimerization detected by microscopy in HeLa cells (8 hr).	E/Anti-E	Negative
A5	OT_ER_ERaERa_1440	Fluorescent protein-fragment complementation assay measuring ERα homodimerization detected by microscopy in HeLa cells (24 hr).	E/Anti-E	Negative
A6	OT_ER_ERaERb_0480	Fluorescent protein-fragment complementation assay measuring ERα heterodimerization with ERβ detected by microscopy in HeLa cells (8 hr).	E/Anti-E	Negative
A7	OT_ER_ERaERb_1440	Fluorescent protein-fragment complementation assay measuring ERα heterodimerization with ERβ detected by microscopy in HeLa cells (24 hr).	E/Anti-E	Negative
A8	OT_ER_ERbERb_0480	Fluorescent protein-fragment complementation	E/Anti-E	Negative

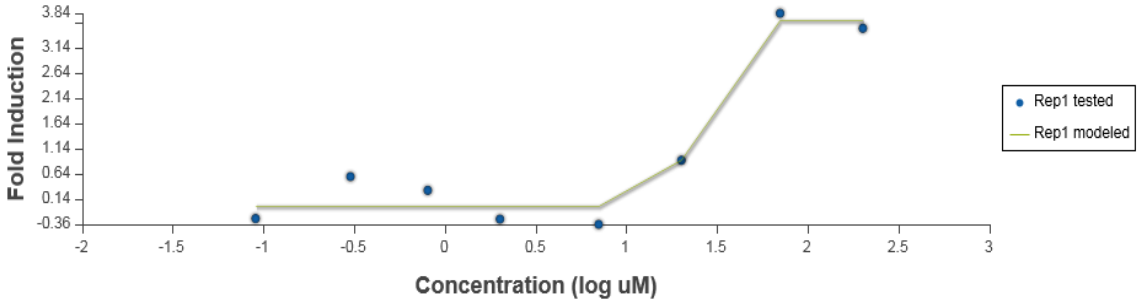
		assay measuring ERβ homodimerization detected by microscopy in HeLa cells (8 hr).		
A9	OT_ER_ERbERb_1440	Fluorescent protein-fragment complementation assay measuring ERβ homodimerization detected by microscopy in HeLa cells (24 hr).	E/Anti-E	Negative
A10	OT_ERa_EREGFP_0120	Fluorescent protein-fragment complementation assay measuring ERα binding to the estrogen response element detected by microscopy in HeLa cells (2 hr).	E/Anti-E	Negative
A11	OT_ERa_EREGFP_0480	Fluorescent protein-fragment complementation assay measuring ERα binding to the estrogen response element detected by microscopy in HeLa cells (8 hr).	E/Anti-E	Negative
A12	ATG_ERa_TRANS_up	Inducible exogenous ERα (ESR1) transcription factor activity via an inducible reporter in HepG2 cells (24 hr).	E	Positive
A13	ATG_ERE_CIS_up	Inducible endogenous ER transcription factor activity via an inducible reporter in HepG2 cells (24 hr).	E	Positive
A14	Tox21_ERa_BLA_Agonist_ratio	Inducible ERα transcription factor activity detected by a betalactamase estrogen response element reporter in agonist mode using HEK293T cells (24 hr).	E	Negative
A15	Tox21_ERa_LUC_BG1_Agonist	Inducible ERα transcription factor activity detected by a luciferaseestrogen response element fusion reporter in agonist mode using BG1 cells (48 hr).	E	Negative
A16	ACEA_T47D_80hr_Positive	ER-dependent cell proliferation in T47D cells	E	Negative
A17	Tox21_ERa_BLA_Antagonist_ratio	Inducible ERα	Anti-E	Negative

		transcription factor activity detected by a betalactamase estrogen response element reporter in antagonist mode using HEK293T cells (24 hr).		
A18	Tox21_ERa_LUC_BG1_Antagonist	Inducible ERα transcription factor activity detected by a luciferaseestrogen response element fusion reporter in antagonist mode using BG1 cells (48 hr).	Anti-E	Negative

Positive assays:

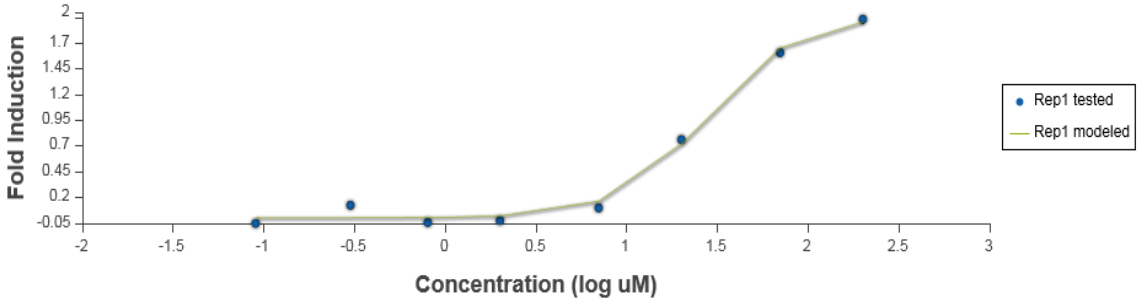
ATG_Era_Trans-up

Inducible exogenuous ERα (ESR1) transcription factor activity via an inducible receptor in hepG2 cells (24hr)



ATG_ERE_CIS_up

Inducible endogenuous ER transcription factor activity via an inducible receptor in hepG2 cells (24hr)



Results and conclusions of the ToxCast ER model (Judson et al, 2015)

Table 38: Results of ER AUC Model Activity

ER AUC Model Activity	Range of Positive Results	1,2,4-Triazole Result	Conclusion
Agonist	0.1-1	0.000127	Negative
Antagonist	0.1-1	0	Negative

- **Androgen activity** (AR pathway incl. androgen receptor transcriptional activation, AR cofactor recruitment and dimerization and AR binding)

  The ToxCast AR biological model integrates 11 in vitro ToxCast/Tox 21 assay endpoints for AR-based pathway.

  8 assays are reported in ToxCast/21 database for 1,2,4-triazole

  7 assays were negative and 1 assay was positive

Table 39: ToxCast/Tox21 Assays on AR pathway

Assay ID (AR biological pathway mode, Kleinsteuer et al, 2017)	Assay Name	Short Assay Description	Androgen (A) or Anti-Androgen (Anti-A) Signal Detection	Result
A1	NVS_NR_hAR	Cell-free radioligand binding assay using LnCAP-cell derived human AR.	A/Anti-A	NT
A2	NVS_NR_cAR2	Cell-free radioligand binding assay using recombinant Sf9-derived chimpanzee AR	A/Anti-A	NT
A3	NVS_NR_rAR	Cell-free radioligand binding assay using rat prostate-derived AR.	A/Anti-A	NT
A4	OT_AR_ARSRC1_0480	Fluorescent proteinfragment complementation assay measuring AR binding to cofactor SRC1 detected by microscopy in HeLa cells (8 hr).	A/Anti-A	Negative
A5	OT_AR_ARSRC1_0960	Fluorescent proteinfragment complementation assay measuring AR binding to cofactor SRC1 detected by microscopy in HeLa cells (16 hr).	A/Anti-A	Negative
A6	ATG_AR_TRANS_up	Inducible exogenous	A	Negative

		androgen receptor (NR3C4) transcription factor activity in HepG2 cells (24 hr).		
A7	OT_AR_ARELUC_AG_1440	Luciferase proteinfragment complementation assay measuring AR binding to the androgen response element detected by microscopy in CHOK1 cells (24 hr).	A/Anti-A	Positive
A8	Tox21_AR_BLA_Agonist_ratio	Inducible AR transcription factor activity detected by a beta-lactamaseandrogen response element reporter in agonist mode using HEK293T cells (24 hr).	A	Negative
A9	Tox21_AR_LUC_MDAKB2_Agonist	Inducible AR transcription factor activity detected by a luciferaseandrogen response element fusion reporter in agonist mode using MDA cells (24 hr	A	Negative
A10	Tox21_AR_BLA_Antagonist_ratio	Inducible AR transcription factor activity detected by a beta-lactamaseandrogen response element reporter in antagonist mode using HEK293T cells (24 hr).	Anti-A	Negative
A11	Tox21_AR_LUC_MDAKB2_Antagonist	Inducible AR transcription factor activity detected by a luciferaseandrogen response element fusion reporter in antagonist mode using MDA cells (24 hr).	Anti-A	Negative

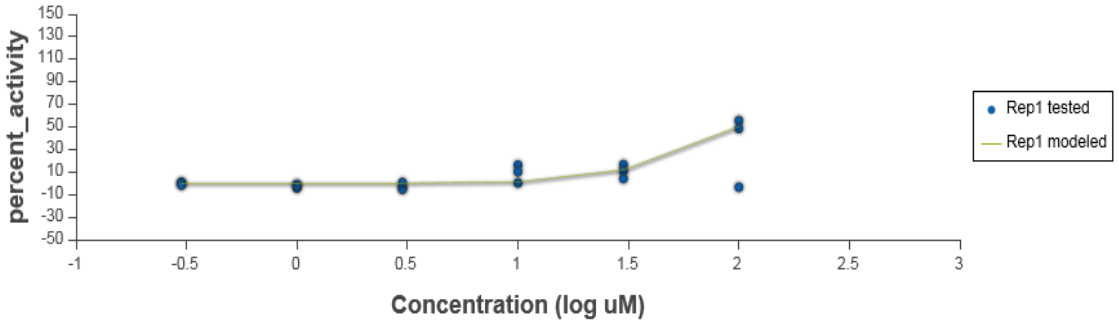
NT=not tested

Positive assay:

OT_AR_ARELUC_AG_1440

Luceferase protein –fragment complementation assay measuring AR binding to the androgen response element detected by microscopy in CHO-K1 cells (24hr) :

- 1 active : positive only at top concentration : concentration close to cytotoxicity threshold of 1,2,4-triazole



Results and conclusions of the toxCast AR model (Kleinstreur et al, 2015)

Table 40: Results of AR AUC Model activity

AR AUC Model Activity	Range of Positive Results	1,2,4-Triazole Result	Conclusion
Agonist	0.1-1	0	Negative
Antagonist	0.05-1	0	Negative

- **Aromatase** :
  - ToxCast high High-throughput Aromatase assays : In 2015 only 2 ToxCast/Tox21 assays (aromatase inhibition) were available of which 1 performed with 1,2,4-triazole which was negative.

Table 41: Results of available aromatase assays in 2015

Assay Name	Short Assay Description	steroidogenesis, S	Result
Tox21_Aromatase_Inhibition	Assay indicates inhibition of aromatase using human breast carcinoma MCF-7 aro ERE cells stablytransfected with a testosterone receptor-responsive luciferase reporter (24 hr).	S	Negative
NVS_ADME_hCYP19A1	Cell-free, biochemical assay of human CYP19A1 activity inhibition	S	NT

NT=Not Tested (In the ToxCast dashboard only studies with multi-concentration testing are shown. Because the screening assay NVS_ADME_hCYP19A1 used only a single, high concentration of 1,2,4-triazole this study was reported as NT. However the result of this study was negative (US EPA ToxCast database).

- **Steroidogenesis** :
- ToxCast High-throughput H295R assays (2016) :

Results of ToxCast steroidogenesis assay

3 hormones (dehydroepiandrosterone, pregnenolone and corticosterone) were excluded from the analysis because many samples contained concentrations below the lower limit of quantification).

Results are negative, meaning that there is no effect (stimulation and inhibition) seen on the 10 measured hormones.

Table 42: Results of available steroidogenesis assays in 2016

Assay Endpoint Name	Short Assay Description	Result
CEETOX_H295R_11DCORT_dn	Assessment of potential to inhibit 11-deoxycorticosterone biosynthesis in H295R cells	Negative
CEETOX_H295R_11DCORT_up	Assessment of potential to stimulate 11-deoxycorticosterone biosynthesis in H295R cells	Negative
CEETOX_H295R_OHPREG_dn	Assessment of potential to inhibit 17α-OH pregnenolone biosynthesis in H295R cells	Negative
CEETOX_H295R_OHPREG_up	Assessment of potential to stimulate 17α-OH pregnenolone biosynthesis in H295R cells	Negative
CEETOX_H295R_OHPROG_dn	Assessment of potential to inhibit 17α-OH progesterone biosynthesis in H295R cells	Negative
CEETOX_H295R_OHPROG_up	Assessment of potential to stimulate 17α-OH progesterone biosynthesis in H295R cells	Negative
CEETOX_H295R_ANDR_dn	Assessment of potential to inhibit androstenedione biosynthesis in H295R cells	Negative
CEETOX_H295R_ANDR_up	Assessment of potential to stimulate androstenedione biosynthesis in H295R cells	Negative
CEETOX_H295R_CORTISOL_dn	Assessment of potential to inhibit cortisol biosynthesis in H295R cells	Negative
CEETOX_H295R_CORTISOL_up	Assessment of potential to stimulate cortisol biosynthesis in H295R cells	Negative
CEETOX_H295R_DOC_dn	Assessment of potential to inhibit deoxycortisol biosynthesis in H295R cells	Negative
CEETOX_H295R_DOC_up	Assessment of potential to stimulate deoxycortisol biosynthesis in H295R cells	Negative
CEETOX_H295R_ESTRADIOL_dn	Assessment of potential to inhibit 17β-estradiol biosynthesis in H295R cells	Negative
CEETOX_H295R_ESTRADIOL_up	Assessment of potential to stimulate 17β-estradiol biosynthesis in H295R cells	Negative
CEETOX_H295R_ESTRONE_dn	Assessment of potential to inhibit estrone biosynthesis in H295R cells	Negative
CEETOX_H295R_ESTRONE_up	Assessment of potential to stimulate estrone biosynthesis in H295R cells	Negative
CEETOX_H295R_PROG_dn	Assessment of potential to inhibit progesterone biosynthesis in H295R cells	Negative
CEETOX_H295R_PROG_up	Assessment of potential to stimulate progesterone biosynthesis in H295R cells	Negative
CEETOX_H295R_TESTO_dn	Assessment of potential to inhibit testosterone biosynthesis in H295R cells	Negative
CEETOX_H295R_TESTO_up	Assessment of potential to stimulate testosterone biosynthesis in H295R cells	Negative

Discussion:

The ToxCast high-throughput steroidogenesis screening assay, which is a modified OECD TG 456 *in vitro* H295R steroidogenesis assay applied in the Karmaus *et al.* study [Karmaus *et al.*, 2016], is in several aspects not of equal value or directly comparable to the results produced by the OECD TG:

- all test samples are evaluated in duplicate rather than triplicate;

- 70% cell viability is accepted instead of 80%;
- concentration-dependent effects were evaluated using the ToxCast Data Analysis Pipeline rather than the OECD guideline recommended Dunnett's Test to identify significant effects in consecutive chemical concentration groups.
- pre-stimulation of the cells with forskolin (strong inducer) to induce steroidogenesis prior to chemical treatment.
- no validation process performed as described in OECD Guidance Document n°34.

The pre-stimulation of the cells with forskolin makes it difficult to find or measure effects of chemicals causing only a weak or moderate increase in hormone levels. This could explain why in Karmaus *et al.* [Karmaus *et al*., 2016] only very few cases of increased hormone levels were found. For 1,2,4-triazole rather an inhibitory effect is to be expected. In view of the eMSCA, the ToxCast steroidogenesis assay can be used to evaluate effects on the inhibition of hormone production, but it will be difficult to measure weak or moderate increase in hormone levels as the cells were already pre-stimulated before the addition of test chemical. The ToxCast steroidogenesis assay with 1,2,4-triazole was performed with a single high nominal concentration (99.9µM) at which no cytotoxicity has been observed. Other response concentrations were not further determined as no significant effects on hormone levels were seen at the maximum tolerated concentration (100µM) using a cutoff ≥1.5 fold change over DMSO controls on a per plate basis.

However in the OECD 456 guideline, the initial run is performed with spaced log 10 intervals with 1000µM being the maximum concentration (f.i.0.001, 0.01, 0.1, 1, 10, 100 and 1000µM), 10 fold higher than the single tested nominal concentration of 100µM in the ToxCast steroidogenesis assays.

Furthermore, in an OECD 456 study, when the substance is soluble, not cytotoxic and the initial run is negative, a second run should be performed to confirm these results. This was not the case in the high-throughput screening for 1,2,4-triazole.

Moreover, the delivery of exact volumes of solutions and samples into the wells during dosing is critical because these volumes determine the concentrations used in the calculations of assay results :

> As part of the ToxCast program, periodic quality control (QC) checks of the stock solutions of test compounds are performed. A notation for a QC check (T0) of a 20 mM stock solution of 1,2,4-triazole indicated a caution, that the measured concentration was lower than the theoretical (5 – 30%). It was confirmed by US EPA that only one stock solution was used to prepare the samples of 1,2,4-triazole used for the ToxCast assays and that during analytical quality control for the ^{1}H-NMR sample only a concentration of 1.3 mM was measured, whereas the expected concentration was 3 mM. This caution flag according to US EPA is thus also applicable for the concentration used in the steroidogenesis assay performed in the ToxCast/Tox 21 program.

No conclusion can be drawn on the stability of the stock solution based on the available data.

Taking the above into consideration, the eMSCA considered that this uncertainity on the exact concentration used for the ToxCast steroidogenesis assay with 1,2,4-triazole is crucial, because it suggests that the negative results obtained for this substance are the outcomes of a measurement with a much lower than expected single concentration (100 µM).

Therefore the eMSCA concluded that it is not unequivocally proven that 1,2,4-triazole does not demonstrate an effect on steroidogenesis in the 10-50 µM to 1000 µM range.. Hence

an *in vitro* steroidogenesis study according to OECD 456 was requested to address this concern.

Conclusion : OECD CF level 1 data

* Estrogen activity :

No or negligible receptor binding affinity

* Androgen activity :

No or negligible receptor binding affinity

* Steroidogenesis :

Unequivocal results : Negative results in ToxCast high throuput H295R assay but quality control analysis for the 1H-NMR suggest that the tested concentration was much lower than the expected single tested nominal concentration of 100µM.

OECD CF Level 2 : *In vitro* assays providing data about selected endocrine mechanisms/pathways

Table 43: Summary of OECD CF Level 2 *in vitro* assays data

Method	Short Method description	Description of results	References
Effect on oestradiol biosynthesis in rat granulosa cell aromatase activity Non-guideline Non GLP	0.25–0.63 x 105 viable immature rat granulosa cells incubated in the presence of human follicle-stimulating hormone (FSH) (100 ng/ml), testosterone (10-7 mol/l) and the test substance (10-5 mol/l) Purity : not reported	Levels of estradiol and progesterone were unaffected No modulating effect on ovarian estrogen biosynthesis	Wickings *et al.*, 1987
In vitro study : Rat embryos Non-guideline	9.5 days aged rat embryos exposed *in vitro* to 500, 1500, 2500, and 5000 µM Solvent : ethanol Purity : not reported	A significant reduction of VYS diameter, crown-rump length, somite number, and total score was found in embryos exposed to triazole 5000 µM Exposure to 1500, 2500, and 5000 µM triazole showed an increase in cellular death at the level of the mesenchyme of the maxillary processes and branchial arches (in particular, at the level of branchial arch II).	Menegola *et al.*, 2001
In vitro study	triazole-derivatives	Triazole itself induced only slight signs of developmental retardation when tested at very high concentration (5000µM) Malformations specifically localised at the level of the pharyngeal apparatus in 100% of embryos exposed to flusilazole, triadimenol and triadimefon after 48 h of culture Hypothesis : perturbation of retinoic acid	Menegola *et al.*, 2005 Used for WoE
In vitro study	OECD 456 H295R Steroidogenesis Assay	1,2,4-Triazole did not alter estradiol and testosterone release in H295R cells	Registration dossier (study report, 2018)

1. Estrogen Activity

No data available.

2. Androgen Activity

No data available.

3. Thyroid Hormone Activity

No data available.

4. Steroidogenesis

Wickings *et al.*, 1987

10^{-5}M of 1,2,4-triazole was screened for its effect on oestradiol and progesterone production by 0.25–0.63 x 10^5 viable immature rat granulosa cell cultures incubated for 48h with hFSH (100ng/ml) and testosterone (10-7M). No cytoxicity was observed at this concentration. Steroid accumulation is expressed as a percentage of accumulation in control cultures (treated with hFSH and testosterone without the test substance). 1,2,4-triazole showed <15% inhibitory effects on oestradiol production. It can be concluded that 1,2,4-triazole has no modulating effect on ovarian estrogen biosynthesis.

eMSCA comments :

It should be however underlined that this study deviates from the validated OECD CF level 2 aromatase study (OPPTS 890.1200, Human Recombinant) :

- Only a single concentration of 10µM was tested while 8 concentrations (range from 10^{-10}M to 10^{-3}M) should be tested in OPPTS 890.1200
- Lack of measurement of testosterone

H295R Steroidogenesis Assay (OECD TG 456) (registration dossier, Study report 2018)

A steroidogenesis assay was performed according to OECD TG 456 to examine whether 1,2,4-triazole has the potential to inhibit some enzymes involved in the steroidogenesis pathway and thus affect T and E2 production.

1,2,4-triazole was tested in 3 individual runs using 24-well plates. H295R cells were exposed for 48h to seven concentrations (0.001,0.01, 0.1, 1, 10,100 and 1000µM). Forskolin, a strong inducer and prorochloraz, a strong inhibitor were used as positive control for resp. induction and inhibition. Doses were administered in DMSO at 0.1% v/v per well. All test concentrations, as well as the control and solvent control were tested in triplicate.

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MIT) assay and hormone analysis was conducted with a Testerone and a Estradiol enzyme Immuno assay : ELISA-kit. 1,2,4-triazole did not interfere with the estradiol and testosterone EIAs. Compared to the basal hormone production, the interference of 1,2,4-Triazole with estradiol and testosterone hormone measurements was 18% and 7%, respectively.

The steroidogenesis study is considered valid as all acceptability criteria were met.

Cell cytotoxicity was not observed up to 1000µM.

In a 1st run no effect was observed on estradiol production. Testosterone production however was significantly inhibited at 0.1µM and significantly induced at 100µM when compared to the solvent control. This run is however considered equivocal (no statistical significant fold change at at least two adjacent concentrations) and thus the test had to be repeated by a 2nd run with refinement of the test concentrations.

A 2nd run was perfomed using 0.03, 0.1, 0.3, 30, 100, 300 and 1000µM of 1,2,4-triazole. This run confirmed the results of the 1st run as to the estradiol production but did not confirm the result on testosterone production. Only a significant inhibition of testosterone production was observed at a concentration of 0.03µM.

Therefore a 3rd run was conducted. The initial 3rd run (a) was performed with the same concentrations of the 2nd run, while OECD 456 prescribes that this run should be conducted using the original test conditions of the 1st run. Therefore another 3rd run (b) was performed using the correct concentrations. In this run no significant effect was seen neither on estradiol nor on testosterone production.

Equivocal results in the 1st run are considered negative if the observed effects are not confirmed in any of the two subsequent runs. Therefore it can be concluded that 1,2,4-triazole had no significant effect on the estradiol and testosterone release in H295R calls and thus did not affect the steroidogenesis pathway.

5. Other hormonal pathways : retinoic acid

Menegola *et al.*, 2001

In an *in vitro* study, whole rat embryos (9.5 days of age) were exposed to 500, 1500, 2500, and 5000 µM triazole for 48h.

At the highest dose a significant reduction of visceral yolk sac diameter (VSY), crown-rump length, somite number, and total score was found in the embryos.

In the exposure group of 2500 and 5000µM, all embryos showed an anemic VYS (significant diference to the control and to the control with etahanol), This effect can be related to generic embryotoxicity.

Embryos exposed to 1500, 2500, and 5000 µM triazole showed an increase in cellular death at the level of the mesenchyme of the maxillary processes and branchial arches (in particular, at the level of branchial arch II).

Menegola *et al.*, 2005 :

A study on fungicides triazole-derivatives (triazole itself was not tested) revealed malformations specifically localised at the level of the pharyngeal apparatus in 100% of embryos exposed to flusilazole, triadimenol and triadimefon after 48h of culture. A fusion between the first and the second pharyngeal arches and the first and the second aortic arches were noted. Whereas, triazole itself induced only slight signs of developmental retardation when tested *in vitro* at very high concentration (5000µM). A hypothesis pathway is the implication of a perturbation of retinoic acid (hindbrain segmentation is defined by the retinoic-acid-controlled differential switching on of specific Hox genes). Reduction and fusion of brachial arches and branchial nerves, (neural crest cells) NCC migration alterations were the abnormalities observed after triazole exposure, same modifications are observed after exogenous retinoic acid exposure.

In vitro studies show the teratogenic activity by different triazole-derivatives. However, only a few and contrasting data are available from experimental and clinical *in vivo* exposure. The cleft palate andcranio facial malformations were present at dose levels which also reveal a maternal toxicity, but it is unlikely that these effects are secondarily mediated by maternal toxicity. The direct implication of perturbations during hindbrain segmentation, NCC migration and brachial arches formation could explain such specific cranio-facial effects.

Conclusion : OECD CF level 2 data

* Estrogen activity :

No information available

* Androgen activity :
No information available

* Thyroid activity :
No information available

* Steroidogenesis :
The steroidogenesis pathway is not affected after exposure to 1,2,4-triazole up to 1mM : no effect on estrogen and testosterone production was observed.

*Development :
In an *in vitro* study, rat embryos exposed to 1500, 2500, and 5000 mM triazole showed an increase in cellular death at the level of the mesenchyme of the maxillary processes and branchial arches (in particular, at the level of branchial arch II).

7.10.1. Endocrine disruption – Environment

OECD Level 3 : *in vivo* assay providing data about selected endocrine mechanism(s)/pathway(s)

No data available

OECD Level 4 : *in vivo* assays providing data on adverse effects on endocrine relevant endpoints

No data available

OECD Level 5 : *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism

No data available

7.10.2. Endocrine disruption - Human health

OECD Level 3 : *in vivo* assay providing data about selected endocrine mechanism(s)/pathway(s)

Steroidogenesis

Zarn *et al.*, 2003 :

Review on *in vitro* and *in vivo* data of azole compounds on sterol 14α-demethylase and aromatase.

Table 44 : Summary of OECD level 3 *in vivo assay data*

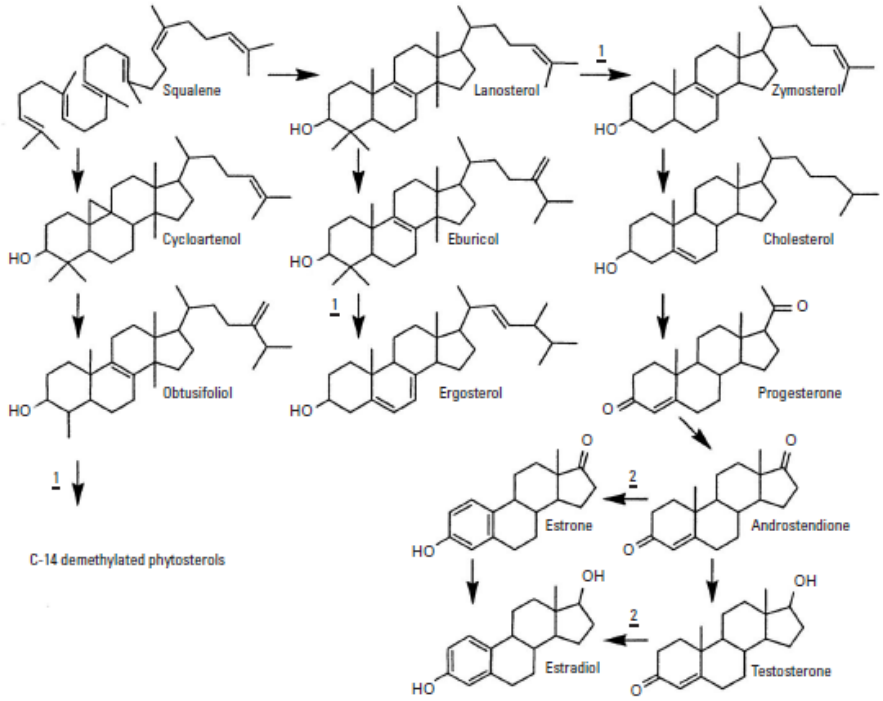
Method (guideline)	Short description of Method	Description of Result	References	Reliability
Review of *in vitro* and *in vivo* data on sterol 14α-demethylase and aromatase	Azole compounds	many azole compounds developed as inhibitors of fungal sterol 14α-demethylase are inhibitors also of mammalian sterol 14α-demethylase and mammalian aromatase with unknown potencies	Zarn *et al*., 2003	Used for WoE

There are 2 classes of azole compounds : triazoles and imidazoles. Their antifungal activity has however the same molecular mechanism: disturbance of the ergosterol synthesis. The targeted enzymes of the azole compounds are the sterol 14α-demethylase and the aromatase.

Sterol 14α-demethylase (encoded by the CYP51 gene) : these enzyme is a member of the family of heme-containing cytochrome P450 enzymes. The sterol 14α-demethylase play different roles depending on the species. In fungi, its reaction leads to an important precursor of ergosterol (essential component of the membranes). In plants, it acts by metabolising obtusifoliol and provides precursors for biosynthesis of phytosterols. And in animals, its reaction is a part of the metabolic pathway leading to the biosynthesis of cholesterol which is the substrate for the production of many other sterols as sex steroid hormones.

Aromatase (encoded by the CYP19 gene) : it's another P450 enzyme involved in the steroidogenesis. Aromatase catalyzes the oxidative demethylation of sterols (demethylates C10) and specifically converts androstenedione and testosterone, resulting in estrone and estradiol, respectively.

Figure 1: Biosynthesis of phytosterols, ergosterol and mammalian sex steroid hormones. Reactions are catalysed by sterol 14α-demethylase (1) and aromatase (2).

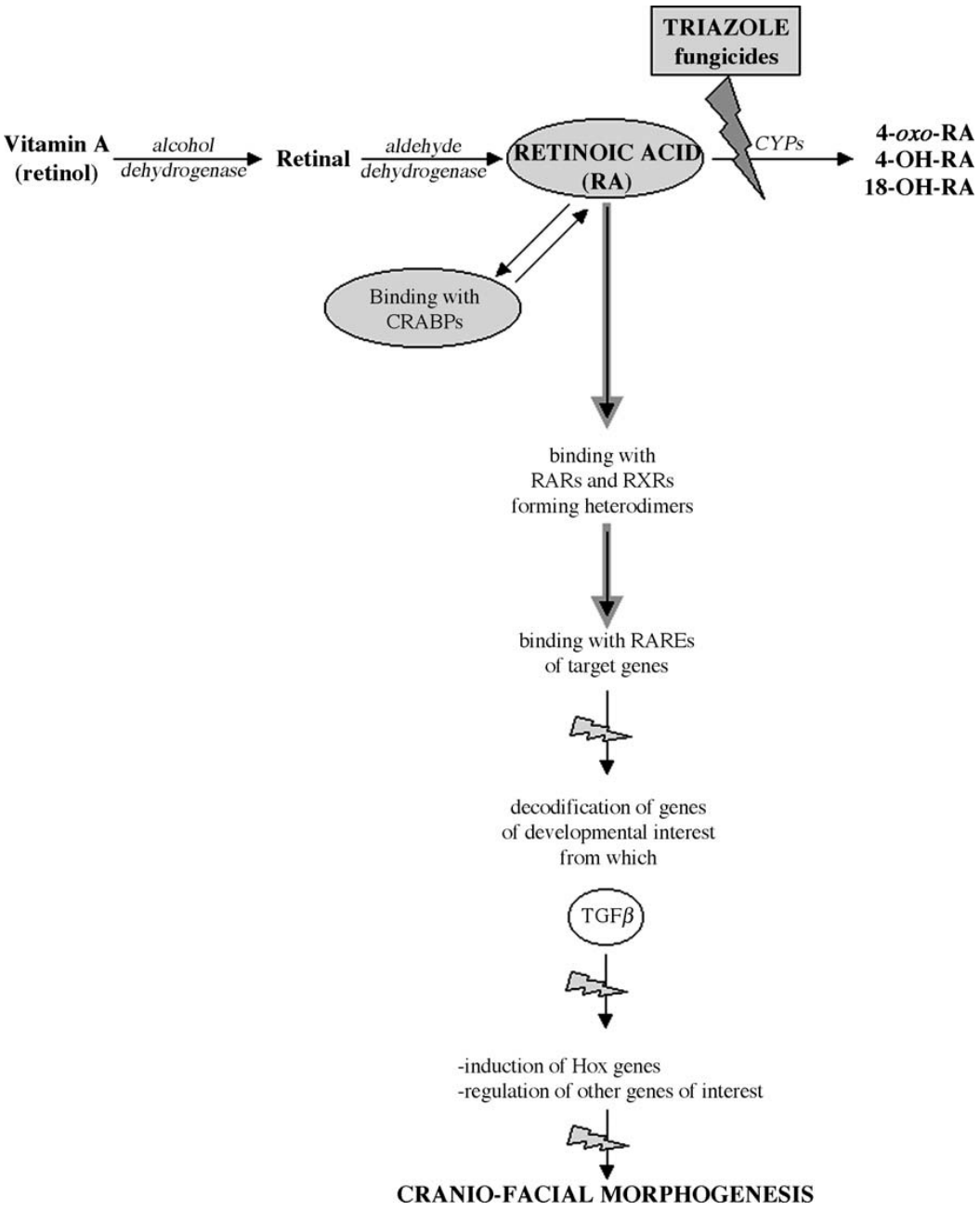


Due to the inhibitory activity of azole compounds on key enzymes involved in sex steroid hormone synthesis, it is likely that effects on fertility, sexual behaviour, and reproductive organ development will occur depending on dose level and duration of treatment of laboratory animals.

Other hormonal pathways : Retinoic acid

Menegola *et al*., 2006 :

Figure 2: Possible pathogenic pathway of triazole-derivative-related teratogenicity



Azole compounds (triazole- and imidazole-derivatives) play a key role as antifungals in agriculture and in human mycoses and, as non-steroidal antioestrogens, in the treatment of oestrogen-responsive breast tumours in postmenopausal women. The antifungal activity results on their capability to cause permeability changes and malfunction of membrane by interfering with steroid biosynthesis.

In vitro studies on effects in mammalian embryos showed that a specific perturbation of the CYP26 mediated degradation of retinoic acid during early embryonic development could be the cause of triazole-derivative-related teratogenic effects. Some tests revealed modifications on the brachial arches as an impaired of the brachial arch morphogenesis in the mouse embryos cultured, a reduction, agenesis and fusion between first and second brachial arches in rat embryos cultured. There are 6 brachial arches and the 2 first are involved in cranio-facial morphogenesis.

In vivo studies on effects in mammal development indicated that treatment of pregnant mice dams with triadimefon (300 mg/kg) during the early development stages induced abnormal hindbrain organisation and abnormal NCC (neural crest cells) localisation at the

level of the brachial arches at midgestation. At the end of gestation, severals modifications were observed as cleft palate, ectopic maxillary cartilage, … . A treatment of fluconazole on GD 10 (175 mg/kg) in mice revealed dose-dependent teratogenic effects as well. The effects observed in these studies correspond to those reported after all-trans-retinoic acide exposure *in vivo*.

Antimytotic triazole-derivatives are teratogenic in rodents using doses not toxic for adults and the mechanism or pathogenic pathway are the same between the *in vitro* and the *in vivo* studies. Moreover, the suggested mechanism involves an abnormal expression of genes controlled by retinoic acid.

Conclusion : OECD CF level 3 data

* Estrogen activity :
No information available

* Androgen activity :
No information available

* Thyroid activity :
No information available

* Steroidogenesis :
WoE : The targeted enzymes of the **azole compounds** are the sterol 14α-demethylase and the aromatase. These enzymes are involved in sex steroid hormone synthesis. Therefore it is likely that effects on fertility, sexual behaviour, and reproductive organ development will occur.

* Other hormonal pathway : retinoic acid
Several teratogenic effects were seen during embryonal development in rats after exposure to **azole-derivates** :
-abnormal hindbrain organisation and abnormal NCC (neural crest cells) localisation at the level of the brachial arches at midgestation
- several modifications (i.e.: cleft ptalate, ectopic maxillary cartilage,…) at the end of gestation
Specific perturbation of CYP26 mediated degradation of retinoic acid considered as a possible hypothesis for the MoA.

OECD Level 4 : *in vivo* assays providing data on adverse effects on endocrine relevant endpoints

Table 45: Summary of OECD Level 4 *in vivo* assay data

Method (guideline)	Short description of Method	Description of Result	References	Reliability
28d Oral toxicity Oral (diet) Non-guideline Non-GLP	CD-1 {[ICR]/BR} mice 15 males and 15 females per dose 0, 50, 250, 500 and 2000 ppm (equal to M : 0, 9, 47, 90, 356 mg/kg bw/d and F : 0, 12, 60, 120, 479 mg/kg bw/d) Purity 99.9%	Testicular degeneration at 356 mg/kg bw/d	Wahle, 2004a	2
30d oral toxicity Oral (gavage) Non guideline Non GLP	Rat 0, 8, 57, 400 mg/kg bw/d	8 mg/kg bw/d based on decrease in adrenal weight	Anonymous, 2006	3
90d repeated dose toxicity Oral (diet) OECD408/US EPA OPPTS 870.3100 GLP	CD-1 {[ICR]/BR} mice 20males and 20 females per dose 0, 500, 1000, 3000 or 6000 ppm (equal to M : 0, 80, 161, 487, 988 mg/kg bw/d and F : 0, 105, 215, 663 and 1346 mg/kg/d) Purity 99.9%	161 mg/kg bw/d : decrease in testicular weight and microscopic testicular changes	Wahle, 2004b	1

90d Repeated dose toxicity Oral (diet) Similar to OECD TG 408 Non-GLP	Wistar rats 15 males and 15 females per dose 0, 100, 500 and 2500 ppm (equal to M : 0, 7.8, 37.9, 212.3 mg/kg bw/d and F : 0, 10.2, 54.2, 266.7 mg/kg bw/d) Purity 99.6%	Up to the dose of 2500 ppm: no significant effect on protein-bound iodine, rectal temperature, and thyroid gland absolute weight. No histopathological indications of treatment-induced alterations up to 2500 ppm for the endocrine glands: pancreas, pituitary, thyroid, adrenals, testicles, ovaries, uterus, and thymus	Registration dossier (Study report, 1979)	2
90d Combined toxicity/neurotoxicity study Oral (diet) OECD TG 424 US EPA OPPTS 870.3100/870.6200 GLP	Wistar rats (Crl:WI[Glx/BRL/Han]IGS BR) 20 males and 20 females per dose 0, 250, 500, 3000, 1000/4000 ppm (equivalent to M: 16, 33, 183, 210 mg/kg bw/d and F: 19, 41, 234, 275 mg/kg bw/d) Purity 99.9%	33 mg/kg bw/day (male) based on decrease of thyroid stimulating hormone (TSH)	Wahle and Sheets, 2004	3
Chronic toxicity study (52 weeks) OECD TG 452 Oral (diet) GLP	Wistar rats (Crl:WI(Han)) 30 rats/sex/group 0, 125, 375, 1000, or 2000 ppm Purity : 98.5 % (12/07); 99.1 % (01/10)	Treatment related lower body weights and lower body weight gains were observed in both sexes at the 2 highest dose groups (bw gain difference of 8-19% at 1000ppm and 8-20% at 2000ppm). The histopathology reveals test-substance–related morphologic changes in brain of both sexes in the highest dose group (decreased population of Purkinje cells within the cerebellar vermis).	Anonymous, 2010	1

		The micropathology examination of reproductive organs revealed no treatment-related effects.		
Developmental toxicity Non-guideline Oral On day 7 to 17 of gestation Non-GLP	rats (Alpk: AP [Wistar-derived]) 10 pregnant rats 0, 25 or 100 mg/kg bw per day Purity : not reported	Maternal observations were restricted to bw on days 1, 7-17 and 22. Offspring observations : litter weights of live pups on postnatal days 1 and 5, and the number of live and dead pups on these days. No specific examination for malformations was conducted. Under the study conditions, no effects were observed on maternal weight gain or on fetuses.	Wickramaratne, 1987	3
Prenatal Developmental Toxicity Study US EPA OPPTS 870.3700 Oral (gavage) On day 6 to 15 of gestation GLP	rats [Bor:WISW (SPFCpb)] 25 pregnant females per dose 0, 10, 30 and 100 mg/kg bw/d Purity : 95.3 %(1) in aqueous 0.5% (w/w) Cremophor EL	100 mg : Decrease body weight gain of dams and foetal weight Increase incidence of runts No effect observed at 30 mg/kg/d	Renhof, 1988a	1
Prenatal Developmental Toxicity Study US EPA OPPTS 870.3700 Oral (gavage) On day 6 to 15 of gestation	rats [Bor:WISW (SPFCpb)] 25 pregnant females per dose 0, 100 and 200 mg/kg bw/d Purity : 94 %(2)	Undescended testes were observed in foetuses at 0, 100 and 200 mg/kg: 2/253 (0,8 %), 11/ 226 (4,9 %) and 6/138 (4,3 %) Also at 200 mg/kg, increased resorptions, cleft palate (4/138) and decreased number of viable foetuses	Renhof, 1988b	1

GLP	in aqueous 0.5 % (w/w) Cremophor EL	NOAEL : 100 mg/kg bw/d for maternal toxicity and <100 mg/kg bw/d for developmental toxicity		
Oral (Stomach Tube) Prenatal Developmental Toxicity Study US EPA OPPTS 870.3700 on days 6 through 28 GLP	Rabbit New Zealand White [Hra:(NZW)SPF] 25 rabbits per dosage group 0, 5, 15, 30 and 45 mg/kg/day Purity : 99.9 % in aqueous 0.5 % (w/w) carboxymethylcellulose (CMC)	Important increase in the mortality rate (20%) was observed at the highest dose (45 mg/kg bw/d). Dose-related increase in the incidence of dead or resorbed conceptuses per litter was already observed at 30 mg/kg bw/d (3.1, 4.7, 4.8, 6.4 and 7.0 respectively at 0, 5, 15, 30 and 45 mg/kg bw/d). This examination may have masked the number of malformations.	Hoberman A.M., 2004	1

28 d oral toxicity study: Wahle, 2004a

Guideline : non-guideline
GLP : non-GLP

Material and methods
Test type : Repeated dose toxicity : oral
Test animals : CD-1 {[ICR]/BR} mice
15 males and 15 females per dose
Age at study initiation: ±8 weeks
Administration/exposure :
- route of administration : oral (diet)
- Duration and frequency of test/exposure period : 28d, daily *ad libitum*
- Doses : 0, 50, 250, 500 and 2000 ppm
equal to M: 0, 9, 47, 90, 356 mg/kg bw/d
F : 0, 12, 60, 120, 479 mg/kg bw/d
- Post exposure observation period : /
- Vehicle : ethanol
- Test substance : 1,2,4-triazole, purity : 99.9 %
The stability, homogeneity and dietary concentrations were confirmed analytically : -diets were prepared weekly and were stable for 7d at
room temperature
-homogenetiy was within acceptable range
-measured test conc. : 96-99% of target conc.

Method :
- Observation for mortality and clinical signs : daily
- Detailed clinical examinations : weekly
- Body weight and food consumption : weekly
- Hematology and clinical chemistry measurements : at termination
- Gross pathological examination
- Weight measurements of selected organs
- Histopathological examination of selected tissues : from the control and the highest dose group

Statistical methods :

- Bartlett's test (Snedecor and Cochran, 1967) was used to evaluate continuous data for equality or homogeneity of variance

- One-way analysis of variance (ANOVA) (Snedecor and Cochran, 1967) followed by Dunnett's test (Dunnett, 1955, 1964)to furher analyse group means

- In the event of unequal variances, and at the discretion of the study director, data were subject to non-parametric procedures consisting of a Kruskal-Wallis ANOVA (Hollander and Wolfe, 1973) followed by the Mann-Whitney-U test for between-group comparisons.

- Frequency data were initially examined for trends; data suggestive of a potential effect were then statistically evaluated using the Fisher exact tests.

- For the Bartlett test, a probability (p) value < 0.001 was considered significant; for all other statistical tests, differences with p values < 0.05 were considered statistically significant.

- All statistical evaluations were performed using software obtained from either INSTEM Computer Systems (Stone, Staffordshire,UK) or SAS Institute Inc. (Cary, NC).

Results and discussion :

No treatment-related effects were seen on survival, clinical signs, body weight, food consumption, haematological or clinical chemistry parameters, organ weights or on glossy observable lesions.

Males in the control and in the highest dose did not show loss of Purkinje cells in any of the cerebellar brain sections.

Some testicular or epididymal effects were observed in the highest dose group.

Table 46: Summary of changes of testicular or epididymal parameters.

	0 ppm	50 ppm	250 ppm	500 ppm	2500 ppm
Testicular degeneration	3	ND	ND	ND	5
Apoptic bodies (testes)	2 (1)[a]	4 (1)	1 (1)	3 (1)	5 (1)
Spermatid degerenation/depletion/asynchrony (testes)	1 (1)	1 (1)	1 (1)	0	5 (1.4)
Focal tubular atrophy (testes)	1 (1)	2 (1)	1 (2)	2 (2)	4 (1.8)
Exfoliated germ cells/debris (epididymides)	0	1 (1)	1 (3)	0	3 (2)

ND : not determined
[a]() : average severity score (1 minimal to 5 severe)

LOAEL = 356 mg/kg bw:d (male); the LOAEL for female mice was not identified.

NOAEL =90 mg/kg bw/d (male) and 479 mg/kg bw/d (female)

30 days oral toxicity study (Anonymous, 2006, US EPA memorandum)

Guideline : Non-guideline
GLP : non-GLP

Material and methods
Test type : Repeated dose toxicity : oral
Test animals : rats : strain : no info reported in the US EPA memorandum, 2006
males and females per dose : no info reported in the US EPA memorandum, 2006
Age and weight at study initiation : no info reported in the US EPA memorandum, 2006
Administration/exposure :

- route of administration : oral (gavage)
- Duration of test/exposure period : 30d, no info is reported in the US EPA memorandum, 2006 on the frequency of the test/exposure period
- Doses : 0, 8, 57, 400 mg/kg
- Post exposure observation period : no info reported in the US EPA memorandum, 2006
- Vehicle : no info reported in the US EPA memorandum, 2006
- Test substance : 1,2,4-triazole, purity : no info reported in the US EPA memorandum, 2006

- The stability, homogeneity and dietary concentrations : no info reported in the US EPA memorandum, 2006

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Statistical methods : no info reported in the US EPA memorandum, 2006

Results and discussion :

Decrease in adrenal weight was seen at 8 mg/kg bw/d. Slight hematology changes were observed at 57 mg/kg bw/d and clinical signs (staggering, tremors, hunched) and decreased body weight at 400mg/kg bw/d.

NOAEL <8 mg/kg/d
LOAEL = 8 mg/kg/d

Comment of eMSCA :
The eMSCA did not obtain the study report but this study was reported in the US EPA memorandum"1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid: Human Health Aggregate Risk Assessment in Support of Reregistration and Registration Actions for Triazole-derivative Fungicide Compounds", 2006.

90d repeated dose toxicity: Wahle, 2004b; JPMR,2008
Guideline : US EPA OPPTS 870.3100
GLP : yes

Material and methods
Test type : Repeated dose toxicity : oral
Test animals : CD-1 {[ICR]/BR} mice
20 males and 20 females (nulliparous and non-pregnant) per dose
An additional group of 15males and 15 females per dose for liver-enzyme analysis
Age : ± 8 weeks
Administration/exposure :
- route of administration : oral (diet)
- Duration and frequency of test/exposure period : 90d, daily *ad libitum*
- Doses : 0, 500, 1000, 3000 and 6000 ppm
equal to M: 0, 80, 161, 487, 988 mg/kg bw /d
F : 0, 105, 215, 663, 1346 mg/kg bw/d
Additional group for liver-enzyme analysis : 0, 3000 and 6000 ppm
- Post exposure observation period : -
- Vehicle : ethanol
- Test substance : 1,2,4-triazole, purity : 99.9 %
The stability, homogeneity and dietary concentrations were confirmed analytically : -diets were prepared every 2 weeks and were stable for
35d at freezer temperature
-homogenety was within acceptable range (<10 %)
-measured test conc. : 94-95 % of target conc.

Method :
- Observation for mortality and clinical signs : daily
- Detailed clinical examinations : weekly
- Body weight and food consumption : weekly
- Hematology and clinical chemistry measurements : at 4 weeks and at termination. In addition, activities of selected hepatic enzymes were measured in the control and the 2 highest dose groups at 4 weeks, and in the control and the highest dose groups at 13 weeks.
- Gross pathological examination
- Weight measurements of selected organs
- Histopathological examination of selected tissues : from the control and the highest dose group

Statistical methods :

- Bartlett's test (Snedecor and Cochran, 1967) was used to evaluate continuous data for equality or homogeneity of variance
- One-way analysis of variance (ANOVA) (Snedecor and Cochran, 1967) followed by Dunnett's test (Dunnett, 1955, 1964)to furher analyse group means
- In the event of unequal variances, and at the discretion of the study director, data were subject to non-parametric procedures consisting of a Kruskal-Wallis ANOVA (Hollander and Wolfe, 1973) followed by the Mann-Whitney-U test for between-group comparisons.
- Frequency data were initially examined for trends; data suggestive of a potential effect were then statistically evaluated using the Fisher exact tests.
- For the Bartlett test, a probability (p) value < 0.001 was considered significant; for all other statistical tests, differences with p values < 0.05 were considered statistically significant.
- All statistical evaluations were performed using software obtained from either INSTEM Computer Systems (Stone, Staffordshire,UK) or SAS Institute Inc. (Cary, NC).
- Additional statistical tests may have been used to evaluate data generated from this study when deemed appropriate by the Study Director

Results and discussion :

No treatment-related effects were observed on mortality, haematological or clinical chemistry parameters.

Treatment related clinical signs were observed, including tremors in both sex at the highest dose group, at these 2 doses yellow staining of the ventrum in males and food spillage in females and at 6000 ppm rough coat in males.

There were some body weight and organ weight changes and body weight gain was also modified (3.1/3.5, 3.6/3.1, 1.7/3.0, 1.1*/2.7, -3.1*/0.9* respectively at 0, 500, 1000, 3000 and 6000 ppm in males/females. p≤0.05)).

Table 47: Organ weight overview

Doses (ppm)	0	500	1000	3000	6000
Males					
Terminal bw (g)	37.3	37.0	36.4	34.9*	31.3*
Abs. brain weight (g)	0.488	0.491	0.476	0.465*	0.445*
Rel. brain weight (%)	1.328	1.378	1.365	1.376	1.462*
Abs. testis weight	0.253	0.247	0.233	0.233	0.219*
Females					
Terminal bw (g)	29.1	28.4	28.4	28.7	26.6*

Brain weight (g)	0.485	0.489	0.483	0.475	0.451*

* : p<0.05

The significant modification of the brain weight was correlated with a decrease of numbers of purkinje cells in the cerebella at 6000 ppm (15 males out of 20 and 10 females out of 18 vs 0 in control group). 9 males out of 11 and 1 female out of 3 showing tremors had also Purkinje cells loss.

An increase of total cytochrome P450 activity was seen at the highest dose group in both sex. In liver tissue, 7-ethoxycoumarin deethylase (ECOD), 7-ethoxyresorufin deethylase (EROD) and aldrin epoxide (ALD) were observed in both sexes at 3000 and 6000 ppm after 4 weeks and at 6000 ppm after 13 weeks. A slight increase of UDP-glucuronyltransferase (GLU-T) activity was also seen at 6000 ppm. These modifications were not correlated with changes in liver weight or histopathology.

Table 48: Summary of the significant clinical chemistry changes.

	0 ppm M/F	3000 ppm M/F	6000 ppm M/F
ECOD (nmol/g*min)	24.2/28.1	35.2**/48.1**	38.7*/56.7**
EROD (nmol/g*min)	1.11/0.70	1.78**/0.91	1.43/1.08*
ALD (nmol/g*min)	47.9/39.4	62.6**/52.8**	62.5**/55.5**

* : p≤0.05 ; ** : p≤0.01

At 6000 ppm absolute testes weights decreased significantly (87% of control). The same decreasing trend, but not significant, was observed at 1000 and 3000 ppm (92% of controls).
Histopathological changes in the testes were observed. These include increased incidence of apoptotic-like bodies in the control group (4/20) and the males at 500 (4/20), 1000 (7/20), 3000 (11/20), and 6000 ppm (12/20)); spermatid degeneration, depletion, and asynchrony (5 /20 and 15/20 of the males at 3000 and 6000 ppm, respectively), and minimal or slight focal tubular atrophy (2/20, 3/20, and 10/20 of the males at 1000, 3000, and 6000 ppm, respectively). Change in the epididymides (increased germ cells and debris in the luminal duct (10/20) and one male with aspermia) at 6000 ppm was considered as secondary to the testicular effects. Minimally increased apoptotic-like bodies and tubular atrophy seen at 1000 ppm were considered treatment-related but not adverse. Testicular atrophy at 1000 ppm was considered to be spontaneous due to their limited tissue distribution (focal and/or u nilateral) and the lack of accompanying spermatid degeneration/depletion/asynchrony.

Table 49: Summary of male reproductive parameters.

	0ppm	500ppm	1000ppm	3000ppm	6000ppm
Number of animals tested	20	20	20	20	20
No abnormality detected	15	16	12	9	1
Mineralisation	1	0	0	0	0
Apoptotic-like bodies	4 (1.0)	4 (1.3)	7 (1.1)	11* (1.3)	12* (1.2)
Spermatid degeneration/depletion/asynchrony	1 (1.0)	0	0	5 (1.4)	15* (2.0)
Tubular atrophy	0	0	2 (1.5)	3 (1.0)	10* (1.8)

() : average severity of animals with lesions : 1 (minimal) to 5 (severe)
* : significantly different from control (p≤0.05)

LOAEL = 487 mg/kg bw:d (males) and 1346 mg:kg bw/d (females)

- ⇨ Males : based on tremors, brain weight, testicular weight and histopathological findings in testes
- ⇨ Females : based on body-weight, body weight-gain, brain weight and histopathological findings in the cerebellum

NOAEL = 161 mg/kg bw/d (males) and 663 mg:kg bw/d (females)

90d repeated dose toxicity, Registration dossier (Study report, 1979)

Guideline : similar to *OECD TG 408 (Repeated dose 90-day oral toxicity in rodents), reliability 2*
GLP : non-GLP

Material and methods
Test type : Repeated dose toxicity : oral
Test animals : Wistar rats
15 males and 15 females per dose
Age and weight at study initiation: 5 to 6 weeks old and mean starting weight of 82g for males and 78g for females
Administration/exposure :

- route of administration : oral (diet)
- Duration and frequency of test/exposure period : 90d, daily
- Doses : 0, 100, 500 and 2500 ppm
  equal to M: 0, 7.79, 37.85, and 212.30 mg/kg bw /d
  F : 0, 10.23, 54.20 and 266.69 mg/kg bw/d
- Post exposure observation period : / 3 months after start of study all the animals were sacricifed to examination.
- Vehicle : A 90 % pre-mix with Ultrasil VN 3 (precipicated highly dispersed silicic acid)
- Test substance : 1,2,4-triazole, purity : 99.6%
  A 90% pre-mix with Ultrasil VN 3 (precipitated highly dispersed silicic acid) was used. In order to compensate the degree of purity, 11% of the pre-mix was added.

Statistical methods : the following were calculated : arithmetic groups means, standard deviations, upper and lower confidence limits on the confidence level of 1 – alpha = 95% and 1 - alpha = 99%
The comparison of the values of the test collective with the control collective was made by means of the significance test (U test) after Mann, Whitney and Wilcoxon, on the significance level of alpha = 5% and alpha = 1%

Results and discussion :

The mortality and the food consumption were regarded but no modifications were observed. In the highest dose group, 2 males and 2 females exhibit slight convulsions. The body weight and body weight gain parameters in the low and mid-dose group were in the same range than the control group whereas in higher group there was a significant lower body weight in the males for the entire study period and in the females in the majority of the observation dates.

The haematology evaluation, made one month after start of study with 5 rats/sex/group, reveals only in males a dose related decrease of haemoglobin and haematocrit (the differences were significant ($p<0.05$) at 2500 ppm and also at 500ppm for haematocrit), and in addition the MCH and MCV value were significantly lower at 2500ppm. At the end of the study, the analysis indicates a statistically significant decrease of haemoglobin, haematocrit, MCH and MCV at 2500 ppm in males. The blood cells counts of the treated animals did not differ significantly from the controls.

The clinical chemistry did not reveal any dose related effect (cholesterol, ALT, ..). One and 3 months after start of study protein-bound-iodine (PBI) was determined and there were no significant differences between control and treated group.

At the highest dose group, the thymus, heart, spleen and testicule weights were significantly lower in males and the same modifications was observed in lungs in the both sexes.

Table 50: Summary of organ weights

	Males				Females			
	0	100	500	2500	0	100	500	2500
BW (in g)	335	342	344	306**	195	195	187	184*
Thyroid	16	16	17	16	13	14	16	15
Thymus	339	323	339	287*	268	233	240	238
Heart	963	938	962	876**	628	631	605	589
Lung	1217	1190	1230	1106*	883	887	847	804**
Liver	11765	12106	12285	11693	6712	7088	6952	9775
Spleen	603	597	578	534*	426	424	421	382
Testis/ovary	3418	3308	3247	3215*	68	69	70	72

* : p<0.05; ** : p<0.01

The necropsy did not reveal any macroscopic modification whereas 2 males of the highest dose group showed signs of moderately defined centrolobular fat infiltration in the liver parenchyma cells.

According to the results, the NOAEL was of 500ppm (corresponding to 37.85 mg/kg bw/d in males and 54.20 mg/kg bw/d in females) and the LOAEL was 2500ppm (corresponding to 212.30 mg/kg bw/d in males and 266.69 mg/kg bw/d in females).

90d Combined toxicity/neurotoxicity study, Wahle & Sheets, 2004, JMPR,2008.

Guideline : OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)/ OECD TG 424 (Neurotoxicity Study in Rodents)
=>With deviations :
After 4 weeks the animals were dosed 4000ppm in the 1000 ppm group

GLP : Yes

Material and methods

Test type : Combined toxicity/neurotoxicity: oral
Test animals : Wistar Hanover rats (Crl:WI[Glx/BRL/Han])
20 males and 20 females per dose
Age and weight at study initiation: approximately 5-weeks old
Fasting period before study: no
Acclimation period: 10 days

Administration/exposure :

- route of administration : oral (diet)
- Test conditions : - Temperature (°C): 19 to 25 °C

- Humidity (%): 30 to 70%
- Air changes (per hr): minimum of 14 air changes per hour
- Photoperiod (hrs dark / hrs light): 12-13 hr of fluorescent light

- Duration and frequency of test/exposure period : 90d, daily
- Doses : 0, 250, 500, 3,000, and 1,000/4,000 ppm (1,000 ppm for four weeks and 4,000ppm thereafter) equal to
- M: 0, 16, 33, 183 and 210 mg/kg bw/dF : 0, 19, 41, 234 and 275mg/kg bw/d
- Post exposure observation period : /
- Vehicle : ethanol
- Test substance : 1,2,4-triazole (CAS 288-88-0), purity : 99.9%, flakes
- The stability, homogeneity and dietary concentrations were confirmed analytically:
  -diets were prepared weakly and were stable for 35d at room temperature
  -homogenetiy was within acceptable range (<10%)
  -measured test conc. : 94-98% of target conc.

Statistical methods :

- Barlett's test to evaluate equlity or homogeneity of variance
- One-way analysis of variance (ANOVA) to analyse groupmeans
- Chi-square and/or Fischer exact tests to evaluate frequency data
- Reapeated-measures ANOVA to analyse continuous data (for the FOB), followed by a one-way ANOVA if there was a significant interaction between dose group and test week
- Dunnett's test : for weeks with a significant treatment effect, to determine chich groups were significantly different from the control groups
- General Linear Modeling and Categorical Modeling (CATMOD)
- Procedures with post-hoc comparisons using Dunett's test and a Analysis of Contrast (SAS) to analyse categorical data in the FOB
- ANOVA to analyse motor and locomotor activity (total session activity and activity for each 10-minute interval)

Results and discussion

No treatment-related effects were observed on food consumption, mortality, haematology and urine analysis parameters.

Tremors were only observed at 1000/4000ppm in 1 female. No other treatment related clinical signs were observed.

Body weight was unaffected up to ≤ 500 ppm in both sexes, and decreased at 3000 ppm (M: 7% and F : 6%) and 1000/4000 ppm (M : 8% and F : 5%), body weight gain decreased in the 3000 ppm group (M : 18% and F : 19%) compared to controls.

No treatment related changes could be observed in the ophthalmologic profile in both sexes in all dose groups. After 12 weeks, retinal degradation was seen at 3000 ppm in 4 out of 20 males and 2 out of 20 females, at 1000/4000 ppm in 5 out of 20 males and females compared to 2 out of 20 in the control group. No significant effect was seen on the number of corpora lutea.

A slight decrease was observed in serum triglyceride concentrations in 3000 ppm and 1000/4000 ppm in males and a slightly increased activity of the hepatic enzymes N-demythylase, O-demythylase, ECOD, EROD and ALD, EH and GS-T and GLU-T. These enzyme alterations indicate an adaptive response of the liver to an increased need to facilitate the metabolism and the excretion of the substance.

Treatment-related changes in T4 and T3 were not observed. A significant decrease in TSH was seen in males at ≥ 500 ppm (74-65% of control). The slight decrease in females was not significant. However, the registrant considered that the decrease in TSH was not toxicologically relevant due to the absence of histopathological findings in the examined

thyroid and changes in T4 and T3 and overall suspectibility perturbation of the rat thyroid after exposure to 1,2,4-triazole.

Table 51: Summary of the thyroid modifications

	0ppm	250ppm	500ppm	1000/4000ppm	3000ppm
Males					
TSH (SD) ng/ml	6.35 (1.36)	5.19 (1.68)	4.68* (1.24)	4.58* (0.94)	4.14* (0.83)
T4 (SD) ug/dL	4.61 (0.39)	4.54 (0.85)	4.37 (0.34)	4.02 (0.59)	3.99 (0.71)
T3 (SD) ng/ml	0.68 (0.08)	0.70 (0.15)	0.69 (0.08)	0.58 (0.12)	0.66 (0.15)
Females					
TSH (SD) ng/ml	7.48 (2.69)	4.36* (1.32)	6.43 (3.31)	4.47* (1.47)	5.27 (1.13)
T4 (SD) ug/dL	2.49 (0.57)	2.89 (0.65)	2.89 (0.80)	2.71 (0.47)	3.22 (0.79)
T3 (SD) ng/ml	0.85 (0.11)	0.78 (0.14)	0.75 (0.19)	0.69 (0.12)	0.84 (0.13)

(* : $p \leq 0.05$)

The necropsy revealed brain modifications in both males and females.

Table 52: Summary of brain changes.

Males	0ppm	250ppm	500ppm	1000/4000ppm	3000ppm
NAD	9/10		8/10	2/10	0/10
Degeneration, axonal				1/10	
Degeneration/necrosis				9/10	10/10
Degeneration, nerve fiber	1/10		2/10	5/10	4/10
Females					
NAD	10/10		10/10	0/10	0/10
Degeneration/necrosis				10/10	10/10
Degeneration, nerve fiber	1/10			1/10	1/10

(NAD : No abnormality detected)

<u>Chronic toxicity study (52 weeks): Anonymous, 2010</u>

Guideline : OECD TG 452 (Chronic toxicity study)
GLP : GLP

<u>Material and methods</u>
Test type : Repeated dose toxicity : oral
Test animals : Wistar rats
For toxicology examination : 20 males and 20 females per dose
Approximately 8 weeks old and mean starting weight of 133-255g for males and 153-191g for females
For neurotoxicology examination : 10 males and 10 females per dose
Approximately 9 weeks old and mean starting weight of 237-305g for males and 168-219g for females
Administration/exposure :

- route of administration : oral (diet)
- Duration and frequency of test/exposure period : 52w, daily
- Doses : 0, 125, 375, 1000 and 2000 ppm
  equal to M: 0, 6.9±2.0, 21±6, 58±16, 113±31 mg/kg bw /d
  F : 0, 8.3±1.4, 26±4, 71±10, 136±22 mg/kg bw/d
- Post exposure observation period : /

- Vehicle : ethanol
- Test substance : 1,2,4-triazole (CAS 288-88-0), purity : 98.5% (12/7) 99.1 (01/10)

Environmental conditions :

- room temperature : 18 to 26 °C
- humidity : 30 to 70%
- daily photoperiod : 12h of light

Methods :

- Observation : general assessment (moribundity, mortality) of all animals at least once daily
  Neurological evaluations : FOB, motor activity : during the week prior to initiation of exposure and again at approximately 3, 6, 9 and 12 months
- Body weight, food consumption : weekly for the first 13w and every 4 ± 1w thereafter on all surviving animals
- Hematology, clinical chemistry and urinalysis : blood and urine were collected from the first 10 rats/sex/group at approximately 3, 6, 10months.
  Hematology parameters : HCT, HGB, WBC, RBC, PLTS, blood clotting measurements, leukocyte differential count, MCH, MCHC, MCV, reticulocyte count, Heinz bodies, erythrocyte morphology, activated partial thromboplastin time, prothrombine time
  Serum chemistry parameters : Ca, Cl, Phosphate, K, Na, ALP, CK, LDH, ALT, AST, GGt, albumin, creat, blood urea nitrogen, total cholesterol, globulin, glucose, total bilirubin, total protein, trig, uric acid.
  Urinalysis parameters : appearance, volume, specific gravity, pH, sediment, protein, glucose, ketones, bilirubin, leukocytes, blood, nitrite, urobilinogen
- Reproductive parameters : estrous cycle staging (10 females/group), male reproduction function (10males/group), ovarian follicle and corpora lutea counts
- Sacrifice and pathology : 20 rats/sex/dose : complete gross examination (cecum, colon, duodenum, esophagus, ileum, jejunum, liver, pancreas, rectum, salivary glands, stomach, tongue, tooth, larynx, lung, nasopharynx, trachea, aorta, bone marrow, heart, lymph node, spleen, thymus, cervix, clitorial gland, epididymides, kidneys, mammary glands, ovary, preputial gland, prostate, seminal vesicle, testicle, urinary bladder, uterus, vagina, adrenal gland, parathyroid, thyroid, brain, eyes, nerve (optic, sciatic), pituitary, spinal cord, bone (femur, rib, sternum), muscle, joint, skin
- Neuropathology : brain (levels 1-8), spinal cord, cauda equine, spinal nerve, optic nerves, peripheral nerves

Statistical methods :

- Bartlett's test to evaluate for equality or homogeneity of variance
- one-way variance analysis (ANOVA) followed by Dunnett's test to analyse group means
- Dunnett’s test was used to compare dose-groups to the vehicle-control group
- Organ weights were evaluated using an analysis of covariance (ANCOVA), with terminal body weight as the covariate
- Chi-Square and/or Fisher’s Exact tests to evaluate frequency data
- FOB (Functional Observational Battery) : continuous data was first analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant interaction between dose group and test week.
  Dunnett's test : for weeks with a significant treatment effect to determine which groups were significantly different from the control group.
  General Linear Modeling and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test : Contrasts Categorical data, respectively.
  ANOVA procedures to analyse motor and locomotor activity
  Repeated-Measures ANOVA to evaluate session activity data, followed by a one-way ANOVA if there was a significant interaction with test occasion.

<u>Results and discussion</u> :

No mortality or clinical signs of toxicity attributable to the test substance. The survival rates were 30/30, 29/30, 27/30, 29/30 and 30/30 for males and 29/30, 30/30, 28/30, 30/30 and 28/30 for females respectively at 0, 125, 375, 1000 and 2000 ppm.

Neurological assessments (functional observational battery and motor activity) indicated no significant effect in males or females at any dietary level.

Treatment related lower body weights and lower body weight gains were observed in both sexes at the 2 highest dose groups (bw gain difference of 8-19% at 1000ppm and 8-20% at 2000ppm).

Food consumption, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, estrous cycle staging, sperm analysis parameters were unaffected.

The histopathology reveals test-substance–related morphologic changes in brain of both sexes in the highest dose group (decreased population of Purkinje cells within the cerebellar vermis).

The micropathology examination of reproductive organs revealed no treatment-related effects.

According to the results, the NOAEL was of 21 mg/kg bw/d in male and 26 mg/kg bw/d in female and the LOAEL was 58 mg/kg bw/d in male and 71 mg/kg bw/d in female.

Developmental toxicity, Wickramaratne, 1987 - JPMR (2008) and US EPA Memorandum (2006)

Guideline : non-guideline
GLP : non-GLP

Material and methods

Test type : Prenatal Developmental Toxicity Study
Test animals : Alpk: AP [Wistar-derived] rats
10 pregnant females per dose group
Age and weight at study initiation: no info reported in the JMPR 2008 and USEPA memorandum, 2006
Fasting period before study no info reported in the JMPR 2008 and USEPA memorandum, 2006
Acclimation period: no info reported in the JMPR 2008 and USEPA memorandum, 2006
Administration/exposure :

- route of administration : oral
- Test conditions : no info reported in the JMPR 2008 and USEPA memorandum, 2006
  - Temperature (°C):
  - Humidity (%):
  - Air changes (per hr):
  - Photoperiod (hrs dark / hrs light):
- Duration and frequency of test/exposure period : from day 7 to day 17 of gestation
- Doses : 0, 25 and 100 mg/kg/day
- Post exposure observation period :-
- Vehicle : not reported
- Test substance : 1,2,4-triazole, purity : not reported

The stability, homogeneity and dietary concentrations : no info reported in the JMPR 2008 and USEPA memorandum, 2006

Results and discussion

No treatment-related effects were observed on maternal weight gain, the number of viable litters, litter size, survival or postnatal-weight gain.
1,2,4-Triazole was not teratogenic in rats as determined by a modified Chernoff-Kavlock assay.
Malformation were not examined.

Prenatal Developmental Toxicity Study, Renhof 1988a – JMPR, 2008
Guideline : EPA, 83-3
GLP : Yes

Material and methods
Test type : Prenatal Developmental Toxicity Study
Test animals : Bor: WISW (SPF Cpb) rats
25 pregnant females per dose group
Age and weight at study initiation: sexually mature, nulliparous, weight: 182 - 213kg
Fasting period before study : not reported
Acclimation period: 1 week
Administration/exposure :
- route of administration : oral
- Test conditions : - Temperature (°C): 21±3°C
  - Humidity (%): ± 55%
  - Air changes (per hr): at least 10 per hour
  - Photoperiod (hrs dark / hrs light): 12-hours light:12-hours dark
- Duration and frequency of test/exposure period : from gestational day 6 to day 15, daily
- Doses : 0, 10, 30 or 100 mg/kg bw/day
- Post exposure observation period : until GD20
- Vehicle : 0.5% aqueous Cremophor-EL emulsion
- Test substance : 1,2,4-triazole, purity : 95,3%, flakes
  The stability, homogeneity and dietary concentrations were confirmed analytically :
  -diets were stable
  -homogenetiy : not necessary as real solution
  -measured test conc. : 95.3%

Statistical methods :
- Distribution-free rank sum test according to WILCOXON (WILCOXON-MANN-WHITNEY-U-TEST), e.g. for weight-gain, number of nidations, foetuses and resorptions
- Chi-squared test (correction after YATES), e.g. for number of runts
- Chi-squared test (correction after YATES or so-called exact test according to FISHER - depending on frequency expected) for numbers of fertilised and pregnant animals.
- Statistical significance has been taken as a probability of 5% or less of this difference occurring by chance.

Results and discussion

Maternal toxicity: no mortality and no treatment-related clinical signs were observed. Body weight gain was statistically significantly reduced (-14%) during pregnancy at the highest dose. Food consumption was unaffected.

Developmental toxicity: at 100 mg/kg, fetal weights were statistically significantly reduced and the number of runts per litter was statistically significantly increased. Malformations were observed in all groups. Microphtalmia/anophtalmia were observed in 5 animals in 5 different litters (1 control, 1 at 10 mg/kg, 3 at 100 mg/kg). False posture of right hind leg was observed in 1 animal at 30 mg/kg. Dysplasia and assymmetry of body of vertebrae and vertebral arches of thoracic spine and abnormal position of one rib was observed in 1 animal at 100 mg/kg.

According to the results, the NOAEL was 30 mg/kg bw/d for maternal and developmental toxicity

Prenatal Developmental Toxicity Study: Renhof, 1988b – JMPR, 2008
Guideline : EPA, 83-3

GLP : Yes

Material and methods

Test type : Prenatal Developmental Toxicity Study

Test animals : Bor: WISW (SPF Cpb) rats
25 pregnant females per dose group
Age and weight at study initiation: sexually mature, nulliparous, weight: 182 - 213kg
Fasting period before study : not reported
Acclimation period: 1 week

Administration/exposure :

- route of administration : oral
- Test conditions : - Temperature (°C) : 21±3°C
  - Humidity (%) : 40%
  - Air changes (per hr) : at least 10 per hour
  - Photoperiod (hrs dark / hrs light) : 12-hours light:12-hours dark
- Duration and frequency of test/exposure period : from gestational day 6 to day 15, daily
- Doses : 0, 100 and 200 mg/kg bw/day
- Post exposure observation period : until GD 20
- Vehicle : 0.5% aqueous Cremophor-EL emulsion
- Test substance : 1,2,4-triazole, purity : 94%

Statistical methods : - Distribution-free rank sum test according to WILCOXON (WILCOXON-MANN-WHITNEY-U-TEST), e.g. for weight-gain, number of nidations, foetuses and resorptions
- Chi-squared test (correction after YATES), e.g. for number of runts
- Chi-squared test (correction after YATES or so-called exact test according to FISHER - depending on frequency expected) for numbers of
fertilised and pregnant animals.

Statistical significance has been taken as a probability of 5% or less of this difference occurring by chance.

Results and discussion

Maternal toxicity: no mortality and no treatment-related clinical signs were observed. Body weight gain was statistically significantly reduced (- 37 %) at 200 mg/kg during pregnancy. The reduction of body weight gain at 100 mg/kg was only 5%. Food consumption was comparable in all groups.

Developmental toxicity: fetal and placental weights were statistically significantly reduced in treated animals and the number of runts per litter was statistically significantly increased. The number of foetuses with skeletal deviations was statistically significantly increased at 100 mg/kg. At 200 mg/kg, the number of surviving foetuses per dam was reduced and the number of resorptions per litter was increased (53.2 % vs 3.9 % in controls). Malformations were observed in all groups. Microphtalmia was observed in 2 animals of the same litter in controls (1 litter/21) (2 foetuses/253). Undescended testicle occurred in 2/253 (0.8%), 11/226 (4.9 %) and 6/138 (4.3%) foetuses at 0, 100 and 200 mg/kg. The incidence per litter was respectively 2/21 (9.5%), 7/19 (36.8%) and 5/25 (20%). Hydronephrosis occurred in 1/253 (0.4%), 1/226 (0.4%) and 7/138 (5.1%) at 0,100 and 200 mg/kg. The incidence per litter was respectively 1/21 (4.8%), 1/19 (5.3%) and 6/25 (24%). Cleft palate was observed at 200 mg/kg in 4/138 (2.9%) fetuses. The incidence per litter was 3/25 (12 %). Long bone dysplasia was observed at 200 mg/kg in 3/138 (2.2%) fetuses. The incidence per litter was 3/25 (12 %). False posture of hind legs was observed in 1 animal at 200 mg/kg. Incidence per fetuses: 0.7%, incidence per litter: 4%.

According to the results, the NOAEL was 100 mg/kg bw/d for maternal toxicity and <100 mg/kg bw/d for developmental toxicity.

Oral (Stomach Tube) Developmental Toxicity Study of 1,2,4-Triazole in Rabbits: Hoberman A.M., 2004 – JMPR, 2008

Guideline : US EPA OPPTS 870.3700
GLP : Yes

Material and methods
Test type : Prenatal Developmental Toxicity Study
Test animals : New Zealand White [Hra:(NZW)SPF] rabbit
25 timed-mated females per dose group
Age and weight at study initiation: 5.5 months, weight: 2.7 - 4.4 kg
Fasting period before study : no
Acclimation period: 1-4d
Administration/exposure :

- route of administration : oral (gavage)
- Test conditions : - Temperature (°C): 16°C to 22°C
  - Humidity (%): 30 to 70%
  - Air changes (per hr): ten changes per hour
  - Photoperiod (hrs dark / hrs light): 12-hours light:12-hours dark fluorescent light cycle
- Duration and frequency of test/exposure period : from day 6 to day 28, once daily
- Doses : 0, 5, 15, 30 and 45 mg/kg/day
- Post exposure observation period : on day of sacrifice
- Vehicle : 0.5% (w/w) carboxymethylcellulose (CMC)
- Test substance : 1,2,4-triazole (CAS 288-88-0), purity : 99.9%, flakes
  The stability, homogeneity and dietary concentrations were confirmed analytically :
  -diets were prepared weekly and were stable for 24h at room temperature (22±5°C) and for 10d at 5±3°C
  -homogenetiy was within acceptable range (A value of 1.2% RSD was obtained which is <10%)
  -measured test conc. : >80% of target conc.

Statistical methods :

- Bartlett's Test to analyse Continuous data, Analysis of Variance when appropriate i.e., Bartlett's Test was not significant ($p>0.001$), followed by Dunnett's Test if analysis of variance was significant to identify the statistical significance of the individual groups.
- Kruskal-Wallis Test was used if analysis of variance was inappropriate, followed by Dunn's Method of Multiple Comparisons(21) to identify the statistical significance of the individual groups if Krukal-Wallis was significant (($p\leq0.05$)
- Fisher's Exact Test was used if there were greater than 75% ties,
- Variance Test for Homogeneity of the Binomial Distribution to analyse Clinical observations and other proportion data

Results and discussion

In the highest dose group (45 mg/kg/d) 5 of the 25 pregnant rabbits were sacrificed due to their moribund conditions.

Treatment related lower body weight gains in dams were observed in the highest dosage group (significant difference with control at GD9-12 ($p\leq0.05$) and GD21-24 ($p\leq0.01$)). Fetal weights (total, male and female) were significantly reduced in the 45 mg/kg/d dosage group.

No treatment-related effects were observed on food consumption.

Adverse clinical observations occurred in the highest dose group. Motor activity, clear perinasal discharge, ptosis, excess salivation and hypernea were singinifcantly increased. These effects were mostly observed in the rabbits that were in moribund conditions. Other

clinical signs were observed in the highest dose group and the rabbits that were sacrificed: scant feces, ungroomed coat, head tilt, lacrimation, flared nostrils and cold touch.

Gravid uterine weights were significantly reduced in the 45 mg/kg/d dosage group (0.46±0.08 versus 0.56±0.10 in the control, p≤0.01).

Several foetuses showed alterations of the urogenital system in the highest doage group where maternal toxicity was observed.

Table 53: Differences in fetal urogenital system:

Dasage group (mg/kg/d)	0	5	15	30	45
Kidneys : low set (%)	0.0	0.0	0.0	0.0	5.3 1.9 d, e**
Kidneys : small (%)	0.0	0.0	0.0	0.0	5.3 1.9 d, e**
Kidneys absent (%)	0.0	0.0	0.0	0.0	10.5 ** 1.3 e**

d. Fetus 8102-1 and 7 had other soft tissue alterations
e. Fetus 8102-4 had other sof tissue alterations
** Significantly different from control group value (p≤0.01)

Conclusion : OECD CF level 4 data

Different toxicological studies with 1,2,4-triazole show several adverse effects on fertility and development in rodents, which could be related to disruption of the endocrine system.

The incidence of cryptorchism was above the historical values at the treated dose groups (100 and 200 mg/kg) in a developmental toxicity study in rats [Renhof, 1988] and an increase of pre- and post-implantation losses was observed.

Statistically significant decrease in epididymal sperm counts in P0 at 3000 ppm (188.6 mg/kg bw/d), decrease in testicular sperm at all doses in P0 and statistically significant decrease of the percentage of normal sperm morphology at 500 ppm (30.9 mg/kg bw/d) and 3000 ppm (188.6 mg/kg bw/d) in P0 were noted in the two-generation reproductive toxicity study in rats [Young and Sheets, 2005]. A delay in sexual maturation in female F1 pups at 250 ppm (17.4-19.3 mg/kg bw/d) and 500 ppm (36.2-38.7 mg/kg bw/d) was also seen.

In the 90-day toxicity study in mice [Wahle, 2004] a statistically significant increase in the number of apoptic-like bodies (at 487 and 988 mg/kg bw/d), disturbances in spermatid development and focal tubular atrophy in the testes (at 988 mg/kg bw/d) were observed.

Statistically significant decrease in the number of recent-cycle and total corpora lutea at 500 ppm (34.4-37.5 mg/kg bw/d) in F1 and a statistically significant increase in total corpora lutea at 3000 ppm (217.9-231.7 mg/kg bw/d) in P0 was found in the 2-generation study in rats [Young and Sheets, 2005]. A similar trend of increase in the number of total and recent-cycle corpora lutea was also observed in the combined subchronic toxicity/neurotoxicity screening study in rats [Wahle and Sheets, 2004] at the two highest doses (3000 ppm (234 mg/kg bw/d) and 1000/4000 ppm (275 mg/kg bw/d)) but the difference was not statistically significant.

Moreover, on basis of the results obtained in the 2-generation study in rats [Young and Sheets, 2005], it is not possible to conclude whether exposure to 1,2,4-triazole above 500 ppm would have caused impaired fertility of the F1 generation as no pups were produced at the higher dose (3000 ppm).

OECD Level 5 : *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism

Table 54: Summary of OECD Level 5 *in vivo* assays data

Method (guideline)	Short description of Method	Description of Result	References	Reliability
OECD TG 416 2-Generation reproduction toxicity study Oral (diet) GLP	Wistar Hannover rats 30males and 30 females per dose 0, 250, 500, and 3000 ppm (in P0 equivalent to 0, 15.4-17.5, 30.9-36.2, 188.6-217.9 mg/kg bw; in F1 equivalent to 0, 16-18.9 and 30-37.5 mg/kg bw) Purity 99.9-101% Dissolved in ethanol before mixing in the diet	At 3000 ppm statistically significant change in weight of thyroid in both sexes, and of spleen, adrenal and ovaries in females, and thymus in males. Decrease in testicular sperm counts at 250 ppm At 218 mg/kg/day decrease in corpora lutea in F0 females At F0: 15 mg/kg/day (male) ↓spleen weight in F1 females At F0: 36 mg/kg/day decrease in corpora lutea in F1 females At 218 mg/kg/day: ↓thyroid weight, and reproductive failure (no viable offspring) At 19 mg/kg/day= ↓spleen weight in F2 female pup. At 31 mg/kg/day: abnormal sperm in F0	Young and Sheets, 2005	1

A Two-Generation Reproductive Toxicity Study in the Wistar Rat with 1,2,4-Triazole, Young and Sheets, 2005

Guideline : OECD TG 416
GLP : Yes

Material and methods
Test type : Two-generation toxicity study
Test animals : Wistar Hannover rats (Crl:WI[G1x/BRL/Han]IGS BR)
30 males and 30 females (nulliparous and non-pregnant) per dose group
Age and weight at study initiation: 9.5 weeks old, weight:
Fasting period before study : no
Acclimation period: 1 week
Administration/exposure :

- route of administration : oral (diet)
- Test conditions : - Temperature (°C): 18°C to 26°C
  - Humidity (%): 30 to 70%
  - Air changes (per hr): ten changes per hour
  - Photoperiod (hrs dark / hrs light): 12-hours light:12-hours dark fluorescent light cycle
- Duration and frequency of test/exposure period : F0 to F2, continuous exposure
- Doses : 0, 250, 500, or 3,000 ppm

Table 55: Mean daily intake of the test substance (in mg/kg bw/d)

	250 ppm	500 ppm	3000 ppm
Premating : P-gen, Male	15.4	30.9	188.6
Premating : P-gen, Female	17.5	36.2	217.9
Premating : F1-gen, Male	16	32	NA
Premating : F1-gen, Female	18.9	37.5	NA
Gestation : P-gen, female	18.6	38.6	231.7
Gestation : F1-gen, Female	17.4	34.4	NA
Lactation : P-gen, Female	19.3	38.7	NA
Lactation : F1-gen, Female	20.3	35.8	NA

- Post exposure observation period : -
- Vehicle : ethanol
- Test substance : 1,2,4-triazole (288-88-0), purity : 99.9-101%, flakes
  The stability, homogeneity and dietary concentrations were confirmed analytically

Statistical methods :

- univariate Analysis of Variance (ANOVA) to analyse parametric data, followed by Dunnett's Test if significant differences
- Kruskal-Wallis test to analyse non-parametric data, followed by Dunnett's Test if significant differences
- Chi-Square Test to analyse nonparametric dichotomous data
- Fisher's Exact Test with the Bonferroni adjustment was used if significance was observed between groups

- frequency of gross lesions was first examined visually, then, in the event of a questionable distribution, by statistical analysis using the Chi-Square and Fisher's Exact tests.
- Comparisons were made at both the 0.05 and 0.01 levels of significance.

Results and discussion :

- BW :

Table 56: BW during premating period (in g)

	0 ppm	250 ppm	500 ppm	3000 ppm
P adults, Male	477.5	465.1	460.6	426.6**
P adults, Female	244.1	244.9	239.5	233.4*
F1 adults, Male	461.9	435.2*	428.4**	/
F1 adults, Female	236.2	227.5	230.8	/

Table 58b : BW during gestation (GD20) (in g)

	0 ppm	250 ppm	500 ppm	3000 ppm
P adults	345.3	340.9	340.0	284.7**
F1 adults	323.8	313.3	311.8	/

Table 58c : BW during lactation (D21) (in g)

	0 ppm	250 ppm	500 ppm	3000 ppm
P adults	284.2	287.4	287.4	/
F1 adults	281.4	267.8*	271.2	/

- Clinical signs : no effects

- Reproductive data :

Table 57: Summary of reproductive data

	0 ppm	250 ppm	500 ppm	3000 ppm
Nb of oestrous cycle : P-gen	3.6	3.8	3.4	3.6
Nb of oestrous cycle : F1-gen	3.7	3.7	3.8	/
Gestation length (d) : P-gen	22.3	22.0	22.2	23.5
Gestation length (d) : F1-gen	22.1	21.9	21.8	/
Total nb of implantations : P-gen	265	310	279	3
Total nb of implantations : F1-gen	304	300	273	/

- Pups examination :

Table 58: Bw pups in g (day 4) and total number of pups born

	0 ppm	250 ppm	500 ppm	3000 ppm
BW pups (nb of pups examined)				
F1 pups	10.4 (22)	9.9 (25)	10.1 (24)	6.7** (2)

F2 pups	10.4 (27)	9.9 (26)	9.9 (24)	/
Tot nb of pups born				
F1 pups	233	279	260	2
F2 pups	280	287	260	/

Preputial separation :

In F1 pups : 40.7, 41.2 and 41.3 respectively at 0, 250 and 500 ppm
In F2 pups : 40.7, 41.8* and 41.5 respectively at 0, 250 and 500 ppm

Vaginal opening :

In F1 pups : 33.4, 35.3**, 35.0 respectively at 0, 250 and 500 ppm
In F2 pups : 33.6, 34.9, 34.2 respectively at 0, 250 and 500 ppm

- Sperm analysis :

Table 59: Sperm analysis

		0 ppm	250 ppm	500 ppm	3000 ppm
Sperm motility (%)	P	76.2	78.9	78.9	78.9
	F1	87.1	87.8	84.5	/
Tot sperm count (epididymides)	P	58.2	57.0	65.7	43.2*
	F1	49.2	/	48.6	/
Tot sperm count (testis)	P	72.0	63.1*	64.4	61.2*
	F1	69.2	/	68.3	/
Sperm morphology (% normal)	P	98.7	98.1	97.0*	65.7*
	F1	98.1	/	97.9	/

eMSCA view :

The available two-generation reproductive toxicity study in rats does not fulfill the requirements of OECD TG 416. More specifically for dietary studies the dose interval should not be more than threefold, whereas in the case of the study performed with 1,2,4-triazole too high interval between the mid and high dose was applied (the selected doses were 0, 250, 500 and 3000 ppm).

Because no doses were tested between 500 and 3000 ppm, it is impossible to conclude whether the observed significant effects on fertility can be considered as a secondary non-specific consequence of the systemic toxicity or not.

Conclusion : OECD CF level 5 data

One 2-generation study with some modifications :

Recommendations of the OECD TG 416 not fulfilled (dose interval should not be more than 3 fold) -> effect at the highest dose but uncertainties between 500 and 3000ppm (±40 and 230 mg/kg bw/d).

Small number of pregnant females obtained at the highest dose (no second generation at this level -> information on the next generation is limited.

Overall conclusion ED HH

The incidence of cryptorchism was above the historical values at the treated dose groups (100 and 200 mg/kg) in a developmental toxicity study in rats [Renhof, 1988] and an increase of pre- and post-implantation losses was observed.

Statistically significant decrease in epididymal sperm counts in P0 at 3000 ppm (188.6 mg/kg bw/d), decrease in testicular sperm at all doses in P0 and statistically significant decrease of the percentage of normal sperm morphology at 500 ppm (30.9 mg/kg bw/d) and 3000 ppm (188.6 mg/kg bw/d) in P0 were noted in the two-generation reproductive toxicity study in rats [Young and Sheets, 2005]. A delay in sexual maturation in female F1 pups at 250 ppm (17.4-19.3 mg/kg bw/d) and 500 ppm (36.2-38.7 mg/kg bw/d) was also seen.

In the 90-day toxicity study in mice [Wahle, 2004] a statistically significant increase in the number of apoptic-like bodies (at 487 and 988 mg/kg bw/d), disturbances in spermatid development and focal tubular atrophy in the testes (at 988 mg/kg bw/d) were observed.

Statistically significant decrease in the number of recent-cycle and total corpora lutea at 500 ppm (34.4-37.5 mg/kg bw/d) in F1 and a statistically significant increase in total corpora lutea at 3000 ppm (217.9-231.7 mg/kg bw/d) in P0 was found in the 2-generation study in rats [Young and Sheets, 2005]. A similar trend of increase in the number of total and recent-cycle corpora lutea was also observed in the combined subchronic toxicity/neurotoxicity screening study in rats [Wahle and Sheets, 2004] at the two highest doses (3000 ppm (234 mg/kg bw/d) and 1000/4000 ppm (275 mg/kg bw/d) but the difference was not statistically significant.

Moreover, on basis of the results obtained in the 2-generation study in rats [Young and Sheets, 2005], it is not possible to conclude whether exposure to 1,2,4-triazole above 500 ppm would have caused impaired fertility of the F1 generation as no pups were produced at the higher dose (3000 ppm).

7.10.3. Conclusion on endocrine disrupting properties

Due to the shortcomings of the *in vitro* data on aromatase (Wickings *et al.*, 1987) and the uncertainty on the exact concentration tested in the the ToxCast H295R steroidogenesis assay, it is currently impossible to conclude on a possible ED MoA (steroidogenesis).

Different toxicological studies with 1,2,4-triazole showed however several adverse effects on fertility and development in rodents, which could be related to the endocrine system**.** The mode of action is unknown and it can not be excluded that it is endocrine mediated. Inhibition of steroidogenesis was further investigated as a a possible mode of action to explain these findings. The results of the **H295R steroidogenesis assay** (OECD TG 456) were negative.

There are shortcomings in the available information, such as deviation from the OECD TG 416 (choice of doses) and the OECD TG 408 and 424 (change of intermediate dose after 4 weeks of exposure) and adverse effects are observed in the available studies.

Furthermore 1,2,4-triazole may be able to act via more than one mechanism. The eventual endocrine disrupting effect in vivo may be a consequence of disturbance of several pathways simultaneously :

- Aromatase: although aromatase is the last step in the steroidogenesis pathway, both mechanistic studies (aromatase OPPTS 890.1200 vs steroidogenesis OECD TG 456) are different because: different cell lines (MCF-7 vs H295R) are used, different endpoints (enzyme activity vs. hormone production) are examined and different read outs (bioluminescence signal vs. steroid hormone levels using HPLC-MS-MS) are used.

- Some of the observed adverse effects on fertility and development in rats could be possibly related to (anti-)androgen MoA

- Adverse effect on reproduction (male and female) and on cerebellar development could be possibly induced by thyroid disruption

Due to the change of the registration dossier scope to intermediate use only, eMSCA concluded that at this moment further testing request to clarify concerns related to endocrine disruption is no longer justified. However the endocrine disruption potential of 1,2,4-triazole, an intrinsic property of a substance, remains unanswered and should be further investigated once there is a change in the scope of the dossier or if new information regarding this endpoint becomes available.

7.11. PBT and VPVB assessment

1) Persistence

1,2,4-triazole is hydrolytically stable and not rapidly degradable (max. 26% degradation at 24 days in a ready biodegradation test (OECD TG 301A) and 1% degradation after 28d in an inherent biodegradation test (OECD TG 302B). In an aerobic degradation study in soil a half life of 8 days (best fit kinetics) or 9.5d (non-linear first order once-compartment kinetics) was determined with NER formation of ≤65% applied radioactivity after 120 days of incubation [Nortox,2000].

In a field soil dissipation study (2004) half-lifes between 6.8 to 28.1d were determined depending on the soil type. PBT assessment is normally not bound to local conditions whereas field studies are particularly dependent on local conditions. Therefore, results from field studies are not directly comparable with laboratory tests or P/vP criteria and can thus be used in a weight of evidence approach(Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment V.3.0, June 2017).

Although degradation half life in soil <120 days, currently available data are not decisive for persistency.

The screening criterion for B as specified in Section 1.1.2 or 1.2.2 of Annex XIII to REACH is not fulfilled. Therefore, the P criterion has not been further investigated.

2) Bioaccumulation

BE CA concludes that based on the experimental log Kow [-0.62 (pH5), -0.71 (pH7), -0.68 (pH9)] the screening criterion as specified in Section 1.1.2 or 1.2.2 of Annex XIII to REACH for B and vB is not fulfilled.

3) Toxicity

Human Health : the substance had a harmonized classification for reproduction : Repr.2, H361d. On 15 March 2019, ECHA's Committee for Risk Assessment (RAC) agreed with the proposal for a more stringent classification (Repr. 1B H360FD) for this substance. This change is however not yet included in the in Annex VI of the CLP Regulation (EC No. 1272/2008).

Environment: Available NOECs for fish and algae>0.01 mg/l

Based on the available human health and environmental data it can be concluded that 1,2,4-triazole meets the T-criterion as specified in Section 1.1.3 of Annex XIII to REACH.

Overall conclusion:

Because the experimental log Kow value was very low, screening criterion for bioaccumulation was not met, hence eMSCA has decided that further evaluation of the P criterion was not necessary in the course of this evaluation. The toxicity criterion (T) is however met.

7.12. Exposure assessment

On 20 June 2019, the eMSCA was informed that the Registrants' submitted an update of the registration dossier in which the status of dossier was changed from full registration (>1000 t/a) to transported and on-site isolated intermediate (1-10 t/a) under strictly controlled conditions. The eMSCA concluded that at this moment there is no or limited exposure to human and environment and there is no need to request futher information under this substance evaluation procedure.

7.13. Risk characterisation

Not assessed.

7.14. References

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7.15. Abbreviations

* : p<0.05

** : p<0.01

♀ or F : females

♂ or M : males

Abs : absolute

ALP : Alkaline phosphatase

ALT : Alanine aminotransferase

Approx. : approximately

AST : aspartate aminotransferase

Conc. : concentration

Bw : body weight

Bwg : body weight gain

FOB : Functional observational battery

GD : gestational day

GGT : Gamma-glutamyltransferase

GLP : Good Laboratory Practice

GPMT : Guinea pig maximisation test

HCT : Hematocrit

HGB : Hemoglobin

ID : intradermal

IV : intravenous

LD50 : lethal dose 50%

LDH : Lactic acid dehydrogenase

LO(A)EL : Lowest observed (Adverse) Effect Level

MCH : Mean corpuscular HGB

MCHC : mean corpuscular HGB conc.

MCV : Mean corpuscular volume

Met. act. : metabolic activation

MoA : mode of action

NA : not applicable

NAD : No abnormality detected

NCC : neural crest cells

ND : not determined

NO(A)EL :No Observed (Adverse) Effect Level
NZW : New Zealand white
OECD : Organisation for Economic Co-operation and Development
PLTS : platelet count
RBC : red Blood Cell
Rel. : relative
S. typh : Salmonella Typhimurium
SD : Sprague Dawley
T3 : triiodothyronine
T4 : thyroxine
TG : test guideline
Tot. : total
TSH : Thyroid stimulating hormone
WBC : White Blood Cell