SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

1,3-diphenylguanidine

EC No 203-002-1

CAS No 102-06-7

Evaluating Member State(s): France

Dated: December 2020

Evaluating Member State Competent Authority

French Agency for Food, Environmental and Occupational Health Safety (ANSES) on behalf French Ministry of Environment
14 rue Pierre et Marie Curie
94701 maisons-Alfort Cedex
(France)
Email: reach@anses.fr

Year of evaluation in CoRAP: 2012

Before concluding the substance evaluation a Decision to request further information was issued on: 26.02.2014
Furthermore, an Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.i test method: OECD TG 443) has been required by ECHA as stated in a Decision on a compliance check dated 22 March 2019.

Further information on registered substances here:

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

DISCLAIMER

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

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Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

1,3-diphenylguanidine (DPG) was originally selected for substance evaluation in order to clarify concerns about:

- CMR : genotoxic potential
- High RCR
- High aggregated tonnage

Other concerns were identified during the evaluation. The additional concerns were:

- Reproductive toxicity
- Skin sensitisation
- Environmental fate
- Exposure of environment
- Other hazard/risk-based concerns: composition of the substance as regards to impurities such as aniline or nitrosamines formed during processes involving high temperature conditions.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A testing proposal was submitted by the registrant in 2010 and examined by ECHA. In July 2012, ECHA sent to the registrant its decision on the proposed tests. The registrant had to performed the following tests by 31 January 2014:
- Long term toxicity to fish: early-life stage toxicity test (OECD TG 2010)
- Sediment simulation testing (OECD TG 308)
- Long-term toxicity to sediment organisms (OECD TG 218)

During substance evaluation, a data gap was identified for this substance according to REACH annex X, section 8.7.3 covering adverse effects on the full range of reproductive endpoints. A decision on compliance check under REACH regulation was sent to the registrant on 22 March 2019 by ECHA to request an EOGRTS, which results should be submitted by 29 July 2021.

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.

The conclusion covers all the concerns identified, with the exception of reproductive toxicity, as the results of the ongoing reproductive toxicity study are needed to draw a final conclusion on this concern.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	x
Harmonised Classification and Labelling	x
Identification as SVHC (authorisation)	
Restrictions	

Other EU-wide measures	
No need for regulatory follow-up action at EU level	

In addition to the conclusion that harmonised classification and labelling is needed, eMSCA intends to prepare a separate regulatory management option analysis (RMOA) in which the appropriate option will be clarified after reception of the requested EOGRTS study under CCH. The RMOA should also cover the risk for the Environment identified for certain exposure scenarios.

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

1,3-diphenylguanidine (DPG) has a harmonised entry in Annex VI to the CLP Regulation where it is classified as:

> Repr. 2 (H361f***), Acute Tox. 4 (H302), STOT SE 3 (H335), Skin Irrit. 2 (H315), Eye Irrit. 2 (H319) and Aquatic Chronic 2 (H411).

Having evaluated the health and environmental hazards in accordance with CLP, the eMSCA proposes to retain the existing harmonised classifications,to add Skin Sens. 1 (H317),and to modify Acute Tox. 4 (H302) to Acute Tox. 3 (H301), Eye irrit. 2 (H315) to Eye Dam. 1 (H318) and Aquatic Chronic 2 (H411) to Aquatic Chronic 3 (H412).

Differences in self classifications for acute toxicity, eye irritation, toxicity to aquatic life and skin sensitisation justify the need for action at Community level:

- Based on recent human data available with DPG, classification as Skin Sens 1 is warranted. Data currently available do not allow sub-categorisation. Indeed, weak positive results are difficult to interpret due to the irritating potential of the substance. It is thus not possible to have a true estimate of human frequency of occurrence of skin sensitisation.

- Based on a new oral toxicity study, a classification as Acute Tox. 3 (H301) instead of Acute Tox. 4 (H302) is considered appropriate

- Based on irreversible effects observed on the eyes in experimental animals, a classification as Eye Dam. 1 (H318) instead of Eye Irrit. 2 (H315) is considered appropriate.

The classification Repr. 2 is proposed to be retained pending clarification of the potential concern on reproductive toxicity based on the results of the requested EOGRTS study under CCH.

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

Not applicable.

5.2. Other actions

Not applicable.

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

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

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Preparation of Annex VI CLH proposal	2022	France
Preparation of RMOA	2022	France

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

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

- CMR: genotoxic potential
- High RCR
- High aggregated tonnage

During the evaluation also other concern were identified. The additional concerns were:

- Reproductive toxicity
- Skin sensitisation
- Environmental fate
- Exposure of environment
- Other hazard/risk-based concerns: composition of the substance as regards to impurities such as aniline or nitrosamines formed during processes involving high temperature conditions.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Acute toxicity	Harmonised classification and labelling to be initiated: Acute Tox. 3 (H301).
Skin and eye irritation	Harmonised classification and labelling to be initiated: Eye Dam. 1 (H318).
Skin Sensitisation	Harmonised classification and labelling to be initiated: Skin sens. 1 (H317).
Mutagenicity	No further action.
Reproductive toxicity	Datagap identified. Ongoing EOGRTS.
Environmental fate properties	No further action.
Environmental hazard assessment	No further action.
High RCR, high aggregated tonnage	RMOA to be initiated

7.2. Procedure

The chemical substance DPG was included in the Community rolling action plan (CoRAP) for evaluation in 2012.

On 29 February 2012, the CoRAP was published on ECHA website and the competent authority of France was appointed to carry out the evaluation.

All the endpoints and uses were evaluated by the eMSCA. The sources of information for the evaluation were the registration data and literature search.

Based on the evaluation of the available data, the eMSCA concluded that there was a need to request further information to clarify the concerns related to substance identity, formation of by products, exposure scenarios, genotoxicity, toxicokinetics, adsorption/desorption, and hydrolysis. Therefore, the eMSCA prepared a draft decision to request further information. The draft decision was submitted to ECHA on 28 February 2013. The decision was agreed by the member state Committee. The final decision was send to the registrants on 26 February 2014.

On February 2016, the lead registrant updated its registration dossier to comply with the mutagenicity test request in the final decision. On 24 January 2017, the lead registrant updated its dossier to comply with the other requests of the draft decision. During substance evaluation, a data gap was identified for this substance according to REACH annex X, section 8.7.3 covering adverse effects on the full range of reproductive endpoints. A decision on compliance check under REACH regulation was sent to the registrant on 22 March 2019 by ECHA to request an EOGRTS, which results should be submitted by 29 July 2021.

The substance evaluation conclusion report was prepared based on the updated registration dossier, and based of the dossier submitted by the new registrant after the publication of ECHA draft decision.

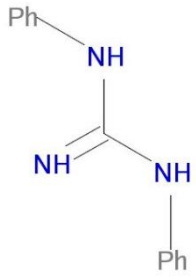
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	1,3-diphenylguanidine
EC number:	203-002-1
CAS number:	102-06-7
Index number in Annex VI of the CLP Regulation:	612-149-00-4
Molecular formula:	$C_{13}H_{13}N_3$
molecular weight range:	211 g/mol
Synonyms:	DPG, denax, guanidine, 1,3-diphenyl-, melaniline, n,n'-diphenylguanidin, sym-diphenylguanidine, vulkazit, vulkacit d, ekaland dpg, mixland+ dpg, acceleratord, denax, nocceler d, usaf ek-1270, usaf b-19, vulcafor dpg, vulkacit d/c, rubator dpg

Type of substance x Mono-constituent ☐ ~~Multi-constituent~~ ☐ ~~UVCB~~

Structural formula:



The information provided in the updated dossiers in term of the composition of DPG, specifications of impurities and analytical data confirm the composition and the presence of impurity aniline at level below 0.1%w/w. However, for some registrants analytical data are not sufficient to confirm the composition of the substance DPG and the concentration of aniline in the substance.

7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Appearance: solid colour: white to pale pink Odour: slight odour The data come from the visual description of the substance and consistent with other handbook and literature data.
Vapour pressure	The effusion method method gives a vapour pressure of 3,7e-10 Pa at 25°C. **Value used for CSA:** $3.7*10^{-10}$ Pa at 25°C
Water solubility	Water solubility is determined by flask method and gives a value of 475mg/L at pH 7 and 325mg/L at pH 11 at 20°C **Value used for CSA**: 475mg/L at pH 7 and 325mg/L at pH 11
Henry's law constant	**Value used for CSA:** $1.64*10^{-10}$Pa.m^3/mol The value of Henry's law constant is $1.64*10^{-10}$Pa.m^3/mol, calculated based on vapour pressure and water solubility.
Partition coefficient n-octanol/water (Log Kow)	-Log Kow=2.42 at pH11 by OCED 107 method - Log Kow>6.2 at pH 9.3 and log kow=4 at pH7.01 by OCED 117 method - Log Kow= 2.89 by KOWWIN The active substance is considered as a slightly surface active substance. Moreover, it has a pka = 10.1 which implies that the substance is in ionised state at environment pH.

	As the OCED 107 method for the determination of partition coefficient is not adapted for ionised or/and surface active substance, the log Kow performed at environmental pH cannot be taken into account. However, as partition coefficient value is not used in risk assessment of DPG, no complementary data is necessary. **Value used for CSA:** not relevant
Flammability	The flammability of DPG (1,3-diphenylguanidine) was performed in a study performed in accordance with EEC A 10 method. Two prelimary tests are performed, no main test is made. DPG is not considered as highly flammable. **Value used for CSA:** not flammable
Flash point	The flash point is only a relevant property for liquid, thus does not need to be done for substances that are solids or gases at room temperature, **Value used for CSA:** not relevant
Autoflammability/self-ignition temperature	The substance has a melting point <160°C, therefore the autoflammability test is not required according to R.7.1.12.1 of the guidance. **Value used for CSA:** not auto-flammable at ambient temperature
Explosive properties	According to theoretical considerations based on chemical structure, DPG has not explosive properties. **Value used for CSA:** non explosive
Oxidising properties	According to theoretical considerations based on chemical structure, DPG has not oxidizing properties. **Value used for CSA:** non oxidizing properties
Granulometry	**Value used for CSA:** 10µm to 10 mm (average 26 µm) Weight of balance approach has been used to determine the particle size distribution of the substance.
Stability in organic solvents and identity of relevant degradation products	Not relevant
Dissociation constant	Publication and review article indicate a constant of dissociation of 10.1 at 20°C **Value used for CSA:** pKa=10.1 at 20°C
Melting/freezing point	The differential scanning calorimetry method gives a melting point of the substance of 149°C. **Value used for CSA:** 149°C This value is consistent with the value of 150°C found in

	the peer review Handbook Merck Index 14th Ed. and CRC Handbook 86th Edition
Boiling point	The differential scanning calorimetry method gives a boiling point of the substance of >250°C. **Value used for CSA:** >250°C The reported boiling point values for 1,3-diphenylguanidine report that this substance decomposes before boiling (at approximately 170°C
Relative density	The pycnometer method gives a tap density of the substance of 0,348g/cm3. Value found in a peer review handbook (Merck Index 14th) gives a pour density of 1.13g/cm3 at 20°C **Value used for CSA:** pour density: 1.13g/cm3
Solubility in organic solvents	DPG is soluble in ethanol, carbon tetrachloride, chlorophorm, toluene and very soluble in ethyl ether.
Surface tension	The surface tension gives by the ring method is 58.8mN/m at 20°C. DPG is a surface active substance. **Value used for CSA:** 58.8mN/m at 20°C
Viscosity	No applicable as the substance is a solid

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

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

7.5.2. Overview of uses

DPG is a synthesis intermediate mainly used in the manufacture of rubber as a vulcanizing agent and vulcanizing accelerator.

Table 7

USES	Use(s)
Manufacture	Manufacture of substances, production of tyres
Formulation and re-packing	Masterbatch production, Formulation of powder and repacking,

	End of life Tyre : Grinding, devulcanization/reclaim, Pyrolisis, coarse shredding, energy recovery; electric arc furnace
Uses at industrial sites	Manufacture of tyres, of general Rubber goods (GRG), use in polymers and as processing aids, use in lubricants
Uses by professional workers	Handling of tyres and technical rubber goods Use in lubricants Use in formulations (coating, adheives, binders, sealants)
Consumer uses	Use of tyres and general rubber goods
Article service life	Usage of tyres (consumers) End of Life Tyre

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 8

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)**		
612-149-00-4	1,3-diphenylguanidine	203-002-1	102-06-7	Repr. 2 Acute Tox. 4 * STOT SE 3 Skin Irrit. 2 Eye Irrit. 2 Aquatic Chronic 2	H361f *** H302 H335 H315 H319 H411	-	-

7.6.2. Self-classification

The following self-classification is proposed in the Classification and Labelling Inventory (September 2020):

Acute Tox. 3 - H301: Toxic if swallowed

Eye Dam. 1 - H318: Causes serious eye damage

Aquatic chronic 2 - H411: Toxic to aquatic life with long lasting effects

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

7.7.1.1.1. Hydrolysis

The study on hydrolysis is summarised in the following table:

Table 9: **Study on hydrolysis**

Method	Results	Remarks	Reference
According to OECD guideline 111 (Hydrolysis as function of pH)	After 5 days at 50°C At pH4: less than 10% hydrolysis, equivalent to half-life greater than 1 year at 25°C. At pH7: less than 10% hydrolysis, equivalent to half-life greater than 1 year at 25°C. At pH9: less than 10% hydrolysis, equivalent to half-life greater than 1 year at 25°C. As the test item was determined to be hydrolytically stable in the tier1 test, no further testing was required.	1(reliable without restriction)	Study Report#1, 2015
Investigation of the hydrolytic properties of 1,3 -diphenylguanidine (0.3 g/L or 0.3 wt.% in water) in relation to the pH value at 80°C.	Half-life (DT50): t1/2 (pH 3.5): at 80 °C; Rate constant: 0 h^{-1} (No abiotic degradation observed) t1/2 (pH 10.5): 168 h at 80 °C	3 (not reliable) supporting study experimental result Test material (EC name): 1,3-diphenylguanidine	Wohlfahrt, R. & Niebergall, H. 1984a Wohlfahrt, R. & Niebergall, H. 1984b Wohlfahrt, R. & Niebergall, H. 1985

The key study (study report#1, 2015) shows that 1,3-diphenylguanidine is hydrolytically stable in water at environmental pH. No hydrolysis took place at 50°C at pH 4; 7 and 9 and neither at 37°C at pH 1.2, indicating that 1,3-diphenylguanidine is hydrolytically stable. The estimated half-life at 25°C of the substance tested is higher than one year at pH 4; 7 and 9. In this context, no degradation product has been investigated in this study.

Additional studies (Wohlfahrt, R. & Niebergall, H.1984 and 1985, Reliability Index (RI)=3), investigated the hydrolytic properties of DPG (0.3 g/L or 0.3 wt. % in water) in relation to the pH value at high temperature (80°C). These additional studies do not investigate the potential hydrolysis of DPG under environmental conditions but allow to identify 1,3-diphenylurea and aniline as hydrolysis products under industrial process (*i.e.* during vulcanization process) at high temperature in contact with water. 1,3-diphenylurea was further hydrolyzed to aniline in both the acidic and alkaline environments.

The eMSCA concludes that the DPG is hydrolytically stable in water under environmental conditions.

7.7.1.1.2. Phototransformation in air

The QSAR data on phototransformation in air are summarised in the following table:

Table 10: Study on phototransformation in air

Method	Results	Remarks	Reference
(Q)SAR	Reaction with hydroxyl radicals at 25°C: Overall OH Rate Constant: =85.3159 x 10^{-12} cm^3/molecule-sec Half Life: =0.125 days, 1.504 hours (12-hour day; 1.5x10^6OH/cm^3)	2 (reliable with restrictions) weight of evidence (Q)SAR Test material (EC name): 1,3-diphenylguanidine	AOPWIN v1.92 model

Based on the data on photochemical degradation in the air, DPG is considered to rapidly degrade in the atmosphere *via* photo oxidation process. The eMSCA can support this conclusion.

7.7.1.1.3. Phototransformation in water

The registrant reports an estimated half-life of DPG in water of approximately 37.5 days (900 hours) using EPI Suite software, and based on the available information, the eMSCA can support this conclusion.

7.7.1.1.4. Phototransformation in soil

The registrant reports an estimated half-life of DPG in soil of approximately 75 days (1800 hours) using EPI Suite software, and based on the available information, the eMSCA can support this conclusion.

7.7.1.2. Biodegradation

7.7.1.2.1. Biodegradation in water

7.7.1.2.1.1. Screening tests

The study on biodegradation in water (screening tests) is summarised in the following table:

Table 11: Screening tests for biodegradation in water

Method	Results	Remarks	Reference
According to OECD guideline 301D (ready biodegradability:closed bottle test)	% Degagradation of test substance: 86 after 14 days (%degradation O_2 consumption) (based on ThOD-NH3) 85 after 28 days (%degradation O_2 consumption) (based on ThOD-NO_3)	1(reliable without restriction)	Study Report#2, 2015

The study (Study Report#2, 2015; Reliability Index (RI) =1) presents the biotic degradation of 1,3-diphenylguanidine following the OECD guideline 301D. In ready biodegradability tests, microorganisms are inoculated into a chemically defined liquid medium containing the test substance as sole carbon and energy source. The 1,3-diphenylguanidine is exposed to microorganisms present in river water, under aerobic conditions for a period of at least 28 days. The biodegradation percentages calculated with $ThOD_{NH3}$ represents the degradation of 1,3-diphenylguanidine. Results of the test show that 85% of the substance was biodegraded at day 28 in the closed bottle, and over 60% biodegration is achieved after approximately 10 days. The test substance therefore fulfilled the 14-day time window criterion for ready biodegradable substances. Hence, it can be concluded that the 1,3-diphenylguanidine is readily biodegradable.

7.7.1.2.1.2. Simulation tests (water and sediments)

No available data

7.7.1.2.2. Biodegradation in soil

No available data.

7.7.1.2.3. Mode of degradation in actual use

The study on mode of degradation in actual use is summarised in the following table:

Table 12: Mode of degradation in actual use

Method	Results	Remarks	Reference
Simultaneous TG/DSCFTIR techniques under nonisothermal conditions	Thermal decomposition of N,N0-diphenylguanidine (DPG) was investigated by simultaneous TG/DSCFTIR techniques under nonisothermal conditions. Online FTIR measurements illustrate that aniline is a major product of DPG decomposition.	2 (reliable with restrictions) Supporting study Experimental Test material (EC name): 1,3-diphenylguanidine	Hu Q. et al., 2012

Guanidine derivatives have been widely used as vulcanization accelerators in rubber industry. 1,3-diphenylguanidine (DPG) has been used as a primary and secondary accelerator in the vulcanization of rubber. Although it is well known that DPG could be broken down at high temperature, leading to the formation of carcinogenic aniline, little is known about the thermal decomposition kinetics of DPG.Thermal decomposition of N,N0-diphenylguanidine (DPG) was investigated by simultaneous TG/DSCFTIR techniques under nonisothermal conditions. Online FTIR measurements illustrate that aniline is a major product of DPG decomposition. The observation that the activation energy depends on the extent of conversion indicates that the DPG decomposition kinetics features multiple processes. The initial elimination of aniline from DPG involves two pathways because of the isomerization of DPG. Mass spectrometry and thin film chromatography suggest that there are two major intermediate products with the major one of C21N3H17. The most

probable kinetic model deduced through multivariate nonlinear regression method agrees well with the experimental data with a correlation coefficient of 0.9998. The temperature-independent function of conversion f(a), activation energy E and the pre-exponential factor A of DPG decomposition was also established through model-fitting method in this research.

Based on the available data, the eMSCA concludes that aniline is a major product of thermal decomposition of DPG.

7.7.2. Environmental distribution

7.7.2.1. Adsorption/desorption

The studies on adsorption/desorption are summarised in the following table:

Table 13: Studies on adsorption/desorption

Method	Results	Remarks	Reference
According to OECD guideline 106 (Adsorption-Desorption using a batch equilibrium method)	Adsorption coefficient: Soil I: log Koc=2.5 at 20.3°C (org.C=1.74%) Soil II: log Koc=2.81 at 20.3°C (org.C=0.67%) Soil III: log Koc=2.95 at 20.3°C (org.C=1.98%) Soil IV: log Koc=3.14 at 20.3°C (org.C=1.66%) Soil V: log Koc=2.91 at 20.3°C (org.C=1.54%) The mean value of the organic carbon-water partition coefficient (Koc) is 807mL/g corresponding to LogKoc = 2.9	1 (reliable without restriction)	Study Report#3, 2015
Study type: QSAR model KOWIN KOCWIN v.2.00 QSAR estimation	Adsorption coefficient: log Koc: ca. 3.21 (estimated data (from MCI)) Koc: ca. 1652 (estimated data (from MCI)) log Koc: ca. 2.43 (estimated data (from log Kow)) Koc: ca. 273.4 (estimated data (from log Kow))	2 (reliable with restrictions) weight of evidence (Q)SAR Test material (EC name): 1,3-diphenylguanidine	KOCWIN v.2.00

With a pKa > 10, 1,3-diphenylguanidine is in cationic form at environmentally relevant pH, and thus has a very high affinity for organic matter and other matrix having a high cation exchange capacity. According to ECHA guidances, the behavior of a substance is based partly on its adsorption / desorption properties. Thus, substances with a Koc below 500 to 1.000 L/kg are generally unlikely adsorbed to sediment. To avoid extensive testing of chemicals, a log Koc (or log Kow) ≥ 3 can be used as a trigger value for sediment effects assessment. In practice a cut-off value for log Kow of 3 can be applied for adsorption potential. We acknowledge that for "classic" organic substances (*i.e.* non polar, non surface active, soluble in water, low adsorptive properties, etc), the Koc should be estimated using read-across or QSPR methods as a first step. In the information provided by the applicant, the adsorption potential of 1,3-diphenylguanidine is estimated by QSAR on the basis of log Kow. The organic carbon-water partition coefficient (Koc) was calculated using KOCWIN v. 2.0. Based on the first-order molecular connectivity index (MCI) and the logKow = 2.89. Koc (estimated from MCI) = 1652 L/kg, logKoc = 3.22, and the Koc (estimated from logKow) =273.4 L/kg; log Koc=2.44. However for ionized substance at environmentally relevant pH like 1,3- diphenylguanidine, substance adsorption is not triggered by the lipophilicity (*i.e.* log Kow of the substance), but by other mechanisms (*i.e.* ionic interaction). Applying QSPR methods for estimating the adsorption potential of 1,3-diphenylguanidine would lead to a probable underestimation of Koc.

A recent study (Study Report#3, 2015; RI=1) has assessed the adsorption/desorption capacity of the 1,3-diphenylguanidine using the OECD 106 guideline (using a batch equilibrium method). Five different types of soils are investigated: soil 1 (Speyer 2.2, loamy sand), soil 2 (Speyer 2.3, sandy loam), soil 3 (Speyer 2.4, loam), soil 4 (Speyer 6S, clay) and soil 5 (Am Fischteich, silt loam). A tested concentration of 1.077mg/l and a soil-to aqueous phase ratio of 1:5 is used for all five soils. After 24h of agitation, aliquots of the aqueous phase were measured with HPLC (LC-MS). Results show that adsorption equilibrium has reached 52.3%, 46.4%, 77,9%, 82% and 71.3% of the applied amount absorbed to soils 1 to 5, respectively. The amount of test item desorbed reached an equilibrium after about two hours of desorption. The mean values for the adsorption and desorption coefficients related to the organic carbon content of the soils, Koc and Kdes,oc were 807 mL/g and 1077 mL/g, respectively. So, Log Koc ranged from 2.5 to 3.13 with five soils displaying arithmetic mean log Koc = 2.9. These results indicate that 1,3 diphenylguanidine does not bind strongly on soil.

Based on the available experimental data and QSAR predictions provided by the registrant, the eMSCA concludes that DPG does not bind strongly on soil.

7.7.2.2. Volatilisation

In the registration dossier, the vapour pressure of 1,3 -diphenylguanidine was evaluated in a study performed in accordance with OECD testing guideline 104 and GLP requirements. The method used is the Knudsen cell effusion method coupled to a microbalance. As the logarithm of the vapour pressure of a pure substance is a linear function of the inverse of the temperature, the vapour pressure is determinated in a limited temperature range (80 -100°C). Three vapour pressure are determinated: at 81°C, P = 6.524Pa; at 90°C P=5.548 Pa and at 100°C P= 5.896 Pa. The vapour pressure of 1,3 -diphenylguanidine extrapolated at 20°C is 7.4e-11 Pa. The hydrosulbility of DPG was found equal to 325mg/L at 20°C (i.e. 1.54 mol/m3) So as a consequence the Henry's law value equals 4.82e-8 $Pa.m^3.mol^{-1}$ at 20°C.

However, not enough information is available to confirm the reliability of this data.

Therefore, the MSCA of France has proposed a calculation of Henry's law constant using the validated values of water solubility 325 mg/L, the validated vapour pressure of 3.7x 10^{-10} Pa and molecular mass of 211.2, which gives a value of 2.4 x 10^{-10} $Pa.m^3.mol^{-1}$.

Value used for risk assessment: Henry's law constant calculated from solubility in water and vapour pressure values is 2.4 x 10^{-10} $Pa.m^3.mol^{-1}$.

7.7.2.3. Distribution modelling

No data available

7.7.3. Bioaccumulation

7.7.3.1. Aquatic bioaccumulation

The studies on aquatic bioaccumulation are summarised in the following table:

Table 14: studies on aquatic bioaccumulation

Method	Results	Remarks	Reference
QSAR EPI Suite	Log BCF =1.577; BCF =37.73 L/kg wet wt (regression-based estimate) Biotransformation half-life =0.068 days (normalized to 10 g fish) Log BAF =1.316 BAF =20.69 L/kg wet-wt (Arnot-Gobas upper trophic)	2 (reliable with restrictions) weight of evidence (Q)SAR Test material (EC name): 1,3-diphenylguanidine	BCFwin Program
Cyprinus carpio aqueous (freshwater) flow-through Total uptake duration: 42 d OECD Guideline 305 C (Bioaccumulation: Test for the Degree of Bioconcentration in Fish)	BCF: < 2 (at 0.1 mg/L (LOQ)) BCF: < 20 (at 0.01 mg/L)	3 (not reliable) supporting study experimental result Test material (EC name): 1,3-diphenylguanidine	MITI 1992

DPG has an estimated log Kow of 2.89. With a pKa > 10, DPG is in cationic form at environmentally relevant pH, and thus has a very high affinity for organic matter and other matrix having a high cation exchange capacity.

A low Bioconcentration Factor (BCF) value (37.73 L/kg $_{\text{wet wt}}$) was estimated by the BCFBAF software using the Arnot-Gobas method. Nevertheless, this method model estimates steady-state BCF (L/kg) values for non-ionic organic chemicals.

In a weight of evidence approach, an estimated log Pow of 2.9, an estimated BCF of 37.73 and a measured BCF for Cyprinus carpio (RI3) of <20 at 0.01 mg/L and <2 at 0.1 mg/L (limit of quantification) indicate that DPG is therefore not expected to bioaccumulate.

Based on this weight of evidence approach, DPG is not likely to bioaccumulate in aquatic organisms.

7.7.3.2. Terrestrial bioaccumulation

No data available.

7.7.4. Secondary poisoning

Based on the available information, there is no indication of a bioaccumulation potential and, hence, secondary poisoning is not considered relevant.

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

The results are summarised in the following table:

Table 15: Short-term effects on fish

Method	Results	Remarks	Reference
Pimephales promelas freshwater static static method : US EPA Ecological Research series 660/3-75009	LC50 (96 h): 4.2 mg/L test mat. (meas. (initial)) based on: mortality (95% CI: 3.2 - 5.6)	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Study Report#4, 1979
Oncorhynchus mykiss (reported as Salmo gairdneri) freshwater static US EPA Ecological Research series 660/3-75009	LC50 (96 h): 11 mg/L test mat. (meas. (initial)) based on: mortality (95% CI: 9.2 - 13 mg/l)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Study Report#5, 1979
Lepomis macrochirus freshwater static	LC50 (96 h): 9.6 mg/L test mat. (meas. (initial)) based on: mortality (95% CI: 7.4 - 12 mg/l)	2 (reliable with restrictions) supporting study experimental result	Study Report#6, 1979

Method: other: static method : US EPA Ecological Research series 660/3-75009		**Test material (EC name): 1,3-diphenylguanidine**	

Study Report#4 (1979; RI 2) assessed in GLP compliance the acute toxicity of DPG on the fish specie *Pimephales promelas* according to the guideline EPA-660/3-75/009 in static conditions. After 48h and 96h of exposure, the LC50 was 6.4 mg/L (CI95% = 5.2 - 7.9), and 4.2 mg/L (CI95% = 3.2 - 5.6; nominal concentration) respectively.

Study Report#6 (1979; RI 2) assessed in GLP compliance the acute toxicity of DPG on the fish specie *Lepomis macrochirus* according to the guideline EPA-660/3-75/009 in static conditions. After 48h and 96h of exposure, the LC50 was 17 mg/L (CI95% = 13 - 22), and 9.6 mg/L (CI95% = 7.4 - 12; nominal concentration) respectively.

Study Report#5 (1979; RI 2) assessed in GLP compliance the acute toxicity of DPG on the fish specie *Oncorhynchus mykiss* according to the guideline EPA-660/3-75/009 in static conditions. After 48h and 96h of exposure, the LC50 was 18 mg/L (CI95% = 13 - 24), and 11 mg/L (CI95% = 9.2 - 13; nominal concentration) respectively.

According to results of the reliable studies, the most sensitive fish specie to DPG is *Pimephales promelas*. As a consequence the following acute toxicity data is taken into account for the risk assessment:

LC50,48h = 6.4 mg/L (nominal concentration)

LC50,96h = 4.2 mg/L (nominal concentration).

7.8.1.1.2. Long-term toxicity to fish

The results of OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test) are summarised in the following table:

Table 16: Long-term effects on fish

Method	Results	Remarks	Reference
According to OECD Guideline 210 (Fish, Early-life Stage Toxicity Test) *Pimephales promelas* Test on embryo and larvae	Based on number hatched: NOEC (34d): 1.3mg/L (nominal concentration) Based on larval mortality: NOEC (34d): 1.3mg/L (nominal concentration) Based on weight: NOEC (34d): 1.3mg/L (nominal concentration) Based on length: NOEC (34d): 1.3mg/L (nominal concentration)	*1 (reliable without restriction)*	Study Report#7, 2014

A reliable study assessed the long term toxicity of DPG to embryo and fish larvae.

Pimephales promelas embryos and larvae were exposed to five concentrations of DPG (0 ; 0.041 ; 0.13 ; 0.41 ; 1.3 ; 4.1 mg/L) for 34 days. Hatching and larval mortality rates, larval length and larval weight were recorded. All fish were considered dead at D9 in the concentration of 4.1 mg/L. No significant difference with the control group was found up to 1.3 mg/L for the four analysed variables. Therefore, the NOEC was considered to be 1.3 mg/L.

According to study results, the following chronic toxicity to fish threshold is taken into account for the risk assessment: NOEC(34d) = 1.3 mg/L (nominal concentration).

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

The results are summarised in the following table:

Table 17: Short-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater static Method: APHA 1975 US EPA Ecological Research series 660/3-75009	EC50 (48 h): 17 mg/L (nominal concentration), based on mortality	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3-**	Study Report#8, 1979

		diphenylguanidine	
Daphnia magna freshwater static Method: UBA-Verfahrensvorschlag "Bestimmung der Schwimmunfaehigkeit beimWasserfloh "Daphnia magna" (EC0, EC50, EC100; statisches System) (Mai,1984)	EC50 (24 h): 62.4 test mat. (meas. (geom. mean)) based on mobility EC50 (24 h): 73.6 mg/L based on mobility	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Study Report#9, 1984

2 reliable studies assessed the short term toxicity of DPG to aquatic invertebrates.

Study Report#8 (1979; RI 2) assessed in GLP compliance the acute toxicity of DPG on *Daphnia magna* according to the guideline EPA-660/3-75/009 in static conditions. After 24h and 48h of exposure, the EC50 was 33 mg. L-1 (CI95% = 28 - 40), and 17 mg/L (CI95% = 14 - 21; nominal concentration) respectively.

Study Report#9 (1984; RI 2) assessed in GLP compliance the acute toxicity of DPG on *Daphnia magna* according to the guideline UBA-Verfahrensvorschlag "Bestimmung der Schwimmunfaehigkeit beim Wasserfloh "*Daphnia magna*" (EC0, EC50, EC100; statisches System) (May,1984) in static conditions. After 24h of exposure, the EC50 was 73.6 mg/L (CI95% = 61.4 - 88.4). It should be quoted that according to the guidelines, the result of reference substance potassium dichromate shows that strain of *Daphnia magna* used for performing the test is not sensitive enough for validate without caution study results.

According to studies results, the following acute toxicity to aquatic invertebrate threshold is taken into account for the risk assessment: EC50,48h = 17 mg/L (nominal concentration).

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

The results are summarised in the following table:

Table 18: Long-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater semi-static OECD Guideline 211 (Daphnia magna Reproduction Test) (Cited as OECD Guideline 202, part 2 (Daphnia sp., Reproduction Test))	NOEC (21 d): 0.6 mg/L based on reproduction LOEC (21 d): 1.9 mg/L based on reproduction	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Study Report#10, 1990

One reliable study assessed the long term toxicity of DPG to aquatic invertebrates.

Study Report#10 (1990; RI 2) assessed in GLP compliance the chronic toxicity of DPG on the *Daphnia magna* according to the guideline OECD202 part 2 in semi-static conditions. After 21 days of exposure, the NOEC and LOEC were 0.6 mg/L (mean measured concentration), and 1.9 mg/L (mean measured concentration) respectively.

According to study results, the following chronic toxicity to aquatic invertebrate treshold is taken into account for the risk assessment: NOEC(21d) = 0.6 mg/L (mean measured concentration).

7.8.1.3. Algae and aquatic plants

The results are summarised in the following table:

Table 19:. Effects on algae and aquatic plants

Method	Results	Remarks	Reference
Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) (algae) freshwater static Method: other: Static method US EPA, 1971, Algae assay procedure : bottle test	EC50 (96 h): 1.4 — 1.7 mg/L based on growth (no. of cells or chlorophyll a) NOEC (96 h): 0.3 mg/L based on growth (no. of cells or chlorophyll a)	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Study Report#11, 1986
Scenedesmus subspicatus (new name: Desmodesmus subspicatus) (algae) freshwater static Method: other: cell multiplication inhibition test according to DIN 38412, part 9	EC50 (72 h): 2.6 mg/L based on biomass EC50 (72 h): 7.5 mg/L based on growth rate EC10 (72 h): 0.013 mg/L based on biomass EC10 (72 h): 2.1 mg/L based on growth rate	3 (not reliable) supporting study experimental result **Test material (EC name): 1,3-diphenylguanidine**	GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, BUA (1992)

The toxicity of DPG to algae and cyanobacteria was assessed in two studies.

Study Report#11 (1986; RI 2) assessed the toxicity of DPG on the algae *Pseudokirchnerella subcapitata* according to EPA guideline (EPA, 1971). After 96h of exposure, the EC50 and NOEC were 1.4 mg/L (nominal concentration), and 0.3 mg/L (nominal concentration) respectively.

BUA (1992; RI 3) cited an unpublished study that assessed in GLP compliance the toxicity of 1,3-diphenylguanidine on the algae *Scenedesus subspicatus* according to guideline DIN 38412 part 9. After 72h of exposure, the EC50 and EC10 were 7.5 mg/L (nominal concentration), and 2.1 mg/L (nominal concentration) respectively.

According to studies results, the following toxicity to algae and cyanobacteria treshold is taken into account for the risk assessment: NOEC = 0.3 mg/L (nominal concentration).

7.8.1.4. Sediment organisms

With a pKa > 10, DPG is in cationic form at environmentally relevant pH, and thus has a high cation exchange capacity. Considering that uses of DPG could induce potential releases of the substance in the aquatic compartment, the exposure of benthic organisms to the substance is expected. A new Koc value has been proposed in the updated CSR using an OECD guideline 106 and given a logKoc = 2.9. Considering the Integrated Testing Strategy (ITS) for toxicity to sediment organisms in Chapter R.7b, no risk assessment for sediment compartment is needed. Nevertheless, as the logKoc of DPG is closed to the threshold value, a PNEC sediment is proposed using the Equilibrium Partitioning method.

7.8.2. Terrestrial compartment

7.8.2.1. Toxicity to terrestrial plants

The results are summarised in the following table:

Table 20:. Effects on terrestrial plants

Method	Results	Remarks	Reference
Avena sativa (Monocotyledonae (monocots)) *Brassica rapa (Dicotyledonae (dicots))* long-term toxicity (laboratory study) Phytotoxicity Test to a Monocotyledonous Plant Species (Avena saliva L.) and a Dicotyledonous Plant Species (Brassica rapa ssp. rapa [DC.] Metzg.)" adopted March, 1984 Substrate: artificial soil	Avena sativa: NOEC (16 d): 316 mg/kg soil dw test mat. (nominal) based on growth Avena sativa: EC50 (16 d): 1169 mg/kg soil dw test mat. (nominal) based on growth Brassica rapa: EC50 (16 d): 358 mg/kg soil dw test mat. (nominal) based on growth Brassica rapa: NOEC (16 d): 100 mg/kg soil dw test mat. (nominal) based on growth	2 (reliable with restrictions) key study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Study Report#12, 1995

One reliable study assessed the long term toxicity of DPG to terrestrial plants.

Study Report#12 (1995; RI 2) assessed the toxicity of DPG on the terrestrial plants species *Avena sativa* and *Brassica rapa* according to the BBA guideline "Phytoxicity test to a monocotyledonous plant species (*Avena sativa L.*) and a dicotyledonous (*Brassica rapa ssp. rapa*) " (adopted in march 1984) - equivalent to guideline OECD 208. After 16 days of exposure: - for *Avena sativa*, the NOEC and EC50 were 316 mg/kg (nominal concentration), and 1169 mg/kg (nominal concentration) respectively; - For *Brassica rapa*, the NOEC and EC50 were 100 mg/kg (nominal concentration), and 358 mg/kg (nominal concentration) respectively.

According to study results, the following toxicity to terrestrial plants threshold is taken into account for the risk assessment: NOEC = 100 mg/kg (nominal concentration); EC50 = 358 mg/kg (nominal concentration).

7.8.3. Microbiological activity in sewage treatment systems

The results are summarised in the following table:

Table 21: Effects on micro-organisms

Method	Results	Remarks	Reference
Activated sludge, industrial freshwater static OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)	EC50 (3 h): 147 mg/L test mat. based on respiration rate (79-208 mg/L)	1 (reliable without restriction) key study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Study Report#13, 1989
other bacteria: Pre-cleaned activated sludge in particle-free communal wastewater (BOD5: 250 mg/l; NH4-N/l: 50-80 mg) Method: other: Quantitative determination of the nitrification rate, colorimetric measurement of the NO2/NO3 concentration; static test system	EC75 (4 h): > 50 mg/L based on nitrification rate	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Tomlinson, T.G. et al., (1966)

Discussion

Two reliable studies assessed the toxicity of DPG to microorganisms.

Study Report#13 (1989; RI 1) assessed in GLP compliance the toxicity of DPG on a non adapted inoculum according to guideline OECD 209. After 3h of exposure, based on measure of microorganisms respiration rate, the EC50 was 147 mg/L (nominal concentration).

Tomlinson et al. (1966; RI 2) investigated the effect of DPG on the nitrification process in municipal waste waters. They incubated purified activated sludge for 2 - 4 hours in residential waste water at various test compound concentrations. The nitrification rate was determined quantitatively by colorimetric mesurement of the NO2- and NO3- concentrations. The effective concentration for decreasing the nitrification rate by 75% in the first stage (NH4 + =>NO2 -) compared to the control was estimated at 50 mg/l (highest tested ineffective concentration).

According to the studies' results, the following toxicity to microorganisms threshold is taken into account for the risk assessment: EC50 = 147 mg/L (nominal concentration).

7.8.4. Non compartment specific effects relevant for the food chain (secondary poisoning)

The results are summarised in the following table:

Table 22: Effects on birds

Method	Results	Remarks	Reference
Agelaius phoeniceus, Sturnus vulgaris and Passer domesticus. Acute oral toxicity Single dose exposition.	LC50 (0 null): > 100 mg/kg bw based on mortality (Single dose)	3 (not reliable) supporting study experimental result **Test material (EC name): 1,3-diphenylguanidine**	Schafer Jr., E.W. et al., (1983) Schafer, E.W., (1972)

This endpoint allows considering potential secondary poisoning issues to birds following chronic exposure to DPG via the fish and earthworm food chains. DPG is not expected to bioaccumulate in fish/earthworm tissues. As a consequence secondary poisoning is not expected as birds will not be exposed to DPG via food consumption.

7.8.5. PNEC derivation and other hazard conclusions

Table 23

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	*PNEC aqua (freshwater): 30 µg/L*	*Assessment factor: 10* *Extrapolation method: assessment factor* *Chronic aquatic toxicity of 1,3-diphenylguanidine is assessed in organisms form three trophic levels:* *-For fish, NOEC=1.3 mg/L (nominal concentration).* *- For aquatic invertebrates, NOEC = 0.6 mg/L (meas. not specified).* *- For algae, NOEC = 0.3 mg/L (nominal concentration).* *According to these results, PNECwater can be determined by applying an assessment factor of 10 to the lowest short term results (i.e. NOEC = 0.3 mg.L-1 for algae). The calculated PNECwater is 30 µg/L.*
Marine water	*PNEC aqua (marine water): 3 µg/L*	*Assessment factor: 100* *Extrapolation method: assessment factor* *Chronic aquatic toxicity of 1,3-diphenylguanidine is assessed in organisms form three trophic levels:* *-For fish, NOEC=1.3 mg/L (nominal concentration).* *- For aquatic invertebrates, NOEC = 0.6 mg/L (meas. not specified).* *- For algae, NOEC = 0.3 mg/L (nominal concentration).* *According to these results, PNECmarine can be determined by applying an assessment factor of 100 to the lowest short term results (i.e. NOEC = 0.3 mg/L for algae). The calculated PNECmarine is 3 mg/L.*
Intermittent releases to water	*PNEC aqua (intermittent releases): 14 µg/L*	*Assessment factor: 100* *Extrapolation method: assessment factor* *Acute aquatic toxicity of 1,3-diphenylguanidine is assessed in organisms from three trophic levels:* *- For fish, the substance is toxic with a LC50,96h = 4.2 mg/L (*measured initial *concentration).* *- For aquatic invertebrates, the substance is harmful with an EC50,48h = 17 mg/L (nominal concentration).* *- For algae, the substance is toxic with an EC50,96h = 1.4 mg/L (nominal concetration).* *According to these results, PNECwater for intermittent release can be determined by applying an assessment factor of 100 to the lowest short term results (i.e. EC50,96h = 1.4 mg.L-1 for algae). The calculated PNECwater_intermittent is 14 µg/L.*

Sediments (freshwater)	*PNEC sediment (freshwater): 2.51 mg/kg sediment dw*	*According to the guidance on information requirements and chemical safety assessment-chapter R10: characterisation of dose-response for environment (ECHA 2008), in the absence of any ecotoxicological data for sediment-dwelling organisms, the PNEC sed may be provisionally calculated using the equilibrium partitioning method (EPM), following the chapter R16.* PNEC aqua=0.03 *mg.L-1; Koc=807 L/kg;* *PNEC sed =2.51 mg/kg dry weight (a conversion factor of 4.6 was used in order to convert the PNEC in dry weight).*
Sediments (marine water)	*PNEC sediment (marine water): 0.251 mg/kg sediment dw*	*According to the guidance on information requirements and chemical safety assessment-chapter R10: characterisation of dose-response for environment (ECHA 2008), in the absence of any ecotoxicological data for sediment-dwelling organisms, the PNEC sed may be provisionally calculated using the equilibrium partitioning method (EPM), following the chapter R16.* PNEC marine=0.003 *mg.L-1; Koc=807 L/kg;* *PNEC sed =0.251 mg/kg dry weight (a conversion factor of 4.6 was used in order to convert the PNEC in dry weight).*
Sewage treatment plant	*PNEC STP: 1.47 mg/L*	*Assessment factor: 100* *Extrapolation method: assessment factor* *Two reliable studies assessed the toxicity of 1,3-diphenylguanidine to microorganisms.* *According to the studies' results, the following toxicity to microorganisms threshold is taken into account for the risk assessment: EC50 = 147 mg/L (nominal concentration).*

Soil	*PNEC soil: 0.404mg/kg soil dw*	*Assessment factor: 1000* *A data is available for a terrestrial plant: Brassica rapa* *EC50-16d = 358 mg/kg ww. This EC50 will be used for the calculation of PNEC. Using this approach, a safety factor of 1000, as for the aquatic compartment, should be applied to the EC50 value obtained with Brassica rapa, as follows:* *PNECsoil = 358 / 1000 = 0.358 mg/kg of wet soil.* *PNECsoil = 0.358 * 1.13 = 0.404 mg/kg of dry soil.* *(A conversion factor of 1.13 was used in order to convert the PNEC in dry weight).* *As only one terrestrial test result is available (earthworms or plants), the risk assessment should be performed both of this test result and on the basis of the outcome of the aquatic toxicity data to provide an indication of the risk.* *As a matter of precaution, the larger PECsoil/PNECsoil ratio determines which further actions should be taken in the framework of the further testing strategy.* *According to the guidance on information requirements and chemical safety assessment - chapter R10: characterisation of dose [concentration]-response for environment (ECHA 2008), in the absence of any ecotoxicological data for soil organisms, the PNECsoil may be provisionally calculated using the equilibrium partitioning method (EPM), following the chapter R16: Environmental exposure estimation.* *PNECaqua= 0.03 mg/L; Koc = 807 L/kg ; Solubility = 325 mg/L ; MW = 211.3g/mol ; Vapor pressure = 1 µPa.* *PNECsoil = 0.485 mg/kg of dry soil. (A conversion factor of 1.13 was used in order to convert the PNEC in dry weight). The PNECsoil calculated from the B. rapa data is the lowest. It is therefore used for risk assessment:* *PNECsoil = 0.404 mg/kg soil dw*
Air	*No hazard identified*	*Due to the low vapour pressure of the test substance no adverse effects are expected. Therefore, no hazard for air is identified.*
Secondary poisoning	*No potential for bioaccumulation*	*Considering that 1,3-diphenylguanidine is not expected to bioaccumulate in fish/earthworms tissue (i.e. log Kow < 3), secondary poisoning issues to birds following chronic exposure is considered as negligible. As a consequence, no PNEC for birds is determined for the environmental risk assessment of the 1,3-diphenylguanidine.*

7.8.6. Conclusions for classification and labelling

Environmental classification justification

Based on the available data on DPG:

- The most sensitive aquatic species are algae, with EC50 = 1.4 mg. L^{-1} and NOEC = 0.3 mg. L^{-1};
- DPG is readily biodegradable;
- DPG is not bioaccumulative.

Thus, DPG warrants to be classified as Aquatic Chronic 3; H412 according to the CLP regulation criteria.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

The data investigating the toxicokinetics of DPG suggest that the substance is readily absorbed from the gastrointestinal tract of rats. The substance is distributed quickly to all tissues examined, metabolized into three major and two minor metabolites (not identified) and excreted in urine and feces. Slower clearance of a minor metabolite was observed in liver, but the significance of this observation is unknown.

DPG is slowly absorbed after dermal application to rats (around 10 % in rats).

7.9.2. Acute toxicity and Corrosion/Irritation

The registrants concluded that the substance is acutely toxic by oral route and shall be classified Acute Tox. 3; H301 "Toxic if swallowed" (LD_{50} of 107-111 mg/kg bw), and based on the available information, eMSCA can support this conclusion.

The registrants concluded that the substance is severely irritating to the eyes and shall be classified according to the CLP regulation Eye Dam. 1, H318 "Causes serious eye damage". Based on the available information, the eMSCA supports this conclusion.

The registrants proposed to remove Skin Irrit. 2, H315 and STOT SE 3; H335 from the current harmonised classification. Classification was agreed during technical committee on classification and labelling (TC C&L) of 1997 based on known irritation in human as no irritation was observed in animals. Based on the pKa value of DPG, solutions of DPG are expected to be alkaline and potentially irritant. It was suggested by the expert from TC C&L that negative results were observed in animals because animals do not sweat and therefore, the alkaline solution arising from DPG and sweat is not formed. Based on the absence of new data since the TC C&L of 1997 and the known irritation potential of DPG in human, the eMSCA is in the opinion that the harmonised classification shall still be applied.

7.9.3. Skin sensitisation

All the publication and study reports as provided by the registrant for DPG and the IUCLID file were taken into account for the evaluation. A literature search was performed in PubMed until September 2017.

DPG has not been classified as a skin sensitiser by TC C&L (harmonised classification, 1997). As a potential concern on skin sensitisation in human has been recently raised in the literature, skin sensitisation potential of DPG has been reassessed.

7.9.3.1. Animal data

A negative Guinea-pig maximisation assay is available with DPG (Confidential, 1995). In this study, DPG was tested in paraffin oil. The study was GLP-compliant and conducted according to OECD TG 406 guideline. The positive control was DNCB and showed a positive response in guinea-pigs (100%). In this study, 5 control and 10 treated female Dunking-Hartley guinea-pigs were used. Intradermal induction concentration was 1% (w/w) in vehicle. The challenge topical concentration was 25% (w/w). The results of the preliminary

assay are not available in the study summary of the IUCLID dossier. **No cutaneous reactions were observed after challenge application**.

7.9.3.2. Human data

Table 24: Summary of the available patch test studies in human with DPG

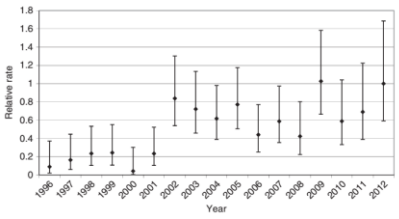
Method	Results	Reference
Epidemiological and clinical patch test studies_**DPG**		
Clinical study 1205 patients (Poland) Patch test, DPG (1%)	744 patients patch tested with DPG Positive reactions: 9.9% The authors records difficulties in most cases to explain the history and distribution of the lesions.	Rudzki et al., 1970
Clinical study 7000 patients suspected of occupational dermatitis (spain, 1978-1988) Patch test, DPG (unknown concentration, no second reading, no information on positive criteria)	Positive results to rubber additives : 686 13 positives reactions to DPG: 2.3%	Conde-salazar et al., 1993
Clinical study 1670 patients (US, Canada; 1981-1988) Patch test, DPG (1%) Reading: 30-60 min + day2 or day3 Positivity : +, ++, +++	316 positives to rubber allergens tested for DPG Positives to DPG: 4.4%	Holness et al., 1997
Retrospective analysis of an Italian database (Italy, 1994-1998) 360 consecutive patients working in healthcare environments and experiencing contact dermatitis Patch test; DPG (1%) Reading: D2 and D4 No information on positive criteria	72 health care personnel with occupational allergic contact dermatitis 2 positive reactions with DPG: 2.8%	Nettis et al., 2002
Retrospective analysis from Information Network of Departments of Dermatology (IVDK), 1995-2001 Patch test, DPG (1%) Positive reactions: +, ++, +++ Reading : D3	No of tested patients: 1455 with occupational contact dermatitis and suspected glove allergy Positive reaction to DPG (+, ++, +++): 1.9% Irritation: 42 patients + : 27 patients ++/+++: 1 patients	Geier et al., 2003

	No significant change in prevalence between 1995 and 2001 (1 to 4%)	
Retrospective analysis of patients from 9 dermatology centres in UK, 1999-2005 British Contact dermatitis society footwear series Patch test, DPG (1%) No information on reading and positive criteria	610 patients tested with DPG 11 positive reactions : 1.80% + 1 irritant and 1 doubtful reaction	Katugampo al., 2005
Retrospective analysis of 1434 patients with suspected allergic contact dermatitis (US, 1994-2006) Patch test, DPG (1%) Positive: +, ++ and +++ Reading: Day 2 and day 4 Relevance: clinical history	31 health care workers tested with DPG 12.9% positive reactions	Suneja et al., 2008
626 patients with suspected allergic contact dermatitis (US, 2007-2009) Patch test, DPG (1%) Reading: Day 2 or 3 and day 6 or 7 Positivity not defined	23 patients with primary allergic contact dermatitis to rubber gloves Positive reaction to carba mix : 20 /23 (87%) 11 were also positive to thiuram mix. 5/5 patch test with DPG were positive to both DPG and carba mix	Cao et al., 2010
Occupational (Leather workers in India) Patch test, DPG (unknown concentration) Reading : days 2, 4 and 7 Unknown criteria for positive patch test	76 of the 472 workers had contact dermatitis 4 workers allergic to DPG : 5.3% Exposed to synthetic rubber gloves	Febriana et al., 2012
Retrospective analysis from Information Network of Departments of Dermatology (IVDK), 2002-2010 93615 patients patch tested Patch test, DPG (1%) Positive reactions: +, ++, or +++	No of tested patients: 2578 patients Patients with positive reaction to DPG: 3% (95% CI: 2.4-3.7). Doubtful: 93 + : 65 patients ++/+++ : 12 patients Authors observed no increase trend identified over the years (patients or health care workers with occupational contact allergy and suspected gloves allergy).	Geier et al., 2012
Retrospective analysis from Information Network of Departments of Dermatology (IVDK), 2003-2012 DKG rubber series	1509 nurses 30 positive reactions : 2% (95% CI: 1.3-2.8)	Molin et al., 2015

Patch test, DPG (1%) Positivity: +, ++ or +++		
Retrospective analysis from IVDK, 2005-2014 female geriatric nurse with contact dermatitis DKG rubber series Patch test, DPG (1%) Positivity : +, ++ or +++	575 female geriatric nurse 9 with positive DPG reaction : 1.6%	Schubert et al., 2016
Retrospective analysis from European Surveillance System on Contact Allergies (ESSCA) network, 2013-2014 29522 patients Patch test, DPG (1%) Reading: second reading performed Positivity: +, ++ or +++	No tested patients: 2331 Positive : 3.26% (95%CI: 2.58-4.06) Irritation : 9.18% +: 2.62% ++/+++ : 0.64%	Uter et al., 2016
Retrospective analysis, NACDG, 2013-2014 screening series of 70 allergens at 13 centers in North America Patch-test, DPG (1%) Reading: first and second reading Positivity: +, ++ or +++	4859 tested patients. 3.8% positive reactions considered clinically relevant (patient's history and clinical examination) +: 2.3% ++/+++ : 0.93%	Dekoven et al., 2017

Table 25: Summary of the available patch test studies in human with carba mix 3% (1% DPG, 1% ZDEC, 1% ZDBC)

Method	Results	Reference
Epidemiological and clinical patch test studies_**CARBA MIX**		
Clinical study 1670 patients (US, Canada; 1981-1988) Patch test, Reading: 30-60 min + day2 or day3 Positivity : +, ++, +++	38% of positive patients to at least one rubber allergens were positive to carba mix	Holness et al., 1997
Retrospective analysis of an Italian database (Italy , 1994-1998) 360 consecutive patients working in healthcare environments and experiencing contact dermatitis	72 health care personnel with occupational allergic contact dermatitis 9 positive reactions with carba mix : 12.5%	Nettis et al., 2002

Patch test; Reading: D2 and D4 No information on positive criteria		
626 patients with suspected allergic contact dermatitis (US, 2007-2009) Patch test, Reading: Day 2 or 3 and day 6 or 7 Positivity not defined	23 patients with primary allergic contact dermatitis to rubber gloves Positive reaction to carba mix : 20 /23 (87%) 11 were also positive to thiuram mix.	Cao et al., 2010
North American contact dermatitis group (2009-2010) Patch test, carba mix	Sample size: 4308 patients Positive reactions to carba mix : 4.6% Only 10% of the case were occupational	Warshaw et al., 2013
Retrospective analysis from UK-wide surveillance scheme (EPIDERM), (UK, 1996-2012) Patch test, carba mix	Patients with allergic contact dermatitis attributed to rubber allergens between 1996 and 2012: 219 Positive reactions to carba mix Decrease incidence of ACD associated with rubber product. Increase relative rate per years with ACD attributed to carba mix mainly from occupational exposure (average annual percentage increase: 10%)  **Fig. 4.** Relative rates (with 95% confidence intervals) by year for allergic contact dermatitis attributed to carba mix and its constituents, as reported to EPIDERM 1996–2012.	Warburton et al., 2015a
Retrospective analysis from European Surveillance System on Contact Allergies (ESSCA) network, 2009-2012 59728 patients Patch test, carba mix	16744 tested with carba mix Positive reactions: 2.29% (95% CI: 2.06-2.52) Statistically significant increase in prevalence of carba mix Decrease prevalence of thiuram mix No increase in prevalence of ZDEC (low prevalence, part of Carba mix)	Warburton et al., 2015b
Retrospective analysis from patch test patient, Odense university hospital 1994-2013 Patch test, carba mix	carba mix : 3.6% A statistically significant trend in the increase in reactions to carba mix without concomitant reactions to other rubber allergen mix was observed.	Mortz et al., 2016
Retrospective analysis from European Surveillance System on Contact Allergies (ESSCA) network, 2013-	% positive to Carba mix: - 2.68% (95%CI: 2.43-3) in all patients (n= 12688) - 3.43% of 15485 consecutive patients in	Uter et al., 2016

2014 29522 patients European multicentric analysis Patch test, Reading: second reading performed Positivity: +, ++ or +++	contributing departments providing special rubber series data (n=7031) - 4.95% of patients tested with special rubber allergens (n=606)	
Retrospective analysis, NACDG, 2013-2014 screening series of 70 allergens at 13 centers in North America Patch-test, DPG (1%) Reading: first and second reading Positivity: +, ++ or +++	4859 tested patients. Carba mix: 229 positive reactions in 4859 patients : 3.4% positive reaction clinically relevant ++/+++ : 54 patients +: 127 patients +/-: 47 patients	Dekoven et al., 2017

Table 26: Summary of case studies with DPG (full-text available, english language)

Subjects (n)	Positive(n)	Concentration (%)	Description	Reference
35	2	1	Shoe contact dermatitis	Adams et al., 1972
8	5 (3 patients ++ or +++ with DPG reacted negatively to carba mix) DPG in gloves	n.g.	Healthcare workers in two different Belgian hospitals Hand eczema related to the use of new latex free sterile gloves (dec 2010- October 2011)	Baeck et al., 2012
105	3	1	Latex free gloves, healthcare workers	Bajaj et al., 1988
1	1	n.g.	Rubber gas mask	Bruze et al., 1994
5	5 (4++, 1+)	n.g.	Rubber gloves	Cao et al., 2010
34	4	n.g.	Agricultural workers	Garcia-Perez et al., 1984
5	1	1	Occupational dermatitis, rubber	Kanerva et al., 1994
46	4	1	n.g.	Kiec-Swierczynska et al., 1995
50	2	n.g.	Occupational workers, rubber industry (tyre and footware)	Kilpikari et al., 1982
61	3	1	Atopic dermatitis patients	Lisi & Simonetti, 1985
1	1(++D2, +++D4)	1	Clothes	Pacheco et al., 2013
5	4 DPG in gloves	Not reported	5 operation-room employees with hand dermatitis	Piskin et al., 2006
16	12 (++ or +++)		Sterile non latex protective gloves	Pontén et al., 2012
15	1	1% and 2%	Rubber boot dermatitis	Ross et al., 1969
50	6	1	50 patients with footwear dermatitis	Sahah et al., 1993
50	1	1	Shoe dermatitis	Suhail et al., 2009

n.g. = not given

7.9.3.3. Summary and discussion

- **Animal data**

Based on the available maximisation test, DPG is not a skin sensitiser.

- **Experimental induction test in human**

One predictive human sensitisation test is available with DPG. Forty-nine human volunteers participated to the study. A series of 12 applications (0.2g as 70% preparation in petrolatum), each of 24 hours duration was carried out during weeks 1, 2, and 3 for induction. Week 4 and 5 were rest periods. For challenge, a series of four applications (0.2g as 70% preparation in petrolatum) on virgin sites was carried out during weeks 6 and 7. Patch testing with 70 % DPG in petrolatum produced no significant positive reactions

following the first induction application. Irritation was noted in 19 out of 49 subjects during subsequent induction exposures. Two subjects showed positive reactions during the 2-week challenge phase at new exposure sites (Study summary from OECD SIDS, unpublished study from Monsanto, 1982). **This study shows that DPG at high concentration is a human skin sensitiser.**

- **Epidemiological and clinical studies based on diagnostic patch test**

A large number of patch tests are available with DPG. DPG was historically tested in US and Canada as part of a mixture which is named carba mix 3% pet. (1% DPG, 1% ZDEC, and 1% ZDBC). Carba mix 3% pet. was introduced for the screening of rubber chemical allergy in 1971 but in 1988 its removal from the baseline series was recommended, due to many irritant reactions. Moreover, the need to check a positive test result by testing the individual components separately and redundancy due to cross-reaction with thiuram mix was identified.

In Europe, DPG is routinely used in rubber test series. Centers that have continued to test carba mix in their baseline series, have reported increasing frequencies of positive reactions in patch tested patients (Pontén et al., 2012; Mortz et al., 2016; Baeck et al.; 2012, Crepy et al., 2016). The increase is suspected to be due to an increase prevalence of allergy to DPG (Warbuton et al., 2015, 2015). The authors suggested that the increase in prevalence allergy might be due to possible increase exposure to DPG in latex-free gloves in health-care workers. In contrast, the retrospective studies of Geier et al., 2012, Molin et al., 2015 and Schubert et al., 2016, concluded that no increase in allergic contact dermatitis to DPG was observed (IVDK database).

For the interpretation of diagnostic patch tests, as DPG is a skin irritant in human, weak positive responses (%+) need to be carefully evaluated and may be considered doubtful (possible false positive). Second reading is for this matter very important. In most of the studies, the severity of the reaction (+ or ++/+++) and the method of reading are not available.

In Geier et al., 2012, retrospective analysis from IVDK network database from 2002 to 2010 gives a frequency of positive (++/+++) reaction to DPG of 0.47% (12 positives in 2578 tested patients). In the previous study investigating data from 1995-2001 (Geier et al., 2003), the rate of positive reactions (++/+++) were < 0.1% (1 out of 1455 tested patients). **An apparent increase in the sensitisation rate of DPG is therefore suggested** by these data but should be interpreted with caution as reading methodology has changed over the years and as no second reading was reported.

In the European multicentre study of Uter et al., 2016, around 0.64% of the positive results to DPG were ++ or +++ and around 70% of the results were considered of doubtful significance or related to irritation (Table 27). The results are obtained from the ESSCA network database from 2013-2014. In previous retrospective analysis of ESSCA network (1996-2012), unfortunately, no results with DPG were available (carba mix only).

Table 27: results obtained in Uter et al., 2016

Allergen	Concentration (%)	n (test)	% +	% ++/+++	% ? +/IR	% positive	95%CI
1,3-Diphenylguanidine*	1.0	2331	2.62	0.64	9.18	3.26	2.58–4.06

In US, in the recent study of Dekoven et al., 2017, the percentage of ++ and +++ reactions were 0.93% (data between 2013 and 2014). Unfortunately, DPG was not tested alone in previous retrospective analysis of the US database. Therefore, it is not possible to conclude if an increase in the frequency of reaction has been observed.

Table 28: results obtained in Dekoven et al., 2017

Substance	n	Positive Reactions	Second Read Code* (%)				Relevance (%)			
			+++	++	+	±	Definite	Probable	Possible	Past
Diphenylguanidine, 1.0% pet	4859	185	4 (2.2)	41 (22.2)	114 (61.6)	24 (13.0)	7 (3.8)	31 (16.8)	88 (47.6)	6 (3.2)

The frequencies of mild to severe reactions (considered as true positives) are reported to be 0.64% in Europe and 0.93% in US in 2013-2014.

The recent published studies suggest that the increase in carba mix contact dermatitis is likely due to an increase in DPG allergy contact dermatitis. Nevertheless, with the available data it is not possible to conclude on an increase in carba mix and on an increase rate of sensitisation to DPG for the following reasons:

- Reading methods of DPG positive reaction has improved over the years;
- Difficulties to compare frequencies of sensitisation between the studies (second reading, % ++/+++ only indicated in few publications; difficulties to interpret doubtful reactions)
- A publication bias cannot be excluded;
- Increase incidence of contact dermatitis to triphenylguanidine has been reported. This substance may be a contaminant / degradation product / alternative to DPG. It has been suggested that triphenylguanidine may be metabolised to DPG in the skin causing positive results in patients already sensitised to DPG (Dahlin et al., 2014).

- **Case reports**

More than 250 publications on DPG contact allergy are available since 1944. Only studies with at least abstract are reported in the table above. In many cases, it is difficult to properly assess the patch test results as concentration tested, control test for toxicity, positivity score, number of reading and clinical relevance were not given.
Nevertheless, the cases provide supporting evidence of the sensitisation potential of DPG.

- **Human exposure**

DPG is manufactured and imported to the EU in amounts of 1 000-10 000 tons/year and is widely used in products on the EU market (ECHA website). According to the CSR (2017), in general rubber goods (e.g. rubber boots), the percentage of DPG is estimated to be maximum 0.23% after vulcanization step. The final residual tonnage of DPG in general rubber goods manufactured in EU is estimated to be 506 t/years. There is no information on the presence of DPG in medical gloves but DPG has been found in these articles in the literature (Hamnerius et al., 2014, Crêpy et al., 2016).

Discussion on the opportunity of a classification
DPG is widely used and is not classified as a skin sensitiser (Annex VI of CLP regulation). The registrant considered that based on the negative results in the guinea-pig (M&K study), positive results observed with DPG in humans were cross-reactions rather than true sensitising effect. Therefore, the registrant did not proposed to classify DPG as a skin sensitiser.

Negative results have been obtained in a M&K animal study. The reliability of the study is difficult to assess as only a summary is available to eMSCA. Nevertheless, animal data do not support a classification.

Based on the available human data, DPG is a known skin irritant that may give false-positive results in human patch test at 1%. During TC C&L, available human data in 1997 were considered weakened by the negative animal studies and no classification was proposed.

Since 1997, substantial number of cases of allergic contact dermatitis have been published with DPG. Methodologies of reading and reporting have been improved over the years (control for irritation, second reading, reporting of the severity of the reaction). These

improvements allow a better distinction between true and false positives. Recent publications focused on medical health care workers in relation to the presence of DPG medical gloves. Moreover, frequency of occurrence in human is reported in several good qualities epidemiological studies (Devoken et al., 2017, Uter et al., 2016; Geier et al., 2012). Taken into account only mild to severe reaction to DPG to avoid taken into account false positive, the frequency rate is considered low to moderate (<1%) in unselected, consecutive dermatitis patients (according to table 3.4.2-b in the CLP guidance of ECHA, 2017). It may be worth noted that the frequency of 0.93% observed in US is near the cut-off of 1% for high frequency. Moreover, as weak positive results should be interpreted with care and are considered doubtful, it is not possible to calculate the true frequency of occurrence of skin sensitisation. Currently, there is no LLNA or *in vitro* data to clarify the potency of DGP, and inform about the subcategorisation. **Therefore, a classification for skin sensitisation in category 1 without sub-categorisation is proposed**.

Litterature data suggest an increase in contact dermatitis to DPG in line with increase exposure. Sub-categorisation of the substance may become possible in case more literature data become available on incidence and exposure to DPG.

7.9.4. Repeated dose toxicity

7.9.4.1. Oral administration

Table 29: Studies on repeated dose toxicity after oral administration

Methods	Results	Remarks	References
14-day range-finding study in rats Range-finding study, GLP Rat (Sprague-Dawley) Oral: feed 5/sex/dose 300, 500, 800, 1500 and 3000 ppm (approx. 36, 56, 73, 119 or 200 mg/kg/day) Examination: mortality, clinical examination, body weight and food consumption, organ weight and gross lesions	Mortality: 2M and 3 F (second week of dosing) at 3000 ppm. Bw: dose related and statistically significant ↓bw gain (> 10% ≥ 500 ppm in males and 800ppm in females). Food consumption: dose related decrease at all dose tested Water consumption: no treatment related effects. Clinical signs at 3000 ppm (Ataxia, piloerection, hunched posture and subdued appearance), one animal with muscular spasm and one with convulsive fits was killed in extremis. ↓ body tone and emaciation at 800 ppm and 1500 ppm. ↑ relative brain weight in all groups (no further details) in males and at 500 and 1500 ppm in females. LOAEL = 300 ppm (eq. to 36 mg/kg bw)	2 (secondary literature from SIDS) Test material: DPG	Confidential, 1980
90-day Repeated Dose Oral Toxicity study in Rats OECD guideline 408, GLP Rat (Sprague-dawley) Oral: feed 15 animals/sex/dose 50, 150, 500 ppm (eq to 4, 11, 37 mg/kg) Haematology, clinical chemistry and urinalysis were performed on week 6 and 13 on 10 rats/sex. The heart, kidneys, liver, lungs, ovaries, prostate gland, spleen, testis, epididymides, thymus, pituitary, adrenals, and thymus were weighed. Organs and tissues were examined for gross lesions. Complete histopathologic examination was performed on all rats in 0 and 500 ppm. The following tissues were examined: adrenal glands, aortic arch, bladder, brain (three sections), eyes, gross lesions, heart, intestines (caecum, colon, duodenum, jejunum, ileum), kidneys, liver, lung/mainstem bronchi, lymph nodes, skin,	**At 500 ppm** Mortality: 1male (week 4 at 500 ppm) and 1 female (control) Bw: ↓bw in males and females. More severe at the beginning of treatment Food consumption: ↓ in males (500ppm) Urinalysis: ↓urine volume in males, slight aciduria in females Clinical signs: no effects Clinical chemistry: increased ALP, AP, sodium levels in males, increase AP, decreased chloride, total protein and calcium levels in females at weeks 6 but not week 13. Haematology: slight increase white blood cells in male and females at week 6 and 13. Histopathological examinations: no effects. NOAEL = 150 ppm (eq to 11 mg/kg)	4 (secondary literature from SIDS) Test material: DPG	Confidential, 1982

spleen, spinal cord/sciatic nerve, stomach, testes (with epididymis and seminal vesicle), thymus, thyroid glands, tongue, trachea and uterus.			
14-day range-finding studies in rats and mice Range-finding study, GLP subacute (oral: feed) Rat (Fischer 344) male/female mouse (B6C3F1) male/female Doses: 0, 250, 500, 750, 1500, or 3000 ppm (22, 45, 65, 121, 200 mg/kg bw in males and 23, 44, 65, 127, 166 mg/kg bw in females) Exposure: 2 weeks Examination : mortality, clinical observation, organ weight and gross examination, no microscopic examination	<u>Rat:</u> - ↓ bw gain (> 10 % at ≥ 1500 ppm in males and 3000 ppm females); - ↓ food consumption (35% less) during first week of treatment in males at 3000 ppm. Improvement during the second week of treatment (increase bw gain and food consumption compare to control); - Clinical signs in males and females (ruffled fur, thin appearance) at 3000 ppm in males and females; - Organ weight and gross lesions: no treatment related effects. <u>Mice:</u> Absence of chemical related toxicity.	2 (reliable with restrictions) Test material: DPG	NTP (1995)
90-day Repeated Dose Oral Toxicity study in Rats OECD guideline 408, GLP Rat (Fischer 344) male/female Subchronic (oral: feed) 250, 500, 750, 1500 and 3000 ppm (17, 32, 50, 100, 181 mg/kg/d in males and 17, 32, 49, 95, 184 mg/kg/d in females) (nominal in diet) Exposure: 13 weeks (ad libitum) Tissues examined were liver, lungs, heart, right kidney, ovaries, prostate glands, seminal vesicles, spleen, right testis and thymus. Other evaluation: haematology and clinical chemistry, sperm mobility, vaginal cytology examination Limitations: - No ophthalmological examination, no assessment of sensory reactivity to stimuli, grip strength and motor activity	**At 3000 ppm** - Mortality: 10/10 females, 6/10 males (no organ specific toxicity), on weeks 4 to 12. - ↓ food consumption (34 to 40% less compare to control, 63% lower than control at the first week of the study indicating poor palatability), ↓ water consumption - ↓ bw (52% of controls in males) - Clinical signs: thin and ruffled fur, discolorations of the tail, ear or vaginal area. In some animals (salivation, seizures, abnormal posture) - Haematology: mild polycythaemia (consistent with dehydration) - clinical chemistry: ↑ bile acids (males), ↓protein, cholesterol, TG, creatinine - relative organ weight in males : ↓ prostate, thymus, ↑heart and spleen relative weight, kidney - microscopic changes in bone marrow, thymus, hypoplasia of uterus, tested, prostate gland, salivary glands **At 1500 ppm** - ↓ food consumption (~15% less than control), ↓ water consumption - ↓ bw gain in males (79% of control) and females (86% of control) - Clinical signs: thin and ruffled fur, discolorations of the tail, ear or vaginal area. In some animals (salivation, seizures, abnormal posture) - haematology: mild polycythaemia (consistent with dehydration) - clinical chemistry: bile acids (males), ↓protein, cholesterol, TG, creatinine	2 (reliable with restriction) Test material: DPG Vehicle: no vehicle	

- bw was recorded only 3 time during the study instead of once a week recommended - only few of the recommended organs were investigated for weight and histopathology - gross necropsy was limited to thinness of the carcass in higher exposure of rats - no statistical analysis provided - no details on histopathological findings	- relative organ weight: ↓ thymus in males, ↑spleen weight in females, ↑kidney rel. weight in males and females - microscopic changes in bone marrow, thymus, hypoplasia of uterus, tested, prostate gland, salivary glands - ↓ sperm motility - ↑length of oestrous cycle **At 750 ppm** - ↓ food consumption (~ 5% less than control) - ↓ bw (92% of control) and females (93% of control) -↑AP (male and females), bile acids (males) - haematology : Dose related ↑ leucocyte, lymphocytes and platelet counts in females - hypoplasia of uterus - ↑length of oestrous cycle **At 500 ppm** - ↑AP (male and females), bile acids (males) - Dose related ↑ leucocyte and lymphocytes counts in females **At 250 ppm:** - ↑AP (male and females), bile acids (males) - Dose related ↑ leucocyte and lymphocytes counts in females LOAEL: 250 ppm equivalent to 17 mg/kg bw/day (male/female)		
90-day Repeated Dose Oral Toxicity study in Mice OECD guideline 408, GLP Mouse (B6C3F1) male/female Subchronic (oral: feed) 250, 500, 750, 1500 and 3000 ppm (38, 75, 114, 231, 457 mg/kg/d in males and 46, 93, 141, 285, 577 mg/kg/d in females) (nominal in diet) Exposure: 13 weeks (ad libitum)	**≥ 3000 ppm** - ↓consumption - ↓ bw gain ↓ bw gain (19% in males and 20% in females) and slight decreased in food consumption (3.9 g/day vs 4.2 g/day in control) ↑ rel. weight: lungs, heart (m) ↑ length of estrous cycle ↓ sperm mobility **1500 ppm** ↓ bw gain (14% in males and 18% in females) **750 ppm**	1 (reliable without restriction) Test material :DPG	

	↓ bw gain (14% in males and 18% in females) (7% in males and females) NOAEL: 500 ppm equivalent to 75 mg/kg bw/day (male/female)		
28-day oral repeated dose toxicity study in Rats + recovery period OECD Guideline 407, GLP Oral: gavage Rat (Crj: CD(SD)) male/female 28-day exposure 10 sex/groups for control and high dose (5 for main study and 5 with recovery). 5/sex/group for mid doses. 0, 10, 30, 90 mg/kg/day (actual ingested) Examinations: clinical signs, bw, food consumption, haematology, blood chemistry, urinalysis, organ weights, histopathological examinations Limitations: Low number of animals at intermediate dose levels (10 and 30 mg/kg), the high dose tested exceed the MTD in females (90% mortality).	**At 90 mg/kg** - Mortality: 1/10 male and 7/10 females (unknown cause of death), weeks 2 to 4. - ↑ clinical signs in both males and females (reversible during recovery period) - ↓ bw gain in males and females (statistically significant) - ↓food efficiency in males (statistically significant), reversible - changes in biochemistry (↓ glucose level in males, ↑ blood urea nitrogen, total bilirubin, AP, ALT, A/G ratio), statistically significant (improvement during recovery) - Urinalysis changes in both sexes (↑ urine volume, ↓specific gravity, ↑ ketone bodies and negative protein) improving with recovery. - Liver: brown - kidney: hydropic changes in renal collecting tubules in both sexes - eardrums turn red in both sexes **At 30 mg/kg** - ↑ salivation in both sexes - ↑ platelet count in females (statistically significant) - ↓ blood glucose level in males (statistically significant), ↑ total cholesterol and triglyceride in females (statistically significant) - Liver: brown, ↓ fatty changes in the liver in males NOAEL: 10 mg/kg bw/day (male/female)	2 (reliable with restriction) Test material : DPG	Confidential (2000)
14-day range-finding study in rats No guideline followed Rat (Sprague- Dawley) male/female Oral: gavage 3 animals/sex/dose 30, 60 and 75 mg/kg bw/d Daily exposure during 2 weeks	**≥ 60 mg/kg** - Mortality: 2/3 males and 3/3 females at 75 mg/kg (day 1 to day 13) and 2/3 males and 1/3 females died at 60 mg/kg (days 5 and 6) - ↓ bw loss (females and males) - ↓ food consumption (females and males) - Prior to death: general neurological clinical signs (lateral recumbency, clonic convulsions, staggering gait, loss of balance, locomotory difficulties, hypoactivity, mydriasis, half-closed eyes and piloerection) **At 30 mg/kg** - ↓bw loss (females)	1 (reliable without restriction) Test material: DPG	Confidential (2010)

	- ↓ food consumption (females) LOAEL: 30 mg/kg bw/day		

Summary and discussion on repeated dose toxicity

In repeated-dose toxicity studies in rats, the main effect observed was a general toxicity consisting in lower body weight gain and food consumption possibly leading to death. No apparent toxicity in target organs was observed in these animals. Effects on body weight and mortality were more severe following gavage than diet administration. The low palatability of the test material may be a contributing factor. Nevertheless, the degree of contribution of the poor palatability is uncertain and other potential mode of action cannot be excluded (e.g. neurological effects on appetite, effects on metabolism). At sub-lethal dose, critical effects were metabolic effects (changes in clinical chemistry and haematological findings). Nevertheless, these findings were not consistent between the studies.

- Oral: diet

Ninety-day feeding experiments in rats and/or mice have been performed in the frame of the US National Toxicology Program (1995). In this study, mortality and marked decrease in body weight gain and food consumption were observed at the highest dose level (~180 mg/kg). No specific organ toxicity was noted. Decreased body weight gain and food consumption were also observed at 750 ppm onwards (~ 50 mg/kg). A dose-related increase in alkaline phosphatase and bile acid concentration was observed in all dose groups. Study reports suggest that these effects could be associated with lower nutrient and water intake at the highest exposures as no lesions in the liver were observed. **The LOAEL was 250 ppm (17 mg/kg).**

In a second 90-day toxicity study in rats, a **NOAEL of 11 mg/kg** was identified based on changes in body weight gain, haematological and clinical chemistry observed at 37 mg/kg (Confidential, 1982).

Mice were less sensitive than rats to DPG. Based on body weight decrease observed at 114 mg/kg, a **NOAEL was identified at 75 mg/kg in both male and female mice**.

The eMSCA noted that as a mark decreased in food consumption was observed in animals fed with DPG, and the dose levels tested in dietary studies may not have been well controlled.

- Oral : Gavage

In a 28-day gavage study (Confidential report, 2000), including a recovery group, decreased food efficiency, body weight and a mark increase in mortality in females were observed at the top dose (90 mg/kg). **The NOAEL was 10 mg/kg based on liver effects in males and haematological changes in females at 30 mg/kg bw**.

There were also two range-finding studies in rats performed by gavage (14-day studies). In these studies, a higher mortality, decreased body weight and food consumption were reported. The NOAEL was 30 mg/kg in both studies.

- Conclusion

A very steep dose-response curve is noted for body weight and mortality effects in animals exposed to DPG. **Overall, a NOAEL of 11 mg/kg bw in rats is retained based on the available 90-day feeding studies. This value is supported by the NOAEL observed in the 28-day gavage study (10 mg/kg) in rats**.

7.9.4.2. Inhalation

No data on the toxicity of DPG following repeated-exposure by inhalation were available.

As DPG is a solid, extrapolation between oral and inhalation routes leads to uncertainties. No data are available on the absorption of DPG by inhalation route but it may be hypothesized that these particles will be absorbed as the substance is highly soluble. Nevertheless, based on particle size distribution of DPG, the substance is mainly inhalable (90% of the particles are between 10 and 45 µm). Thus, only a small proportion of the test material is expected to be respirable and will get into the deep lung.

7.9.4.3. Justification for classification or no classification

No specific target organs have been identified and the classification for repeated dose toxicity is not considered relevant.

7.9.5. Mutagenicity

All the publication and study reports as provided by the registrant for DPG and the IUCLID file were taken into account for the evaluation. A literature search has also been performed in pubmed until September 2016.

At the end of the substance evaluation of DPG in year 2012, further investigations have been requested in order to clarify the potential mutagenicity of the compound (ECHA decision of 26.02.2014).

In February 2016, the registrants have updated the dossier with the following studies:
- Ames assay with S9 from rat, hamster and human;
- *In vitro* comet assay in rat and hamster;
- *In vivo* combined micronucleus and comet assay

The existing and new submitted studies are described and discussed below:

Table 30: Summary of *in vitro* mutagenicity studies with DPG

Method	Concentration/ cytotoxicity	Results	Observation and remarks	References
Direct damage to DNA				
Ames test Non-guideline Non-GLP Method of Mortelmans, 1986 *S. thyphimurium* TA 1535, TA 1537, TA 98, TA 100 Limitations: dose higher than the max. recommended dose of 5000 µg/plate, only 4 strains instead of 5, 2-aminoanthracene only used as positive control with S9-mix	± rat and hamster S9 mix 0, 100, 333, 1000, 3333, 6667 and 10 000 µg/plate Cytotoxicity at 10000 µg/plate	Non-mutagenic with and without Rat S9-mix Equivocal in strain TA98, 100, 1535 and 1537 with Hamster S9-mix with and without pre-incubation	2 (reliable with restriction) Test material DPG Vehicle: no data	Mortelmans et al., 1986
Ames test Similar to OECD 471 GLP *S. thyphimurium* TA 1535, TA 1537, TA 98, TA 100 , *E.coli* WP2 uvrA Limitations : 2 aminoanthracene only used as positive control with S9-mix	± rat S9 mix Cytotoxicity: above 625µg/plate with metabolic activation; above 1250µg/plate for TA1535, 2500µg/plate for TA100, 98, 1537 and *e.coli*)	Non-mutagenic with and without Rat S9-mix	2 (reliable with restriction) Test material: DPG Solvent: DMSO	Confidential, 2000a and b
Ames test OECD 471 GLP *S. thyphimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102	±S9 mix of rat and hamster Cytotoxicity: without S9: 1500 µg/plate Rat S9: TA1535, TA 1537 : 5000 µg/plate ; TA98, TA 100, TA 102: 3000 µg/plate ; Hamster S9: 3000µg/plate	Negative with and without Rat S9-mix **Positive** in strain TA100 with Hamster S9-mix with and without pre-incubation	1 (reliable without restriction) Test material: DPG Vehicle: DMSO	Confidential, 2014

Ames test OECD 471, GLP *S. thyphimurium*, TA 98, TA 100 With and without pre-incubation Limitations : no historical control data, only 2 instead of 5 strains recommended	With Human S9mix Cytotoxicity: strong toxicity above 1500 µg/plate	negative with Human S9 mix	2 (reliable with restriction) Test material: DPG Vehicle: DMSO	Confidential, 2015
Mammalian cell gene mutation assay Non guideline Non GLP Litton Bionetics Inc. standard protocol of Mouse Lymphoma Forward Mutation Assay Mouse lymphoma L5178Y cells Limitations: no data on the purity of the test material, no results details in the endpoint study record of the study, only short treatment period	With and without rat S9-mix 4-h treatment period 16.4, 32.8, 65.6, 131, 188 µg/ml without S9, 32.8, 131, 188, 375, 525 µg/ml with S9	negative with and without metabolic activation	2 (reliable with restriction)	Confidential, 1979
***Ex vivo* alkaline comet assay** Non-guideline, GLP Rat and hamster Hepatocytes Limitations: - no repetition of the assay, -no 24h exposure -Higher concentrations should have been tested - positive results in hamster with DPG disregarded following positive control being negative, -Study claimed to be ex-vivo but rather *in vitro* study - negative control outside Historical control values in the main study -although methylmethane sulphonate was positive, the positive control 2-acetamino-fluorene was not positive in the main study in hamster and is more relevant for this type of substance than methylmethane sulfonate	3-h treatment Rat hepatocytes: 125 – 62.5 – 31.25 µg/L Hamster Hepatocytes : 125 – 62.5 – 31.25 (high cytotoxicity observed at 500 µg/L)	**Negative in rat** **Equivocal in hamster**	2 (reliable with restriction) Test material: DPG Vehicle: DMSO	Confidential, 2016a

Damage at chromosomal level				
Mammalian chromosome aberration test OECD 473 GLP Chinese hamster lungs cell	With and without rat liver S9 mix 6+18-h treatment and 24h treatment: 60, 100, 200, 400 µg/plate Cytotoxicity: 50% cell growth inhibition at 192 µg/L with S9 and 232 µg/mL without S9	Negative With and without rat S9 mix	1 (reliable without restriction) Test material: DPG Vehicle: 1% CMS-Na	Confidential, 2000c

Table 31: Summary of *in vivo* mutagenicity studies with DPG

Method	Dose	Results	Observation and Remarks	References
13-week study combined with in vivo micronucleus Non guideline method: NTP protocol based on McGregor et al. (1990) Fundam Appl Toxicol, 14, 513-522, GLP B6C3F1 Mouse oral feed Erythrocytes of peripheral blood A modification of the technique described by MacGregor et al. (1990) was used. At the termination of the 13-week toxicity study, blood was obtained from 5 male and 5 female mice and smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. The frequency of micronuclei was determined in 2000 normochromatic erythrocytes (NCEs) in each of 5 animals per dose group. The criteria of Schmid (1976) were used in defining micronuclei. The frequency of micronucleated PCEs was analyzed by a statistical software package (ILS, 1990) that employed a one-tailed trend test across dose groups and a t-test for pairwise comparisons of each dose group to the concurrent control. Deviations: no positive controls	0, 250, 500, 750, 1500, 3000 ppm equivalent to 0, 38, 75, 114, 231, 457 mg/kg bw in males and 0, 43, 93, 141, 285, 577 mg/kg bw in females No mortality observed during the study.	Negative in males In females, a significant increase in micronucleated normochromatic erythrocytes was noted in the 750 ppm group. Because the trend test for the female data did not yield a significant P value (P>0.025) and the increase in micronucleated normochromatic erythrocytes was noted in only one exposure group, the female mouse data were judged to be equivocal. Data on historical controls were submitted by the Registrants, these result obtained in the mice micronucleus could be considered within the range of historical control. Nevertheless, it is worth noting that the publication considers that "because the MN studies reported here were conducted by a variety of technicians and scorers over a period of several years, the range of historical control values may not provide a useful basis for judging the result of a single study".	2 (reliable with restriction) Test material:DPG	NTP, 1995

***In vivo* micronucleus test combined to comet assay in the liver and stomach in rats** Similar to OECD guideline 489 and 474, GLP 1 daily treatment for 3 days, sampling at 2-6hours after the third treatment Sprague-Dawley rat Oral : gavage 5 males/group Histopathological analysis and quantification of cleaved Caspase-3 on the pieces of stomach sampled on animals used in the main assay were performed. Limitations: - positive control shall have been aromatic amine (e.g. 2-AAF) to confirm the sensitivity of the comet assay	20, 40, 80 mg/kg bw Mortality at 200 mg/kg bw At 125 mg/kg bw, no mortalities but animals had difficulties to move	Micronucleus study: negative. No proof of bone marrow exposure but high doses tested. Liver comet assay: negative **Stomach comet assay: positive** Histopathological investigations show no apoptose/necrose and conclude actual genotoxicity.	2 (reliable with restriction) Test material: DPG Vehicle: CMC	Confidential, 2016b

7.9.5.1. *In vitro* mutagenicity

Bacterial reverse mutation test

DPG was tested in a reverse mutagenic assay (Ames) performed using *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537. Negative results were obtained with rat S9-mix but equivocal results were obtained with hamster S9-mix *(*Mortelmans, 1986).

DPG was negative in reverse mutagenic tests performed using five strains bacteria of *S. typhimurium* TA98, TA100, TA1535 and TA1537 *E.coli* WP2 uvrA with and without S9mix from rats (Confidential, 2000a and b).

Consistant to previsous findings, by means of the Ames test in compliance with OECD TG 471 *(*Confidential, 2014), the substance was negative without metabolic activation and with Rat S9 mix but had a mutagenic activity in presence of Hamster S9-mix in strains TA98 and TA100. According to the study report, this specific metabolic activation by hamster liver S9-mix, rich in N-Acetyl Transferases, is in favour of the formation of the highly reactive nitrenium ions from the aromatic amine moiety of DPG.

In the confidential, 2015 study, DPG was tested with metabolic activation by a microsomal liver fraction of human origin in TA 98 and TA100. Under these experimental conditions, no mutagenic activity was revealed in both strains TA98 and TA100 in presence of human S9-mix.

Mammalian gene mutation assay

This study evaluated the ability of DPG to induce mutation in the L5178Y TK+/- mouse lymphoma cell line (Confidential, 1979). DPG did not induce a significant increase in mutations at the TK locus in L5178Y mouse lymphoma cells in the condition of the study.

***In vitro* Comet assay**

A mammalian alkaline comet assay was performed *in vitro* on primary rat and hamster hepatocytes (Confidential, 2016a). By comparing data in the rat and the hamster, this study aimed at assessing a systemic hazard in more or less sensitive species in order to evaluate the relevant transposition to Human. No genotoxicity activity was observed in rat hepatocytes. However, in a first assay positive results in hamster were obtained with DPG but were disregarded because the positive control benzidine was not positive in this assay. Furthermore, the dose levels chosen in this study were not high enough to detect potential mutagenic effects.

***In vitro* mammalian chromosome aberration test**

In vitro chromosomal aberration tests were conducted using DPG on CHL/IU cell strains derived from female Chinese hamster lungs (Confidential, 2000c). Under the test conditions, the chromosomal aberration induction for DPG on CHL/IU cells was negative.

7.9.5.2. *In vivo* mutagenicity

The potential *in vivo* clastogenic activity of DPG was tested using a combined *in vivo* micronucleus test in bone marrow and comet assay in the liver and stomach in male rats (Confidential, 2016b). The limitation of the study is the choice of the positive control. Indeed, positive control such as 2-AAF would have been more relevant to prove the sensitivity of the comet assay to detect this familly of substances.

No statistically significant increase in the number of micronuclei was noted in the study. Nevertheless, no proof of systemic exposure was noted. Indeed, no statistically significant decrease in the ratio PCE/NCE was obtained, despite the overall toxicity induced by the test item.

In male rats liver, no statistically significant increases in the percentage of DNA in tail were observed in the comet assay.

In the stomach, a statistically significant dose-related increase in the percentage of DNA in tail was noted. Moreover, at the top dose (80 mg/kg/day), the percentage of DNA in tail was above the maximum value observed in historical data for negative control (27.22% vs. 19.03%). In order to discriminate between necrosis and/or apoptosis and an actual genotoxic potential of the test item, a histopathological study was performed, followed by a specific quantification of apoptosis. No significant necrosis and/or apoptosis was shown. This finding suggested that the increase in the percentage of DNA in tail may probably be due to an intrinsic genotoxic potential rather than a cytotoxic interference.

7.9.5.3. Discussion

The requested Ames test confirmed the positive results observed with Mortelmans, 1986 when using hamster S9-mix (SIEF DPG/MLPC international, 2014a). In contrast, when performed with rat S9-mix, or human S9-mix (confidential, 2000a and b, SIEF DPG/MLPC international, 2014a and b), the Ames test elicited no biologically significant effect. **This indicates that the hamster may be the most sensitive species to the potential genotoxic activity of the metabolites of DPG.**

In the required combined oral *in vivo* micronucleus/comet assay, the *in vivo* micronucleus gives **negative response**. However, in this study no proof of exposure was obtained although toxic effects were observed in the animals (difficulties to move). **The *in vivo* comet assay in stomach and liver cells isolated from male rat, demonstrated a positive genotoxic effect in the stomach, in particular at high dose.** In contrast, **no increase in primary DNA damage was observed in the liver**. Therefore, a local hazard cannot be excluded at high dose. **Local effects observed in the stomach may be indicative of a direct genotoxicity potential of DPG but this is not in line with the negative results obtained in the *in vitro* genotoxicity studies without metabolic activation**. One of the hypothesis would be the formation of reactive compounds at low pH (such as stomach). Low pH are also observed in bladder and aromatic amines are known carcinogens in bladder due to the formation of reactive compounds. Nevertheless, there is no data to confirm this hypothesis and to extrapolate to potential mutagenic effects in bladder.

With regard to the systemic exposure, negative results were obtained in the *in vivo* comet assay in the liver. In the other hand, positive results were obtained using hamster S9-mix. This indicates that hamster may be the most sensitive species to the potential activity of DPG. A systemic *in vivo* effect in hamster could not be totally excluded.

In order to further elucidate the differences between species the consortium has performed a comparative *in vitro* comet assay in both rat and hamster liver hepatocytes showing equivocal results in hamster due to study limitations.

In conclusion, **positive results were obtained at the site of contact in the *in vivo* comet assay which were not consistent with the *in vitro* genotoxicity database**. Furthermore, there are still uncertainties on the systemic genotoxic potential of the substances due to species differences observed in the studies. Although species-sensitivity differences cannot be rulled out, the eMSCA concludes that DPG has a low genotoxic potential in humans and rats.

Finally, eMSCA agrees not to require anymore the toxicokinetics study or a comparative *in vitro* study on potential differences between rats, human and hamster metabolism because it would not clarify the direct potential effects of the substance.

7.9.5.4. Justification for classification or no classification

Overall, based on the new available studies, **DPG had a low capacity to induce heritable mutations. There are still uncertainties in the mutagenic potential of**

DPG due to positive results in stomach and clear species differences in mutagenic potential. Nevertheless, although positive Ames assays were observed with hamster S9 mix, negative results using both rat and human S9 mix are reassuring. In conclusion, based on the available database and weight-of-evidence, no classification is warranted for DPG.

7.9.6. Carcinogenicity

No data available.

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

Table 32: Studies on sexual function and fertility effects

Method	Results	Remarks	Reference
OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test) Rat (Sprague-Dawley) male/female oral: gavage 5, 15 and 25 mg/kg/day (nominal conc.) Premating time in males: 4 weeks before pairing	***At 25* mg/kg/day**: lower body weight gain NOAEL (Parental, developmental): 15 mg/kg bw/day NOAEL (reproductive) : > 25 mg/kg bw/day	1 (reliable without restriction) Test material: DPG	Confidential, 2010
Repeated Dose 90-day oral toxicity study in rodent Similar to OECD TG 408 Rat (F344/N); Mice (B6C3F1) Oral: diet 10/sex/dose/strain 0, 17/17, 32/32, 49/50, 100/95, 181/184 mg/kg bw/d in males and females rats, respectively 0, 38/46, 75/93, 114/141, 231/285, 457/577 mg/kg bw/d in males and females, respectively See table 7.9.4.1	See table 7.9.4.1 for detailed results **RAT** NOAEL for reproductive effects = 32 mg/kg bw/day **MICE** NOAEL for reproductive effects = 231 mg/kg bw/day	2 (reliable with restriction) Test material: DPG Purity: 99%	NTP, 1995
Non-guideline sperm morphology and male fertility study CD-1 mice Oral: gavage Males treated during 8-weeks premating period 25 males/group 0, 0.06, 0.25, 1, 4, 16 mg/kg bw per day	**Toxicity toxicity** No effects on mortality, bw, organ weights. No histopathological findings in testis. **Sperm morphology** ↑ normal sperm head with folded tail but no effect on number of abnormal sperm heads with a folded tail	2 (reliable with restriction) Test material: DPG Purity: 99.9% Vehicle: acetic acid solution	Confidential, 1989

Dose levels chosen according to Bempong's study Examinations: bw, weigh of testes, epididymis, seminal vesicles, kidneys, liver, heart and spleen, histopathological examination of testis, sperm morphology 11 males for fertility evaluation (0, 4, 16 mg/kg) Examinations: number of implantation sites, number of early and late resorptions, number of live, dead, grossly visible malformations Limitations: - Dose tested were too low as no toxicity was observed at the highest dose	**Fertility study** Increase in early and late resorption and dead fetuses. According to the authors, post-implantation losses were inside historical control value for this strain of mice		
Non-guideline seminal cytology, testicular development and fertility investigation in mice and hamster Hybrid mice and Syrian golden hamsters Oral route 4-8 mg/kg 15-week exposure Limitations - no GLP status - Low purity according to the correspondence with the authors	- ↑ in the frequency of sperm abnormalities in mice and hamsters from week 4. - ↓in sperm count and testes weight from week 5 - irregularly shaped seminiferous tubules in mice - ↓ fertility index and number of implants per pregnant female mice (not time dependant) - ↑ frequency of early or late dead foetuses per litter at the 5th and 7th week of dosing at the high dose levels	3 (unreliable) Test material: DPG Purity: not reported Vehicle: 0.025% acetic acid	Bempong, 1983

Table 33: Studies on developmental toxicity

Method	Results	Remarks	Reference
Rat (Sprague-Dawley) oral: gavage 25 rats/groups 5, 25 or 50 mg/kg/day (actual ingested) Exposure: days 6-15 of gestation (once daily) EPA Health Effects Test Guidelines 560/6-82-001	<u>Maternal toxicity:</u> At 50 mg/kg bw/day: ↓bw gain Severe clinical signs <u>Developmental toxicity:</u> At 50 mg/kg bw/day: ↑ post-implantation loss ↓ mean fetal weight No teratogenicity ↑ Reduced ossification (bent ribs) NOAEL (maternal toxicity and fetotoxicity): 25 mg/kg bw/day	1 (reliable without restriction) Test material : DPG	Confidential, 1986

<table>
<tr>
<td>rat (Sprague-Dawley)
oral: gavage
5 rats/group
10, 50, 100, 150 or 200 mg/kg/day (actual ingested)
Exposure: day 6-15 of gestation (once daily)
Range-finding study</td>
<td><u>Maternal toxicity:</u>
At ≥ 150 mg/kg/day:
Only one animal at 100 mg/kg bw/day survived until scheduled sacrifice.

At 50 mg/kg/day:
No mortality
Bw loss during first three days and decreased bw gain

<u>Developmental effects:</u>
At 10 and 50 mg/kg bw/day
No adverse effect on intra-uterine survival</td>
<td>2 (reliable with restrictions)
Test material: DPG</td>
<td>Confidential, 1985</td>
</tr>
<tr>
<td>mouse (ICR-JCL)
oral: gavage
19/20 mice/groups (except 7 females at dose 10 mg/kg)
0.25, 1, 4 or 10 mg/kg/day (actual ingested)
Exposure: days 0-18 of gestation (once daily)
Sacrifice: day 18
Details on study design: Since all non-pregnant mice given 15 mg/kg bw/day died within six days, 10 mg/kg/day was chosen as the highest dose.
equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)
Limitations:
- Lack of reporting: no data on environmental condition, weight of the animals at study initiation
- Only 7 animals dosed at 10 mg/kg/day
- only uteri examined post mortem)</td>
<td>Maternal toxicity: No effects on maternal bw
(no more detail available).
At 10 mg/kg/day: ↓ number of implants (statistically significant)

<table><tr><td>Dose (mg/kg)</td><td>No. Of pregnant mice</td><td>Total No. implants (average no. implants±SEM)</td></tr><tr><td>0</td><td>20</td><td>253 (12.7± 0.3)</td></tr><tr><td>0.25</td><td>19</td><td>229 (12.1± 0.7)</td></tr><tr><td>1.0</td><td>20</td><td>266 (13.3± 0.5)</td></tr><tr><td>4.0</td><td>20</td><td>261 (13.7± 0.3)</td></tr><tr><td>10.0</td><td>7</td><td>79 (11.3±0.4)a</td></tr></table>
No treatment related effects on developmental toxicity</td>
<td>2 (reliable with restrictions)
Test material: DPG
Vehicle: Carboxymethyl cellulose</td>
<td>Yasuda Y and Tanimura T, 1980</td>
</tr>
</table>

Table 34: Specific investigations: other studies

Method	Results	Remarks	Reference
Type of effects studied: estrogenic activities (*in vitro*) *In vitro* 10^{-3} to 10^{-7} mol/l (analytical conc.) Exposure: 4h (1 time)	Sixteen kinds of vulcanizing agents and vulcanization accelerators (e.g. DPG) used for food contact rubbers and their metabolites were tested. All the vulcanizing agents and	2 (reliable with restrictions) Test material: DPG	Ogawa et al., 2006

DPG induced by the S9-mix was tested for its estrogenicity activity using the yeast two-hybrid assay.	vulcanization accelerators and their metabolites did not display any estrogenicity.		

7.9.7.1. Effects on sexual function and fertility

Based on OECD TG 421, with a reliability klimisch score of 1, no effects were observed in rats on mating, fertility, gestation or delivery at any dose levels of 5, 15 or 25 mg/kg bw. Male and female pups from the group treated at 25 mg/kg/day had lower mean body weight gain over the lactation period. **There were no effects of treatment with DPG on sperm analysis, organ weights, macroscopic post-mortem examination and microscopic examination at any dose-level**. However, only 4 weeks pre-mating period was performed and a low number of animals was used in the study (screening test).

In a NTP 13-week sub-chronic toxicity study (1995), with a reliability score of 2, in male and female F344/N rats and B6C3F1 mice, vaginal cytology and sperm motility evaluations were performed on all rats in the 0, 500, 750, and 1500 ppm groups and in all mice in the 0, 250, 750 and 3000 ppm groups.
In rats, at 3000 ppm, treatment related mortality (4/10 males and 10/10 females), marked body weight gain and food consumption were observed in male and female rats. At 1500 ppm, although no mortality occurred, marked body weight gain decrease and decrease in food consumption was observed. At 750 ppm, only slight body weight decrease was observed. **Female rats exhibited uterine hypoplasia and a prolonged reproductive cycle in the 750 ppm group (ca. 49 mg/kg bw/day) and 1500 ppm group (ca. 95 mg/kg bw/day) compared with control animals**. In the male rats, only animals in the 1500 ppm group (ca. 100 mg/kg and day), showed diminished sperm motility. Alterations in the reproductive organs (e.g. secretion depletion of the prostate, epididymal hypospermia, reduced spermatogenesis) were occasionally found in the males of the 3000 ppm group (eq. to 181 mg/kg bw/day).

In mice, marked decreased in bw gain was observed at 750ppm (ca. 114 and 141 mg/kg bw per day in male and females, respectively) and 3000 ppm groups (ca. 457 and 577 mg/kg bw/day in male and females, respectively). At the highest dose, prolonged oestrous reproductive cycle was observed in females and decreased in sperm motility in males. **Fertility effects (sperm mobility, prolonged reproductive cycle and uterine hypoplasia) may be an unspecific secondary consequence of reduced feed consumption**. Nevertheless, at 50 mg/kg bw per day no feed restriction was reported in rats and mice. Consequently, it is difficult to consider that feed restriction is entirely responsible for the reproductive effects observed, and the lack of reproductive effects of DPG cannot be ruled out based on these results.

In a non-guideline, non-GLP study (Confidential, 1989), male sperm morphology and fertility study in mice, with a reliability of 2, DPG (purity 99.9%) was administered by daily gavage to groups of 25 male CD-1 mice at dose levels of 0, 0.06, 0.25, 1, 4 and 16 mg/kg/d during a 8-week pre-mating period. Females were not dosed at any time during the study. Within 24 hours after the last treatment, 9 to 13 males, randomly taken from each group were killed and subject to gross examination at autopsy. A selected number of organs were weighted and preserved. Sperm abnormality evaluation was performed in the selected males from the control and 16 mg/kg dose group. The remaining males in the control, 4 and 16 mg/kg bw/d groups were mated with non-dosed females. Reproductive performance, necropsy findings and litter data were recorded. No differences were found between control and dosed groups in body weight gain during the dosing period, macroscopic observations and organ weights at necropsy. Microscopic examination of the testes in the 16 mg/kg/d group, did not show any effect due to DPG

dosing when compared to the control group. **Sperm abnormality evaluation in the 16 mg/kg/d group showed a slight but statistically significant increase (5% versus 2% in control) in sperm with folded tails but normal heads**. However, since the total number of abnormal sperm cells as well as the number of specified sperm abnormalities were similar in control animals, the observed increased number of sperm cells with folded tails is considered of doubtful biological relevance. Male and female fertility as well as reproduction performance were comparable in the groups examined (0, 4 and 16 mg/kg/d). The dose were chosen according to the Bempong study (see summary below) but are not appropriate for risk assessment and classification and labelling as no toxicity was observed in males at the highest dose tested.

Bempong et al., 1983 analysed the effects of DPG (purity not reported, probably of low purity) on seminal cytology, testicular development and fertility. The reliability of the study was 3 (lack of details on compound purity, mode of administration, number of treated animals/dose, food and water consumption, clinical signs and body weight, poor statistical evaluation and no GLP). Dose-levels of 4 and 8 mg/kg bw/day DPG, administered by the oral route up to 15 weeks, induced a time- and dose-dependent increase in the frequency of sperm abnormalities in both mice and hamsters from week 4, **a significant decrease in sperm count and testes weight from week** 5, and **irregularly shaped seminiferous tubules in mice**. **The fertility index and the number of implants per pregnant female mice were decreased in a dose-dependent fashion, but the effect did not seem to be time-dependent**. The frequency of early or late dead foetuses per litter was significantly increased at the 5th and 7th week of dosing at the high dose levels.

The available *in vivo* studies show inconsistent results on the capability of DPG to cause adverse effects to reproduction. **Since the discussion on the harmonised classification of DPG, a new screening OECD 421 is available. On this basis, the registrant proposed in its dossier the withdrawal of the classification Repr. 2 H361f. MCSA FR does not agree with this proposal as limitations in the screening study (number of animals, only 4-week pretreatment period of males) does not permit to clarify the reproductive concern of DPG.** Indeed, pathological changes in female reproductive organs have been observed in published studies at dose level not associated with feed restriction and, in absence of a EOGRTS, an effect on fertility cannot be totally excluded. Thus, the available information is not sufficient either for classification as reproductive toxicant category 1 or for risk assessment. Therefore, the requested EOGRTS is needed to clarify the concern and to cover the standard information requirement.**The EOGRTS should be submitted by 29 July 2021.**

7.9.7.2. Developmental toxicity

In female rats and mice, fetotoxic, but not teratogenic, effects were seen after the oral administration of maternotoxic doses. In the rat developmental toxicity study, the NOAEL for fetuses was set at 25 mg/kg bw. In the mouse developmental toxicity study, the NOAEL was set at ≥ 10 mg/kg for fetuses.

7.9.7.3. Justification for classification or non classification

No effects were observed in the two developmental toxicity studies available with DPG. Thus, no classification for developmental toxicity is proposed.

With regard to fertility and sexual function, DPG is classified as Repr. 2 H361f in the current entry of the CLP regulation. This classification was adopted by the Commission Working Group on the Classification and Labelling of Dangerous Substances in July 1997 (ECBI/32/97) based on sperm and fertility effects reported by Bempong et al., 1983.

During the evaluation, the registrant proposed to withdrawn the classification Repr. 2, H361f based on the results of a new OECD 421 study. **Due to the pathological changes in female organs observed at dose not associated with feed restriction, an effect on fertility cannot be totally excluded at present. Moreover, a datagap has been**

identified for this endpoint. Therefore, the classification of DPG for reproductive toxicity should be reviewed taking into account the results of the ongoing EOGRTS study.

7.9.8. Hazard assessment of physico-chemical properties

No specific hazard identified.

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

7.9.9.1. Selection of the critical DNELs for DPG (as proposed by eMSCA pending upcoming EOGRTS study)

Table 35

CRITICAL DNELS/DMELS					
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**DNEL/ DMEL**	**Justification/ Remarks**
Sub-chronic toxicity Systemic effect-Long-term	90- day toxicity study	NOAEL = 11 mg/kg	NOAEL	Inhalation: 0.16 mg/m^3 Dermal: 1.1 mg/kg bw	Workers
Sub-chronic toxicity Systemic effect-Long-term	90- day toxicity study	NOAEL = 11 mg/kg	NOAEL	Inhalation: 0.032 mg/m^3 Dermal: 0.16 mg/kg bw Oral: 0.016 mg/kg bw	General population
Reproductive toxicity	No DNELs: datagap need to be address first				
Skin sensitisation	No quantitative DNEL could be derived. Nevertheless, classification of the substance is needed as a RMM for this endpoint of concern.				

7.9.9.2. WORKERS

<u>Inhalation-systemic effect – long term</u>

Critical effects: Metabolism, haematological changes

Dose descriptor: NOAEL observed in rat, 90-day toxicity study of 11 mg/kg.

Correction of respiratory volume for relevant duration: For 8h of exposure, the respiratory volume of rats and humans are 0.38 m^3/kg bw and 6.7 m^3/ person, respectively. The respiratory volume light activity for worker (8h exposure) is 10 m^3/person: 1/0.38 x 6.7/10

Inhalation and oral absorption: 100% by default

<u>Default assessment factors:</u>

Interspecies: 2.5

Intraspecies: 5 (workers)
Exposure duration: 2 (DNEL is based on a subchronic toxicity study)
Dose-response: 1 (the starting point is a NOAEL)
Quality of the database: 5 (data gap for reproductive toxicity and absence of studies by inhalation)

Dermal systemic effect – long term
Critical effects: Metabolism, haematological changes
Dose descriptor: NOAEL observed in rat, 90-day toxicity study of 11 mg/kg.
Dermal absorption: 10% by default.

Default assessment factors:

Interspecies: 2.5*4 (allometric scaling)
Intraspecies: 5
Exposure duration: 2
Dose-response: 1
Quality of the database: 1

7.9.9.3. GENERAL POPULATION

Inhalation-systemic effect – long term

Critical effects: Metabolism, haematological changes
Dose descriptor:
NOAEL observed in rat, 90 day toxicity study of 11 mg/kg.
Correction of respiratory volume for relevant duration: For 24h of exposure, the respiratory volume of rats is 1.15 m^3/kg bw.
Inhalation absorption: 100% by default

Default assessment factors:

Interspecies: 2.5
Intraspecies: 10 (general population)
Exposure duration: 2 (DNEL is based on a subchronic toxicity study)
Dose-response: 1 (the starting point is a NOAEL)
Quality of the database : 5 (datagap for reproductive toxicity potential of DPG and no studies by inhalation)

Dermal systemic effect – long term

Critical effects: Metabolism, haematological changes
Dose descriptor: NOAEL observed in rat, 90 day toxicity study of 11 mg/kg.
Dermal absorption: 10% by default

Default assessment factors:

Interspecies: 2.5*4 (allometric scaling)
Intraspecies: 10
Exposure duration: 2
Dose-response: 1
Quality of the database: 1

Long-term oral systemic effect

Critical effects: Metabolism, haematological changes

Dose descriptor: NOAEL observed in rat, 90-day toxicity study of 11 mg/kg.

Default assessment factors:

Interspecies: 2.5*4 (allometric scaling)
Intraspecies: 10
Exposure duration: 2
Dose-response: 1
Quality of the database: 1

7.9.9.4. Selection of critical DNEL/DMEL for DPG by products

A potential concern was identified on the potential exposure of workers and consumer to DPG-by products via the environment or consumer products. The major by-products of DPG consist of aniline, N-nitroso-diphenylurea and phenyl guanidine depending on the conditions. Aniline is the only chemical with an harmonised classification and toxicological data. Worst-case concentration of aniline in articles was provided by the lead registrant in the dossier and a risk assessment for all uses was performed. **The risk related to the two other by-products has not been evaluated. No data on the concentration of these by-products in articles, toxicological profile was available.**

Aniline (EC no. 200-539-3) has the following harmonised classification (index number 612-008-00-7):
Carc. 2 ; H351: suspected of causing cancer
Muta. 2; H341: suspected of causing genetic defects.
Acute Tox. 3*; H331 : Toxic if inhaled.
Acute Tox. 3* ; H311 : Toxic in contact with skin.
Acute Tox. 3*; H301 : Toxic if swallowed.
STOT RE 1; H372**: Causes damage to organs through prolonged or repeated exposure.
Eye Dam. 1; H318: Causes serious eye damage.
Skin Sens. 1; H317: May cause allergic skin reaction.
Aquatic Acute 1; H400: Very toxic to aquatic life.

For risk assessment, the long-term DNEL for systemic effects by inhalation used by the registrant was 7.7 mg/m3 (equivalent to 2 ppm), which correspond to the 8h-TWA value recommended by SCOEL, 2016. eMSCA considers this value appropriate. As aniline may also be absorbed by dermal route a long-term DNEL of 1 mg/kg for systemic effects by dermal route has also been derived for workers.

Table 36: DNEL for aniline used for worker risk assessment

Route	Type of effect	Risk characterisation type	Hazard conclusion (see section 5.11)
Inhalation	Systemic effects - long term	Quantitative	DNEL (Derived No Effect Level) = 7.7 mg/m³
Dermal	Systemic effects - long term	Quantitative	DNEL (Derived No Effect Level) = 2 mg/kg bw/day
	Local effects - long term	Qualitative	Medium hazard (no threshold derived)
Eye	Local effects	Qualitative	Medium hazard (no threshold derived)

For the general population, DNEL has been derived from worker DNEL.

Consumer DNEL inhalation= worker DNEL inhalation/2 (factor 5 to 10) and *10/20 for respiratory volume correction from worker to consumer.

Consumer DNEL dermal = worker dermal/2 (factor 5 to 10)

Consumer DNEL oral = same value as DNEL consumer dermal.

Table 37 DNEL for aniline used for general population risk assessment

Route of exposure and type of effects	Risk characterisation type	Hazard conclusion (see section 5.11)
Inhalation: Long term, Systemic	Quantitative	DNEL (Derived No Effect Level) = 1.93 mg/m³
Oral: Long term, Systemic	Quantitative	DNEL (Derived No Effect Level) = 1 mg/kg bw/day

For consumer, the proposal of the registrants to derived DNEL for workers to consumer may underestimate the risk as children may be more sensitive than workers. Therefore, an additional safety factor of 10 could be proposed for the general population.

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

The substance has harmonised classification and labelling entry in Annex VI of the CLP Regulation (human health only): Acute Tox. 4; H302*, Skin Irrit. 2; H315, Eye irrit. 2; H319, STOT SE 3; H335, Repr. 2; H361f***.

In their dossier, the registrants proposed to modify current DPG classification for acute toxicity (Acute Tox. 4; H302 by Acute Tox. 3; H301), to remove classification for skin irritation (Skin Irrit. 2; H315), to change classification for eye irritation (Eye. Dam. 1 instead of Eye irrit. 2), and to remove classification for reproductive toxicity (Repr. 2; H361f).

eMSCA agrees to change Acute tox. 4; H302 by Acute Tox. 3; H301 and Eye Irrit. 2; H319 to Eye Dam. 1; H318 but disagrees with the removal of classification Skin Irrit. 2; H315 and Repr. 2; H361f.

Indeed, based on human data, DPG has been shown to be a skin irritant, therefore, eMSCA disagrees that absence of effects observed in animal could justify the removal of the skin irritation classification. Based on recent publication of relevant human data of contact dermatitis following DPG exposure, a classification Skin Sens. 1, H317 is warranted.

Moreover, with regard to the withdrawal proposal of the classification Repr. 2, H361f, due to the pathological changes in female organs, observed at dose not associated with feed restriction, an effect on fertility cannot be totally excluded. Classification and labelling for this endpoint need to be reassessed with the results of the new ongoing EOGRTS study. Meanwhile, current harmonised classification and labelling is considered appropriate.

Overall, the proposed revision of the current harmonised classification is summarised in the table below.

Table 38: Existing and proposed classification according to CLP regulation

	Classification	
	Hazard Class and Category Code(s)	**Hazard statement Code(s)**
Current Annex VI entry	Acute Tox. 4 Skin Irrit. 2 Eye irrit. 2 STOT SE 3 Repr. 2	H302* H315 H319 H335 H361f
eMSCA proposal	**Retain** Skin irrit. 2 STOT SE 3 Repr. 2 **Modify** Acute Tox. 3 Eye Dam. 1 **Add** Skin Sens. 1	**Retain** H315 H335 H361f **Modify** H301 H318 **Add** H317

* minimal classification

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Not assessed.

7.10.2. Endocrine disruption - Human health

No conclusion on endocrine disrupting properties can be made as a datagap on reproductive toxicity has been identified. The endpoint will be reassessed following analysis of the ongoing EOGRTS study.

7.11. PBT and VPVB assessment

Persistence:

According to study report#2 (2015) in the study OECD guideline 301D (ready biodegradability:closed bottle test) presented in the section 7.7.1.2.1.1, DPG is readily biodegradable. Therefore, the substance does not fulfil the persistence criterion (P).

Bioaccumulation:

As presented in the section 7.7.3.1, the estimated log Kow for DPG is 2.89 which is below the cut-off value of 3 for bioaccumulation. So, DPG is not likely to bioaccumulate in aquatic organisms.

Toxicity:

The lowest NOEC value is 0.3 $mg.L^{-1}$ for algae species. Therefore DPG is not considered to fulfil the T criteria for the environment.

The substance does not meet the criteria for P/vP or B/vB, so no further assessment on the T criterion for human health is needed.

Conclusion:

Based on the assessment described in the subsections above the submitted substance DPG is not a PBT / vPvB substance.

7.12. Exposure assessment

7.12.1. Human health

7.12.1.1. Workers

The table below described exposure scenarios related to: manufacturing of DPG, formulation and re-packaging, manufacturing of articles (tyres and rubber goods), use of tyres, storage of used tyres before recycling, tyres recycling (End of Life Tyres, ELT), re-use of ELT and use of General Rubber Goods (GRG) articles.

Table 39: Main exposure scenarios for workers

Exposure Scenario name	PROC	
Manufacture of substances	PROC 3, 8a, 8b, 9, 14, 15	
Manufacture of Masterbatches - continuous process	1, 8b, 9, 14, 15, 21	PC32: polymer preparations and compounds Technical function: processing aid
Masterbatch production - internal mixture	5, 8a, 8b, 9, 14	
Formulation and re-packaging	8a, 8b, 5, 9	
End of Life tyre : Coarse shredding	8a, 8b, 21	
Formulation - End of life Tyre : Grinding (ambiant)	8a, 8b, 24	
End of Life Tyre : Grinding cryogenic	8a, 8b, 24	
Formulation - End of Life Tyre : Pyrolysis	8b, 2, 22	
Formulation - End of Life Tyre : Energy recovery : cement kiln	8b, 21, 22	
Formulation - End of Life Tyre : Energy recovery : other	8b, 21, 22	
Formulation - End of Life Tyre : Electric arc furnace	8b, 21, 22	
Formulation - End of Life Tyre : Devulcanization/reclaim	8b, 21, 22	
USE AT INDUSTRIAL SITE		
Manufacture of General Rubber Goods (Grad PD, C, GC & Mixland)	5, 8b, 9, 10, 13, 14, 21	SU 11, manufacture of rubber products
Manufacture of Tyres (Grad PD, C, GC & Mixland)	5, 9, 10, 14, 21	SU 11, manufacture of rubber products
ARTICLE SERVICE LIFE (WORKERS)		
Garage owner – tyres change	21	
End of life tyre: ELT pre-processing storage	21	AC1: vehicles
ELT articles: installation of shock absorbing tiles	21	AC 10: rubber articles
Installation of synthetic turf infilled with granules	21	AC 10: rubber articles
GRG articles – conveyor belt	21	AC10a: rubber articles: large surface area articles

The exposure assessment was based on the registration dossier as provided by the lead registrant. CHESAR 3.1 with in-built ECETOC TRA was used for Tier I modelisation and RISKOFDERM and ART for Tier 2.

7.12.1.2. Consumers

Table 40: Main exposure scenarios for consumers

Exposure Scenario n°	Exposure Scenario name	Article category	Product category
Consumer use of tyres (vehicles)		AC10	PC32
ELT articles – shock absorbing tiles (children < 3 years)		AC10a	PC32
ELT articles – synthetic turf related to subsequent service life (children < 6-11 years)		AC 10a	PC32
GRG articles – consumer Children use of balloons GRG		AC 10b	PC32

The same scenario were also derived for aniline risk assessment.

7.12.2. Environment

The following exposure scenarios were addressed in the registration dossiers and these were assessed as part of the substance evaluation.

Exposure assessment of DPG

We described in the following table exposure scenarios related to: manufacturing of DPG, formulation and re-packaging, manufacturing of articles (tyres and rubber goods), use of tyres, storage of used tyres before recycling, tyres recycling (End of Life Tyres, ELT), re-use of ELT and use of General Rubber Goods (GRG) articles.

Table 41: Exposure scenario and release factors for DPG:

Exposure Scenario n°	Exposure Scenario name	Environmental Release Categories (ERC)	Source of release factors considered
1	Manufacture of substances -	1	Environmental monitoring
2	Manufacture of Masterbatches - continuous process	3	Justification based on confidential information provided by the registrant
3	Masterbatch production - internal mixture	3	Not correctly justified
4	Formulation and re-packaging	2	Not correctly justified
5	Manufacture of General Rubber Goods (Grad PD, C, GC & Mixland)	6d	Values of SPERC from ETRMA
6	Manufacture of Tyres (Grad PD, C, GC & Mixland)	6d	Values of SPERC from ETRMA
7	Consumer use of tyres	10a	Default values of ERC From the R16 guidance
8	Garage owner - tyres change	10a	Default values of ERC From the R16 guidance
9	End of Life Tyre : ELT pre-processing storage	10a	Default values of ERC From the R16 guidance
10	End of Life tyre : Coarse shredding	3	Default values for waste

			treatment processes from the R18 guidance
11	End of life Tyre : Grinding (ambiant)	3	Default values for waste treatment processes from the R18 guidance
12	End of Life Tyre : Grinding cryogenic	3	Default values of ERC From the R16 guidance
13	End of Life Tyre : Pyrolysis	3	Default values of ERC From the R16 guidance
14	End of Life Tyre : Energy recovery : cement kiln	3	Default values for waste treatment processes from the R18 guidance
15	End of Life Tyre : Energy recovery : other	3	Default values for waste treatment processes from the R18 guidance
16	End of Life Tyre : Electric arc furnace	3	Default values of ERC From the R16 guidance
17	End of Life Tyre : Devulcanization/reclaim	3	Default values of ERC From the R16 guidance
18	ELT articles – installation of shock absorbing tiles	10a	Default values of ERC From the R16 guidance
19	ELT articles – installation of synthetic turf fields	10a	Default values of ERC From the R16 guidance
20	ELT articles - shock absorbing tiles	10a	Default values of ERC From the R16 guidance
21	ELT articles - synthetic turf fields	10a	Default values of ERC From the R16 guidance
22	General Rubber Goods articles - conveyor belt	11a	Default values of ERC From the R16 guidance
23	General Rubber Goods articles - ballons	10a	Default values of ERC From the R16 guidance

Relevant details for exposure scenario

For the scenario 1: "Manufacture of DPG", an environmental monitoring process has been performed to assess the quantity of DPG released in the local conditions of one manufacturing plant.

Some measurements have been considered to assess the emissions of DPG in freshwater:

- The flow rate of the river has been measured by the national water company downstream from the effluent discharge. More than 100 measurements have been performed during the 1995-2005 period, distributed all over the year excepted in January (no measure in January) were flow rate can be assumed to be high. According to ECHA guidance R16, the 10th percentile, corresponding to the low flow rate, has been used for calculations.

The flow rate of the discharged waste water was measured daily. Measurements from January 2009 to August 2010 have been considered. The 90th percentile has been considered for calculations.

- The concentrations of DPG in effluent have been measured. Monitoring of the waste effluent of the plant, which is discharged into the river, has been performed over 10 days in October 2016. The 90th percentile of release DPG has been calculated and chosen for release rate value in assessment approach.

All data presented above take into account realistic measures, considering for all parameters the worst case values to cover the risk in all meteorological conditions

and support a realistic environmental risk assessment.

For the soil compartment, the default worst case value of ERC from the R16 guidance is considered.

For the air compartment, no monitoring data is available. However, the releases of DPG in the atmosphere correspond to the loss of material passing through the sleeve to the dust collector of the air drying and grinding filter. Thus, the local release of DPG has been estimated.

For the scenario 2, (corresponding to ERC 3) the release factor to water was estimated to 0%. Justifications and the detailed description of the existing process were provided by the registrant. The information provided were considered sufficient by the eMSCA of FR to justify the estimated release factor.

For the scenario 3 (corresponding to ERC 3) and scenario 4 (corresponding to ERC2) the release factors to water were estimated to 0% without proper justifications (not supported by SPERC, monitoring or other arguments). Except for processes in closed systems and controlled conditions, zero emission to water cannot be considered as a standard situation. In fact, in the assumption made in the CSR, non-negligible emissions to water are considered with default release factors of 2% for ERC 2 and 0.2% for ERC 3. So, the evaluating MSCA cannot support the risk assessment proposed in the CSR. **eMSCA intends to prepare a regulatory management option analysis (RMOA) in which the conditions of release of DPG into the Environment (limit value) will be discussed**.

For scenario 5 and 6, the generic exposure scenario used is described in the following website: http://www.etrma.org/activities/chemicals/reach/exposure-scenarios.

The European Tyre and Rubber Manufacturers' Association (ETRMA) has developed relevant SPERC for substances included in tyre and rubber products, those exposure scenario are validated by competent authorities.

For scenario 7, 8, 9, 12, 13 and 16 to 23, the estimated local release factors correspond to default values of ERCs set in the guidance "R16: Environmental exposure assessment" (February, 2016, page 74-75).

For scenario 10, 11, 14 and 15, the parameters to derive the environmental release rates correspond to the default release factors set in the guidance "R18: Exposure scenario building and environmental release estimation for the waste life stage" (October, 2012, p53-54).

Exposure assessment of 1,3-diphenylguanidine by-product:

The major by-products of 1,3-diphenylguanidine have been characterized in different studies, and consist of aniline, N-Nitroso-diphenylurea and phenyl guanidine depending on the conditions [(1), (2), (3), (4)]. Among these degradation products, aniline is the only classified chemical [(1)]. Based on this information, it was proposed to address the issue of the impact of degradation products of 1,3-diphenylguanidine by performing a chemical safety assessment on aniline as the sole degradation product. This approach is reasonable, as aniline is the only classified substance among the different by-products anticipated to be produced via 1,3-diphenylguanidine degradation.

From existing literature [(2), (5)], aniline appears during vulcanisation process (high temperature process). In order to cover all emissions, each exposure scenario and activity where aniline is present is assessed from vulcanisation process (first apparition of aniline) to General Rubber Goods and tyres articles including

valorisation steps. Exposure scenario and release factors are summarized in the following table.

Table 42: Exposure scenario and release factors for aniline (by-product of DPG).

Exposure Scenario n°	Exposure Scenario name	Environmental Release Categories (ERC)	Source of release factors considered
1	Manufacture of General Rubber Goods (Grad PD, C, GC & Mixland)	6d	Values of SPERC from ETRMA
2	Manufacture of Tyres (Grad PD, C, GC & Mixland)	6d	Values of SPERC from ETRMA
3	Consumer use of tyres	10a	Default values of ERC From the R16 guidance
4	Garage owner - tyres change	10a	Default values of ERC From the R16 guidance
5	End of Life Tyre : ELT pre-processing storage	10a	Default values of ERC From the R16 guidance
6	End of Life tyre : Coarse shredding	3	Default values for waste treatment processes from the R18 guidance
7	End of life Tyre : Grinding (ambiant)	3	Default values for waste treatment processes from the R18 guidance
8	End of Life Tyre : Grinding cryogenic	3	Default values of ERC From the R16 guidance
9	End of Life Tyre : Pyrolysis	3	Default values of ERC From the R16 guidance
10	End of Life Tyre : Energy recovery : cement kiln	3	Default values for waste treatment processes from the R18 guidance
11	End of Life Tyre : Energy recovery : other	3	Default values for waste treatment processes from the R18 guidance
12	End of Life Tyre : Electric arc furnace	3	Default values of ERC From the R16 guidance
13	End of Life Tyre : Devulcanization/reclaim	3	Default values of ERC From the R16 guidance
14	ELT articles – installation of shock absorbing tiles	10a	Default values of ERC From the R16 guidance
15	ELT articles – installation of synthetic turf fields	10a	Default values of ERC From the R16 guidance
16	ELT articles - shock absorbing tiles	10a	Default values of ERC From the R16 guidance
17	ELT articles - synthetic turf fields	10a	Default values of ERC From the R16 guidance
18	General Rubber Goods articles - conveyor belt	11a	Default values of ERC From the R16 guidance
19	General Rubber Goods articles - ballons	10a	Default values of ERC From the R16 guidance

The evaluating MSCA validates the approach adopted to assess emissions of by-products of DPG and supports the exposure scenario proposed for aniline as by-product.

7.12.3. Combined exposure assessment

Not evaluated

7.13. Risk characterisation

Environmental risk characterisation

The evaluating MSCA concludes to a lack of reliable information concerning the emission levels linked to some stages of the DPG life cycle. This situation does not allow to carry out a comprehensive and realistic assessment for the registered substance.
The evaluating MSCA proposes to draw the attention of the control authorities on these critical life cycle stages by suggesting a measurable threshold value beyond which the risk is not acceptable. The aim of this approach is to give to competent authorities a concrete risk management tool detailed due to a follow-up risk management option analysis (RMOA).

Worker and consumer risk characterisation

Worker contributing scenarios leading to RCR > 1 were identified for some PROC (5, 8b, 9, 21) in some exposure scenarios.

For some of these exposure scenarios, due to the conservativeness of the modelling approach and remaining options for additional RMMs to be applied, no human health concern is expected even if the DNELs proposed by eMSCA are lower than the ones used by registrants.

For some other exposure scenarios, only a Tier I assessment tool was used and refinement with Tier II tools would be necessary to conclude on potential risk (using DNELs proposed by eMSCA). In case refinement with a Tier II tool would not be sufficient, further refinement of RMM would be necessary.

The human health risk assessment presented in this document is provisional pending the reception of the EOGRTS study.

With regard to aniline risk assessment, RCR < 1 were calculated and no further risk management is needed.

7.14. References

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7.15. Abbreviations

AC: Article category

BW: body weight

CI: Confidence interval

CLP: classification, Labelling, packaging

CMC: Carboxymethyl cellulose

CMR: Cancerogen, mutagen, reprotoxic

CoRAP: Community Rolling action plan

DPG: 1,3-diphenylguanidine

eMSCA: evaluating Member state competent authority

ELT: End of Life Tyres

EOGRTS: Extended One-generation Reproductive toxicity study

GLP: Good Laboratory Practice

GRG: General Rubber Goods

LLNA: Local lymph node Assay

M&K: Magnusson and Kligman

RCR: Risk characterisation ratio

TC C&L: Technical committee on classification and labelling

ZDBC: Zinc dibutyl dithiocarbamate

ZDEC: Zinc Diethyldithiocarbamate