CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

1,3-diphenylguanidine

EC Number: 203-002-1

CAS Number: 102-06-7

Index Number: 612-149-00-4

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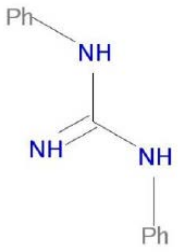
TABLES

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1,3-diphenylguanidine
Other names (usual name, trade name, abbreviation)	Guanidine, N,N'-diphenyl-, dpg, diphenylguanidine, ekaland dpg, denax, vulkacit d, dfg, n,n'-diphenylguanidin, mixland+ dpg, , sym-diphenylguanidine, vulkazit, , denax dpg, n,n'-diphenylguanidine, guanidine, 1,3-diphenyl-, rubator dpg, alchem dpg, melaniline, 1,3-diphenylguanide dpg, 1,3-difenilguanidina, kumac d, ekaland dpg c, 1,3-di-o-phenylguanidine, guanidine, 1,3-diphenyl ; melaniline, soxinol d
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	203-002-1
EC name (if available and appropriate)	1,3-diphenylguanidine.
CAS number (if available)	102-06-7
Other identity code (if available)	-
Molecular formula	$C_{13}H_{13}N_3$
Structural formula	
SMILES notation (if available)	N=C(Nc1ccccc1)Nc2ccccc2
Molecular weight or molecular weight range	211 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	98-100%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP) – January 2022
1,3-diphenylguanidine	Mono-constituent	Repr. 2 - H361f *** Acute Tox. 4 * - H302 Eye Irrit. 2 - H319 STOT SE 3 - H335 Skin Irrit. 2 - H315 Aquatic Chronic 2 - H411	Acute Tox. 3 - H301 Acute Tox. 4 - H302 Skin Irrit. 2 - H315 Eye Irrit. 2 - H319 Eye Dam. 1 - H318 STOT SE 3 - H335 Repr. 1B – H360FD Repr. 2 - H361f Aquatic Chronic 2 - H411 Aquatic Chronic 3 - H412

The impurities do not have an impact on classification.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	612-149-00-4	1,3-diphenylguanidine	203-002-1	102-06-7	Repr. 2 Acute Tox. 4 * Eye Irrit. 2 STOT SE 3 Skin Irrit. 2 Aquatic Chronic 2	H361f *** H302 H319 H335 H315 H411	GHS08 GHS07 GHS09 Wng	H361f *** H302 H319 H335 H315 H411			
Dossier submitters proposal	612-149-00-4	1,3-diphenylguanidine	203-002-1	102-06-7	**Retain** Eye Irrit. 2 **Modify** Repr. 1B Acute Tox. 3 Eye Dam. 1 Aquatic chronic 3 **Add** Skin Sens. 1A STOT RE 2	**Retain** H315 **Modify** H360 FD H301 H318 H412 **Add** H317 H373 (nervous system)	**Retain** GHS 08 GHS 07 **Add** GHS 06 GHS 05 **Modify** Dgr **Remove** GHS 09	**Modify** H360 FD H301 H318 H317 H412 **Add** H317 H373 (nervous system)		Oral: ATE=110 mg/kg	
Resulting Annex VI entry if agreed by RAC and	612-149-00-4	1,3-diphenylguanidine	203-002-1	102-06-7	Repr. 1B Acute Tox. 3 STOT SE 3 STOT RE 2 Skin Irrit. 2	H360 FD H301 H335 H373 (nervous system)	GHS 08 GHS 06 GHS 05 GHS 07 Dgr	H360 FD H301 H335 H373 H315		Oral: ATE=110 mg/kg	

COM					Eye Dam. 1 Skin Sens. 1A Aquatic chronic 3	H315 H318 H317 H412		H318 H317 H412			

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Harmonised classification proposed	Yes
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	Harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Harmonised classification proposed	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

1,3-diphenylguanidine (DPG) was introduced in the Annex I by Commission Directive 98/98/ EC of 15 December 1998 adapting to technical progress for the 25 time Council Directive 67/548/EEC. The classification was agreed by the commission Working group on the classification and labelling of Dangerous Substance (TC C&L) in 1997 (ECBI/32/97). The precise basis for this classification is not available.

The current harmonised classification (CLP00) is:

- Acute Tox. 4* - H302
- Skin Irrit. 2 – H315
- Eye Irrit. 2 – H319
- STOT SE 3 – H335
- Aquatic chronic 2 – H411
- Repr. 2 – H361f***

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The available data on 1,3-diphenylguanidine, as presented in this report, support the following changes / added classifications with:

- Repr 1B – H361FD instead of Repr. Cat. 2; H361f***
    - There is no requirement for justification for this hazard class.

- Acute Tox. 3 – H301 instead of Acute Tox. 4* – H302 (Xn, R22)
    - Reason for a need for action at Community level: Change in existing entry due to changes in the criteria (minimal classification). Moreover, differences in self-classification are noted despite harmonized classification exists for this hazard class (about 30% of notifications for Acute Tox. 3 and 70% for Acute Tox 4).

- Eye Dam.1 – H318 instead of Eye Irrit. 2 – H319
    - Reason for a need for action at Community level: Change in existing entry due to new interpretation/evaluation of existing data

- Aquatic chronic 3 instead of Aquatic chronic 2
    - Reason for a need for action at Community level: change in existing entry due to new data.

- Skin Sens. 1 - H317
    - Reason for a need for action at Community level: Disagreement of DS with current self-classification. There is no harmonized or notified classification for this hazard class although human data justify classification as Skin Sens. 1.

- STOT RE 2
    - Reason for a need for action at Community level: Disagreement of DS with current self-classification. There is no harmonized or notified classification for this hazard class although experiment data justify classification as STOT RE 2.

The need for updating the harmonized classification is also highlighted in the French Substance Evaluation (SEv) Conclusion document on DPG (Anses, 2020).

5 IDENTIFIED USES

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

Table 5: Summary of DPG uses (Anses, 2020)

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, Pyrolysis, 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, adhesives, binders, sealants)
Consumer uses	Use of tyres and general rubber goods
Article service life	Usage of tyres (consumers) End of Life Tyre

6 DATA SOURCES

All the data available in the registration dossier (last modification taken in account: 30/09/2021) and study reports provided by the registrants have been used in the CLH report.

A bibliographic search was performed based on pubmed, scopus and winley databases. For skin sensitization, a generic search was carried out with the terms “1,3-diphenylguanidine OR DPG OR diphenylguanidine”. The date of the requests was 05/03/2022. For reproductive toxicity, the following keywords were added “fertility”, “toxic to reproduction” and “reproduction”. The date of the requests was 06/04/2022. For neurotoxicity, the following keywords were added “neurotoxicity”, “neurotoxic”, “neuro”. The date of the requests was 08/09/2022.

The time frame selected for the publication search was from 01/2017 to 03/2022. The data published before 2017 had already been collected and analyzed during the evaluation of the substance by France (Anses, 2020). These data were also used in the CLH report.

7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Appearance: solid colour: white to pale pink Odour: slight odour	Anses, 2020	Visual description of the substance consistent with other handbook and literature data.
Melting/freezing point	149°C	Anses, 2020	Obtained by the differential scanning calorimetry method. 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	> 250°C	Anses, 2020	Obtained by the differential scanning calorimetry method. The boiling point values report that the substance decomposes before

Property	Value	Reference	Comment (e.g. measured or estimated)
			boiling (at approximately 170°C)
Relative density	1.13 g/cm^3	Anses, 2020	The pycnometer method gives a tap density of the substance of 0,348g/cm^3. Value found in a peer review handbook (Merck Index 14th) gives a pour density of 1.13g/cm^3 at 20°C.
Vapour pressure	3.7*10^{-10} Pa at 25°C	Anses, 2020	Effusion method
Surface tension	58.8 mN/m at 20°C	Anses, 2020	DPG is a surface active substance.
Water solubility	475 mg/L at pH 7 and 325 mg/L at pH 11	Anses, 2020	Water solubility is determined by flask method
Partition coefficient n-octanol/water	Not relevant	Anses, 2020	- Log Kow = 2.42 at pH11 by OECD 107 method - Log Kow > 6.2 at pH 9.3 and log Kow = 4 at pH 7.01 by OECD 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 OECD 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.
Flash point	Not relevant	Anses, 2020	The flash point is only a relevant property for liquid at room temperature
Flammability	Not flammable	Anses, 2020	Study performed in accordance with EEC A 10 method. Two preliminary tests are performed, no main test is made. DPG is not considered as highly flammable.
Explosive properties	Non explosive	Anses, 2020	According to theoretical considerations based on chemical structure, DPG has not explosive properties.
Self-ignition temperature	Not auto-inflammable at ambient temperature	Anses, 2020	The substance has a melting point <160°C, therefore the autoflammability test is not required according to R.7.1.12.1 of the REACH guidance.
Oxidising properties	Not oxidizing	Anses, 2020	According to theoretical considerations based on chemical structure, DPG has not oxidizing properties.
Granulometry	10 µm to 10 mm (average 26 µm)	Anses, 2020	Weight of balance approach has been used to determine the particle size

Property	Value	Reference	Comment (e.g. measured or estimated)
			distribution of the substance.
Stability in organic solvents and identity of relevant degradation products	Not relevant	Anses, 2020	
Dissociation constant	pKa=10.1 at 20°C	Anses, 2020	Publication and review article indicate a constant of dissociation of 10.1 at 20°C
Viscosity	No applicable	Anses, 2020	The substance is a solid

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this CLH proposal.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

According to the French SEv conclusion document (Anses, 2020), the data investigating the toxicokinetics of DPG suggest that the substance is readily absorbed from the gastrointestinal tract of rats. According to Ioannou & Matthews (1984), the substance is distributed quickly to all tissues examined, metabolized into three major and two minor metabolites (not identified). Most of the dose of DPG was excreted in the urine and feces at approximately equal amounts within 24 hour after oral or intravenous administration. Greater than 99% of the DPG dose was cleared into the urine and feces within 3 days after administration.

Slower clearance of a minor metabolite was observed in liver, but the significance of this observation is unknown.
A study conducted by Shah et al. (1985) demonstrated that DPG is slowly absorbed after dermal application to rats (around 10 % in rats within 5 days).

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 7: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
OECD Guideline 401 (Acute Oral Toxicity) GLP Gavage Vehicle : Corn oil Reliability : 1 Key study	Rats, Crj:CD(SD)IGS, both sexes 6 weeks of age 5 rats/sex/dose	DPG Purity: 99.9%	0, 50, 65, 85, 110 and 143 mg/kg bw Single oral administration Observation period: 14 days	Males: LD50 = 111 (95% CI: 86-161) mg/kg bw Females: LD50 = 107 (95% CI: 70-177) mg/kg bw **Acute Tox. 3**	Unpublished study report (2000) Please see annex I for more details
Equivalent to OECD Guideline 401 (Acute Oral Toxicity) Not GLP Gavage Vehicle : Corn oil Reliability : 3 (no data on body weights, purity not specified, contradictory results between the mortality table of females presented in annex I and the LD50 calculated)	Rats, Sprague-Dawley, both sexes 7 weeks of age 10 rats/sex/dose	Soxinol D (DPG) Purity not specified	100, 130, 170, 220, 284, 385, 500, 650, 845, 1000 mg/kg bw Observation period : 14 days	Males: LD50 = 460 (95% CI : 320-662) mg/kg bw Females: LD50 = 384 (95% CI : 309-477) mg/kg bw **Acute Tox. 4**	Unpublished study report (1977a) Please see annex I for more details
No guideline followed Not GLP Oral route Vehicle : olive oil Reliability: 3 (doses not specified, purity not specified)	Mice, ddY, both sexes 5 weeks of age 10 animals / dose	DPG Composition /purity not specified	6 increasing dose levels No more precisions. Observation period: 14 days	Males: LD50 = 150 mg/kg bw Females: LD50 = 211 mg/kg bw **Acute Tox. 3**	Hasegawa R *et al.* (1989)
No guideline followed Not GLP Gavage Vehicle: Corn oil Reliability: 3 (no data on protocol, test conditions, statistical test)	Rats, Sprague-Dawley, both sexes 5 animals (2 or 3 males and females) per dose	DPG Composition /purity not specified	251, 316, 398, 501, 631 mg/kg bw Observation period: 14 days	Males/females LD50 = 350 mg/kg bw **Acute Tox. 4**	Unpublished study report (1977b) Please see annex I for more details

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
No guideline followed Not GLP Oral route Vehicle: propylene glycol Reliability: 3 (data not sufficient for independent interpretation)	Rats, strain not specified, sex not specified 7 animals per dose	DPG Composition /purity not specified	No data	LD50 = 500 mg/kg bw **Acute Tox. 4**	Dieke SH *et al.* (1947)
No guideline followed Not GLP Oral route Vehicle: not specified Reliability: 3 (data not sufficient for independent interpretation)	Mice, strains not specified, sex not specified	DPG Composition /purity not specified	No data	LD50 = 290 mg/kg bw **Acute Tox. 3**	Arkhangel'skaya LN *et al.* (1964, 1963 and 1968)
No guideline followed Not GLP Oral route Reliability: 3 (data not sufficient for independent interpretation)	(1) Rats, strain not specified, sex not specified (2) Mice, strain not specified, sex not specified (3) Rabbits, strain not specified, sex not specified No data on the number of animal per dose	DPG Composition /purity not specified	No data	(1) Rats: LD50 = 323 mg/kg bw **Acute Tox. 4** (2) Mice: LD50 = 258 mg/kg bw **Acute Tox. 3** (3) Rabbits: LD50 = 246 mg/kg bw **Acute Tox. 3**	Vlasyuk MG *et al.* (1978)
No guideline followed Not GLP Gavage Vehicle: CMC (carboxymethyl cellulose) Reliability: 4 (secondary literature)	Rats, Sprague-Dawley, both sexes 5-6 animals (2 or 3 males and females) per dose	DPG Composition /purity not specified	700, 800, 900, 1000 mg/kg bw Observation period: probably 24h	Males/females LD50 = 850 mg/kg bw **Acute Tox. 4**	Unpublished study report (1954) Cited in OECD SIDS (2002)
No guideline followed Not GLP	Mice, strains not specified, sex not specified	DPG Composition /purity not	No data	LD50 = 520 mg/kg bw **Acute Tox. 4**	McCormick WE *et al.* (1971)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Reliability 4 (secondary literature)		specified			

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

No human data are available.

Numerous studies on the acute oral toxicity of DPG have been carried out in various species. Only one study in rats is considered acceptable.

In a study conducted according to OECD TG 401, DPG was administrated by gavage to groups of five males and five female rats (Crj:CD(SD)IGS), at the concentrations of 50, 65, 85, 110 and 143 mg/kg bw. The animals were subsequently observed for 14 days. No mortalities were noted at the doses of 50 mg/kg bw in males and females and at 65 mg/kg bw in males. 1/5 females died at 65 mg/kg bw, 2/5 males and 2/5 females died at 85 mg/kg bw, 3/5 males and 3/5 females died at 110 mg/kg bw, and 3/5 males and 3/5 females died at 143 mg/kg bw. Most of the deaths occurred one to three hours after administration. The LD50 (95% confidence interval) established was 111 (CI95%: 86-161) mg/kg bw for the males and 107 (CI95%: 78-177) mg/kg bw for the females according to the Probit method. The study is considered fully reliable by DS and rated as a key study (unpublished study report, 2000).

Other studies available are of lower quality or are not considered acceptable due to missing information. The LD50 in the other rats studies were in the range between 323 and 850 mg/kg bw (Unpublished study report, 1977a, unpublished study report (1977b), Dieke SH *et al.* (1947), Arkhangel'skaya LN *et al.* (1963 and 1968), Vlasyuk MG *et al.* (1978), unpublished study report (1954) Cited in OECD SIDS (2002)) and the LD50 in the acute mice studies was reported to be between 150 and 520 mg/kg bw (Hasegawa R *et al.* (1989), Arkhangel'skaya LN *et al.* (1964, 1963 and 1968), Vlasyuk MG *et al.* (1978), McCormick WE *et al.* (1971)). One study conducted in the rabbits, established a LD50 of 246 mg/kg bw (Vlasyuk MG *et al.*(1978)).

10.1.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No 1272/2008, classification for acute oral toxicity is required for substances with ATE (acute toxicity estimate), based on LD_{50}, below 2000 mg/kg bw.

Exposure Route	Category 1	Category 2	Category 3	Category 4
Oral route (mg/kg bw)	ATE ≤ 5	5 < ATE ≤ 50	50 < ATE ≤ 300	300 < ATE ≤ 2000

The studies available lead to several LD50, but in their entirety, the results are consistent. According to the Guidance on the application of CLP criteria 3.1.2.3.2 "*In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification*"

The lowest LD50 was obtained in the most recent and well-conducted study in rats with a LD50 of 107 mg/kg in females within the range of Acute Tox. 3 criteria (50-300 mg/kg bw) (Unpublished study report, 2000). The LD50 obtained in two studies although with some limitations in mice also support a classification in this category (LD50 < 300 mg/kg bw). There is no indication that sex greatly influence the toxicity of DPG.

Difference in purity of the test material and strain may explain the differences in results in the rat studies but data on purity and strain are lacking in some studies to make a firm conclusion.

Overall, based on the CLP criteria, DPG should be classified as Acute Tox. 3 (H301). It is proposed to assign an ATE of 110 mg/kg bw for acute oral toxicity based on the most reliable and most recent study which was associated with the lowest LD50.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Regarding the data available, a classification to **Category 3 H301: Toxic if swallowed** is warranted with an **ATE of 110 mg/kg bw**.

10.2 Acute toxicity - dermal route

Not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Not assessed in this dossier.

10.4 Skin corrosion/irritation

Not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Table 8: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Similar to OECD TG 405 (for the unwashed group) GLP not specified Reliability: 2 (Individual scores not provided) Key study	Rabbits, New Zealand White 6 rabbits: -unwashed group: 2 males, 1 female -washed group: 1 male, 2 females	Soxinol D (DPG) No vehicle Purity: 99.6%	Application: 0.1 g of the test substance on the conjunctival sac of the one side of lower eyelid.	Observations conducted at 1, 24, 48, 72 hours after the administration for the washed group, and 96 h, 1, 2, 3, 4, 5, 6 weeks for the unwashed group. ***Unwashed group : 3 animals*** **Mean scores (24h, 48h, 72h) in 3 animals:** Corneal opacity: 2, 2, 2 Iritis: 1, 1, 1 Conjunctival redness: 2, 2, 2 Conjunctival chemosis: - at 24h: score 3 in 1 animal and score 2 in 2 animals - at 48h and at 72h score 2 in 2 animals and score 1 in 1 animal Conjunctival discharge: -At 24h: score 1 in 1 animals, score 2 in 2 animals. -At 48h: score 2 in all animals. -At 72h: score 1 in 2 animals, score 2 in 1 animal. Local reactions at the conjunctivae disappeared by 3 weeks and iris congestion by 4 weeks. Corneal opacity at week 3: grade 3 6 weeks after administration: cornea opacity still observed (grade 1). Arterialisations of the cornea surface were observed. ***Washed group not described as not***	Unpublished study report, 1988

				relevant for classification	
No guideline followed Draize test Not GLP Reliability: 3 (not acceptable for classification purposes: scores not comparable to classification criteria)	Rabbits, New Zealand albino 6 animals	DPG Purity: not specified No vehicle	Control not specified 100 mg of test substance applied The test substance was not rinced.	Observation period: 1, 24, 48, 72, 120 and 168 hours and up to 21 days. <u>24h after exposure:</u> Very slight to slight corneal cloudiness, iris showed little or no reaction to light, severe erythema (necrosis), slight to moderate edema, copious discharge containing whitish exudate (with blood in 3 instances). Gradual improvement until 14 days <u>14 days after exposure:</u> Effects were not fully reversible in 2 animals. <u>21 days after exposure:</u> All scored zero.	Unpublished study report, 1977a
No guideline followed Draize test Not GLP Reliability: 3 (not acceptable for classification purposes: score not comparable to classification criteria, 0.02 g tested instead of 0.1 g recommended)	Rabbits, New Zealand white 6 animals	DPG Purity: not specified No vehicle	Control not required. 20 mg of substance applied. The test substance was not rinsed.	Observation period: 1, 24, 48, 72, 120 and 168 h <u>24h after exposure</u>: Areas of barely perceptible corneal dullness, the reaction of the iris to the light was sluggish, severe erythema, very slight edema, copious discharge containing slight whitish exudate Effects reversible by 168h	Unpublished study report, 1977b

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Three studies assessing the eye irritation potential of DPG in New Zealand white rabbits are available. No OECD guidelines are followed in the studies and the studies are not GLP compliant or GLP status is not specified.

In the first study, 6 rabbits were separated in two groups: 3 animals in the washed group and three animals in the unwashed group (Unpublished study report, 1988). The washed group is not detailed further as not relevant for classification (but details are provided in Annex I). In the unwashed group, 0.1 g of DPG was applied on the conjunctival sac of the one side of lower eyelid. The eyes with administration of DPG were not washed and the other eyes were treated as control. Observations were conducted at 1, 24, 48, 72 hours and another 96 hours, 1, 2, 3, 4, 5, 6 weeks after the administration. Local reactions were numerically recorded following the Draize standards. Given the protocol is close to OECD TG 405 and well described, this study is considered as acceptable and as a key study for classification purpose.

At 24h after administration, all animals presented corneal opacity (grade 2), iris congestion (grade 1), conjunctiva redness (grade 2), conjunctiva chemosis (grade 2 in two animals, grade 3 in one animal), conjunctiva discharge (grade 1 in one animal, grade 2 in two animals). At 48h after administration, all animals presented corneal opacity (grade 2), iris congestion (grade 1), conjunctiva redness (grade 2), conjunctiva chemosis (grade 1 in one aminal, grade 2 in two animals), conjunctiva discharge (grade 2). At 72h after administration, all animals presented corneal opacity (grade 2),

iris congestion (grade 1), conjunctiva redness (grade 2), conjunctiva chemosis (grade 1 in one animal, grade 2 in two animals), conjunctiva discharge (grade 1 in two animals, grade 2 in one animal).

After 3 weeks, iris congestion was still seen in one animal (grade 1). All animals displayed corneal opacity until the end of the study. Arterialisations of the cornea surface were observed in all animals from 1 week until the end of the study. Therefore, the effects were not fully reversible at 21 days post-exposure.

The two remaining studies are considering as supportive evidence as the protocol is not clearly provided. DPG was administrated to New Zealand White rabbits at the doses of 20 mg (Confidential, 1977b) and 100 mg (Confidential, 1977a). Animals clearly displayed irritation reactions that were fully reversible within 168 hours (Confidential, 1977b) or 21 days (Confidential, 1977a).

10.5.2 Comparison with the CLP criteria

According to table 3.3.1 of the CLP regulation, criteria to classify a substance for irreversible effects on the eye (category 1) are the following:

A substance that produces:

(a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(b) in at least 2 of 3 tested animals, a positive response of:

(i) corneal opacity ≥ 3 and/or

(ii) iritis > 1,5

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

According to table 3.3.2 of the CLP regulation, criteria to classify a substance that have the potential to induce reversible eye irritation (category 2) are the following:

Substances that produce in at least in 2 of 3 tested animals, a positive response of:
(a) corneal opacity ≥ 1 and/or
(b) iritis ≥ 1, and/or
(c) conjunctival redness ≥ 2 and/or
(d) conjunctival oedema (chemosis) ≥ 2
calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

In the key study (Unpublished study report, 1988), the mean scores at 24, 48 and 72 hours are the following:

- Corneal opacity: all 3 animals with a score of 2 [do not fulfil criteria for Eye. Dam. 1 but fulfils Eye Irrit. 2]
- Iritis: all 3 animals with a score of 1 [do not fulfil criteria for Eye. Dam. 1 but fulfils Eye Irrit. 2]
- Conjunctival redness: all 3 animals with a score of 2 [do not fulfil criteria for Eye. Dam. 1, but fulfilled Eye. Irrit. 2]
- Conjunctiva chemosis: at 24h: score 3 in 1 animal and score 2 in 2 animals, at 48h and at 72h score 2 in 2 animals and score 1 in 1 animal [not comparable with CLP criteria, in the absence of individual data]

Corneal opacity as well as arterialisations of the cornea surface were observed in all three animals beyond 21 days and up to the end of the observation period at 6 weeks.

Even if the mean scores only fulfil classification criteria as Eye Irrit. 2 as the effects observed were not fully reversible within 21 days, it can be concluded that DPG meets the classification criteria for Eye Dam. Category 1, serious eye damage.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Classification of DPG as Eye Dam. 1; H318, is warranted.

10.6 Respiratory sensitisation

Not assessed in this dossier.

10.7 Skin sensitisation

Table 9: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD Guideline 406 (Skin Sensitisation) GLP Maximization method of Magnusson and Kligman Vehicle: paraffin oil Reliability: 2 (higher number of animals recommended in case of negative results according to revised guideline)	Guinea pig, Dunkin-Hartley, female Control group = 5 females Treated group = 10 females	DPG Purity: please see 99.9% Vehicle: paraffin oil	**Day 1: Intradermal injection** 6 intradermal injections (1% DPG in treated group) **Topical application:** **Day 7:** Treatment with sodium laurylsulfate (10%) **Day 8:** topical application (48h occlusive dressing). Treated group: 25% (w/w) in the vehicle. Control group: vehicle. **Challenge phase:** **Day 22:** in both groups: 25% (24h topical occlusive application) **24 and 48h post retention:** observation to evaluate cutaneous reactions No histological examination performed. Use of positive controls treated with dinitro-2,4-chlorobenzene in a previous study.	No clinical signs or mortalities observed **End of the induction period (day 10):** irritation in control and treated groups. **Challenge application**: no cutaneous reaction observed 24 and 48 hours after removal of the dressing. **Conclusion:** no classification possible (too few animals tested for negative results)	Unpublished study report (1995)

Table 10: Experimental induction test in humans

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Induction study in human volunteers	DPG Concentration: 0.2 g as 70% No data on purity Vehicle : petrolatum	49 volunteers without known previous exposure to DPG. Induction phase : series of 12 applications (each of 24 hours duration) carried out during weeks 1, 2, and 3 Rest period : weeks 4 and 5 Challenge phase: series of 4 applications carried out during weeks 6 and 7.	Patch testing with 70 % DPG in petrolatum produced no significant positive reactions following the first induction application. 19 of the 49 subjects displayed irritation during subsequent induction exposures. Two subjects (4%) displayed sensitization positive reactions during the 2-week challenge phase.	Unpublished study from Monsanto, (1982) cited by SIDS, 2002

Table 11: Human diagnostic patch test data

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Selected dermatitis patients.	DPG No further information	283 patients wearing gloves in the workplace with chronic hand eczema included in the study	32 % of the patients reacted to personal gloves or gloves allergens **5.3 %** positive reactions to DPG.	Clément *et al.* (2021)
Retrospective study (January 2016 to March 2020, USA) Unselected dermatitis patients	DPG No further information	108 children between 0 to 17 year-of-age patch-tested during the time study. 33 children patch-tested with DPG.	2 positive reactions **(6.1%).** Age 0 to 5 years: 0 reaction (9 children tested) Age 6 to 12 years: 1 reaction: 6.7% (15 children tested) Age 13 to 17 years: 1 reaction: 11.1% (9 children tested)	Tam *et al.* (2021)
Retrospective study (2000 to 2019, Germany, Switzerland, and Austria) Unselected dermatitis patients	DPG Concentration: 1% Vehicle: petrolatum Purity: no data	212646 patients patch-tested during the study period. Of these, 1361 (0.6%) had been working as painters at the time of patch testing (737, 54.2% with occupational dermatitis). 274 male painters with occupational dermatitis patch-tested with DPG Reading at day 3 Reactions scored as +, ++ or +++	9 positive reactions to DPG **(3.3%)** (no details on the grade of reaction). Occupationally used protective gloves were patch tested in four of nine patients sensitized to DPG, and only one patient reacted positively.	Schubert *et al.* (2021)
Retrospective study (2009 to 2018, Germany, Switzerland, and Austria) Selected dermatitis patients	DPG Concentration: 1% Vehicle: petrolatum Purity: no data	119417 patients patch tested during the study period. 625 patients with presumed shoe dermatitis identified. 467 patients patch-tested with DPG. Reading at day 3 Reactions: +, ++ or +++	**2.6 %** positive reactions to DPG.	Traidl *et al.* (2021)
Retrospective study (2005–2016, Finland) Work place study with selected workers with known dermatitis	DPG No further information	978 healthcare patients tested during the study period. 242 cases of allergic contact dermatitis identified.	84/978 patients reacted to rubber chemicals (34.7% of the allergic contact dermatitis cases). 1 patient **(0.1%)** reacted to DPG.	Aalto-Korte *et al.* (2020)
Retrospective study (January 2010 to	DPG Concentration:	804 cutting metalworkers exposed to metalworking fluids	Cutting metalworkers [95%CI]: **1.9%** [0.9–3.6]	Schubert *et al.* (2020a)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
December 2018, Germany, Austria, Switzerland) Work place study with selected workers with known dermatitis	1% Vehicle: petrolatum Purity: no data	(MWF) on a daily basis, 2197 mechanics without routine exposure to MWF and 355 other metalworkers not exposed to MWF. Reading at day 3 Reactions: +, ++, or +++	reacted to DPG. Mechanics [95%CI]: **2.2%** [1.5–3.1] reacted to DPG. Other metalworkers [95%CI]: **4.7%** [2.3–8.5] reacted to DPG.	
Retrospective study (January 2013 and December 2017, Germany) Unselected dermatitis patients	DPG Concentration: 1% Vehicle: petrolatum Purity: no data	654 patients with suspected occupational dermatitis and patch tested. Occupational allergic contact dermatitis was diagnosed in 113 (17.3%) patients. A total of 306 different types of occupationally used gloves were patch tested “as it is” in 404 patients. Reading at day 3 Reactions: +, ++, or +++	13 gloves (3.8%) containing DPG. Not a single patient in the cohort displayed an occupationally relevant patch-test reaction to DPG tested at 1% in pet.	Schubert *et al.* (2020b)
Retrospective study (2013 to 2016, North American Contact Dermatitis Group) Unselected dermatitis patients	DPG Concentration: 1% Vehicle : petrolatum And Carba mix 3% (1% DPG, 1% ZDEC (zinc diethyldithiocarbamate), 1% ZDBC (zinc dibutyldithiocarbamate)) Vehicle: petrolatum Purity: no data	10457 patients patch tested to both carba mix and DPG. Reactions: +, ++, or +++ Only doubtful reactions with a final interpretation of “allergic/positive” were included. Final reading at day 5, 6, 7, or 8	610 patients had at least one reaction to either carba mix alone (n = 292), DPG alone (n = 128), or both (n = 190). Total reactions to carba mix : 482 Total reactions to DPG: 318 (3%) - 34 (10.7%) reactions: doubtful +/− - 206 (64.8%) reactions: + - 71 (22.3%) reactions : ++ - 7 (2.2%) reactions: +++ **(78/10457 patients with reactions ++/+++, 0.7%)** Reaction to DPG alone: 128 (1.2%) - 14 (10.9%) reactions doubtful - 88 (68.8%) reactions: + - 25 (19.5%) reactions:	Warshaw *et al.* (2020)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			++ - 1 (0.8%) reaction: +++ **(26/10457 patients with reactions ++/+++, 0.2%)** 39.4% of carba mix-allergic patients were also positive to DPG, and 59.7% of DPG-allergic patients were also positive to carba mix.	
Retrospective study (September 2010 to December 2017, Belgium) Work place study with selected workers with known exposure to rubber gloves and dermatitis	Carba mix 3% (1% DPG, 1% ZDEC, 1% ZDBC) Concentration: 1% DPG Vehicle : petrolatum Purity: no data	44 caregivers patients with hand dermatitis after wearing rubber gloves and who had positive patch test reactions to at least one sterile synthetic rubber glove. Reading at days 2 and 4 Reactions: +, ++ or +++ ?+ : doubtful	On 44 patients patch-tested : 84% reacted positively to carba mix: 37 positive reactions 86% reacted positively to DPG: 38 positive reactions - 18 reactions + (47%) - 10 reactions ++ (26%) - 10 reactions +++ (26%)	Dejonckheere *et al.* (2019)
Work place study with selected workers with known exposure to gloves (rubber gloves), soaps, alcoholic hand disinfectants and hand creams provided at the hospitals. (September 2014 to January 2015, Sweden)	DPG Concentrations : 1%and 2%Vehicle : petrolatum Purity: no data	474 health-care workers patch-tested and fully investigated. 360 of 474 (76%) with history of hand eczema, and 114 of 474 (24%) with no history of hand eczema. In the hand eczema group: - 311 of 360 (86%) reported having had hand eczema at some time during the preceding 12 months, - 49 of 360 (14%) reported having had hand eczema prior to but not during the past 12 months. This group was not included in the analyses. Reading at days 3 or 4 and 7 Reactions: +, ++ or +++ ?+ : doubtful	<u>DPG 1%:</u> 8/311 positive reactions (2.6%). - 4 reactions: + - 1 reaction: ++ - 3 reactions: +++ **(4/311 reactions ++ and +++, 1.3%)** 0 positive reactions in the group of 114 patients with no history of hand eczema. <u>DPG 2%:</u> 7/311 positive reactions (2.3%) - 2 reactions: + - 2 reactions: ++ - 3 reactions: +++ **(5/311 reactions ++ and +++, 1.6%)**	Hamnerius *et al.* (2018)
Retrospective study (14 years, Tunisia) Work place study with selected workers with known	DPG No further information	308 patients with occupational allergic contact dermatitis identified during the study period. Of whom, 38 patients had	7.94 % positive reactions.	Henchi *et al.* (2018)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
dermatitis to rubber		occupational rubber-related allergic contact dermatitis. 24 patients patch-tested with DPG.		
Work place study on selected workers (2015-2016) Work place study with selected workers with known dermatitis caused by rubber accelerators.	DPG No further information	9 health-care workers patients with hand eczema caused by rubber accelerators confirmed by positive and relevant patch test reactions from 2015 to 2016. Reading at days 2, 3 or 4 and 10 Reactions: +, ++ or +++ Irritant, doubtful and negative test reactions interpreted as negative.	5/9 patients reacted to DPG. Among them, 2 patients with atopic dermatitis. - 2 reactions: ++ - 3 reactions: +++	Crepy *et al.* (2017)
Retrospective study (January 2013 to December 2014, North American Contact Dermatitis Group) Unselected dermatitis patients	DPG Concentration : 1% Vehicle: petrolatum Purity: no data	4871 patients patch-tested with screening series of 70 allergens. Reactions: ± (weak/ doubtful), +, ++, +++	4859 patients tested with DPG. **3.8%** positive reactions to DPG considered clinically relevant (patient's history and clinical examination): - 2.3% patients: + - **0.93% patients : ++/+++**	Dekoven *et al.* (2017)
Selected dermatitis patients	DPG No further information	18 female patients aged from 14 to 22 year-old with itching and erythematous to purple-coloured eczematous lesions on both feet, patch-tested. General population (Shoes) Reading at day 2 and day 4	One patient **(5.5%)** with positive reaction to DPG: ++	Hulstaert et al. (2017)
Retrospective study (2013-2014, Switzerland, Germany). Dermatitis patients (selected or unselected, status unclear from publication (patch-tested to DPG due to standard practices or specific suspicion)	DPG Concentration : 1% Vehicle : petrolatum Purity: no data	29 522 patients patch-tested with the baseline series, 2870 patients patch-tested with a special rubber series (group RT), among them 2331 patients tested with DPG. Reading at day 3 and 5 Reactions: +, ++ or +++ ? +/IR: probably doubtful reaction, not clearly explained in the protocol	**3.26%** positive reactions: - 2.62% reactions: + - 0.64% reactions: ++/+++ - 9.18% reactions: ?	Uter *et al.* (2016)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Retrospective study (2005-2014, Germany) Work place study with selected workers with known dermatitis	DPG Concentration: 1% Vehicle : petrolatum Purity: no data	743 female geriatric nurses with occupational contact dermatitis (OCD) in comparison to 695 geriatric nurses without occupational contact dermatitis, patch tested with allergen series. 575 patients tested with DPG. Reading at day 3 Reactions: +, ++ or +++	9 patients reacted to DPG: **1.6%** No information on the number of geriatric nurses with OCD and without OCD who reacted to DPG.	Schubert *et al.* (2016)
Retrospective study (1994 to 2013, Denmark) Selected dermatitis patients	DPG No further information Purity: no data Carba mix 250 µg/cm2 (DPG, purity ≥96%),, ZDEC purity ≥96%, ZDBC purity ≥96%)in equal proportions)	9741 patients patch tested. 579 (5.9%) had at least one positive reaction to a rubber allergen. Patch testing with single allergens included in the mix was performed in 250 of the 351 patients who reacted to carba mix. Reactions: +, ++ or +++ Readings at day 3/4 and day 7	9717 patients tested with carba mix. 351 (3.6%) patients reacted to carba mix. 250 patients tested with DPG. 38 patients reacted to DPG : 15%	Mortz *et al.* (2016)
Retrospective study (2003 to 2012, Germany) Work place study with selected workers with known dermatitis	DPG Concentration : 1% Vehicle: petrolatum Purity: no data	Patch test results from 2248 nurses with occupational contact dermatitis (OCD) compared with those of 2138 nurses without occupational contact dermatitis 1509 nurses with OCD patch-tested with DPG. Reading at day 3 Reactions: +, ++, or +++	30 positive reactions: **2%** (95% CI: 1.3-2.8) No information on the number of nurse without OCD who reacted to DPG.	Molin *et al.* (2015)
Work place study with selected workers with known dermatitis related to the use of rubber gloves	DPG No further information	8 healthcare worker patients with hand eczema due to the use of rubber gloves, diagnosed by patch test. Reading at day 2 and 4 Reactions: +, ++ ,+++	5 patients (62 %) reacted to DPG, respectively: - 2 patients : + - 2 patients : ++ - 1 patient : +++ 3/8 (37%) had reactions ++ and +++	Baeck *et al.* (2013)
Workplace study, unselected workers	DPG No further information	Assessment of the prevalence of occupational allergic contact dermatitis (ACD) in tannery workers and patch-tested.	76 of the 472 workers had contact dermatitis diagnosed by patch-test. 4 workers allergic to DPG,	Febriana *et al.* (2012)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Reading at days 2, 4, and 7	present in synthetic rubber gloves: **5.3% of workers with contact dermatitis, 0.8% of all workers**	
Retrospective study (2002–2010, Germany) Work place study with selected workers with known dermatitis related to protective gloves (no specification of rubber gloves)	DPG Concentration : 1% Vehicle : petrolatum Purity: no data	93615 patients patch tested Reading at day 3 Reactions: +, ++, +++	On 2578 patients with occupational contact dermatitis and suspected glove allergy patch-tested, 77 patients [**3%** (95% CI: 2.4-3.7)] reacted to DPG. Patients with positive reaction to DPG: - 93 reactions : doubtful or irritant - 65 reactions : + - 12 reactions **(0.47% of patients):** ++/+++ Authors observed no increase of trend over the years (patients or health care workers with occupational contact allergy and suspected gloves allergy)	Geier *et al*. (2012)
Work place study with selected workers with known dermatitis	DPG No further information	Patients patch-tested with a baseline series and rubber chemical series, and the patients' own gloves. Occupational exposure (surgical operating theatre personnel) 16 patients with occupational contact dermatitis caused by rubber chemicals and sterile polyisoprene synthetic gloves described. Reactions: ?+, +, ++, +++ Reading at day 3 or day 4 and day 7	12 patients on the 16 patients described reacted to DPG (**75%):** - 3 reactions : +++ (2 with atopic dermatitis) - 9 reactions : ++ (2 with atopic dermatitis)	Pontén *et al.* (2012)
Retrospective study (May 2007, to May 2009, USA) Selected dermatitis patients	DPG No further information	626 patients with suspected allergic contact dermatitis (ACD) patch tested. 23 patients were found to present with primary hand/wrist dermatitis due to currently relevant contact allergy to 1 or more chemicals in the rubber gloves they were wearing. 5 patients patch tested with DPG	All patients reacted to DPG : - 1 patient : + - 4 patients (80%): ++	Cao *et al.* (2010)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Readings at day 2 or 3 and 6 or 7.		
Retrospective study (January 1, 2000, through December 31, 2007, USA) Selected dermatitis patients	DPG Concentration : 1% Vehicle not specified Purity: no data	771 patients with suspected allergic contact dermatitis from rubber and patch tested with rubber allergens. 759 patients tested with DPG.	55 positive reactions: **7.5%.** - 17 relevant macular erythema reactions - 11 questionable relevant macular erythema reactions - 17 relevant mild or moderate reactions - 8 questionable relevant mild or moderate reactions - 1 past relevant mild or moderate reactions - 1 relevant severe reaction 28 irritant reactions	Bendewald *et al.* (2010)
Selected dermatitis patients (April 2002 to March 2003, Pakistan)	DPG Concentration : 1% Vehicle : petrolatum Purity: no data Carba Mix Concentration : 3% Vehicle : petrolatum	50 patients with suspected allergic contact dermatitis from footwear and 50 controls patch-tested with 20 allergens. Reading at 1 hour, day 2 and day 4 and day 7 Reactions: ?, +, ++, +++	1 patient reacted to DPG : **2%** No reaction in controls 5 patients reacted to carba mix alone : 10% 5 (10%) patients who were patch test positive to carba mix exhibited co sensitivity to DPG, which is one of the ingredients present in carba mix (positive reaction to DPG when tested at the same time than carba mix).	Suhail *et al.* (2009)
Retrospective study (July 1994 to June 2006) Work place study with selected workers with known dermatitis	DPG Concentration : 1% Vehicle : petrolatum Purity: no data	1434 patients with suspected allergic contact dermatitis patch-tested. 31 healthcare workers tested with DPG. Occupational exposure : gloves Readings at 2 and 4 days. Reactions: ±, +, ++, +++	**12.9%** reactions to DPG (no information on grading of reactions).	Suneja *et al.* (2008)
Work place study with selected workers with known dermatitis due to the	DPG No further information	5 patients with work-related allergic hand dermatitis due to the use of rubber gloves.	4 patients reacted to DPG (80%). This percentage is a overestimation as the patients	Piskin *et al.* (2006)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
use of rubber gloves (April to June 2005)		Diagnostic patch test performed.	already had occupational rubber-related allergic contact dermatitis.	
Retrospective study (1999 to 2005, UK) Selected dermatitis patients	DPG Concentration: 1% Vehicle : petrolatum Purity: no data	Patients patch tested with footwear allergens. 610 patients tested with DPG	- 11 positive reactions: **1.80%** - 1 irritant reaction identified: 0.16% - 1 doubtful reaction identified: 0.16%	Katugampola *et al.* (2005)
Retrospective study (1995–2001, Germany) Work place study with selected workers with known dermatitis due to the use of protective gloves (not specification of rubber gloves)	DPG Concentration : 1% Vehicle: petrolatum Purity: no data	67188 patients patch tested 1455 patients with occupational contact dermatitis and suspected glove allergy and patch-tested with DPG. Reading at day 3 Reactions: +, ++, +++	**1.9%** reacted to DPG. Irritation: 42 patients (Erythematous reactions) - 27 reactions: + - 1 reaction: ++/+++ (0.07%)	Geier *et al.* (2003)
Retrospective study (1994 to 1998, Italy) Work place study with selected workers with dermatitis	DPG Concentration : 1% Vehicle : petrolatum Purity: no data	360 consecutive patients, working in healthcare environments with skin disease localized to their hands, wrists and forearms, in whom finally, on the basis of anamnesis, clinical examination and test results, contact dermatitis was diagnosed. Patients patch tested with a standard series, 'health' screening and rubber allergens Reading at day 2 and day 4	2/360 positive reactions to DPG: **0.6%**	Nettis *et al.* (2002)
General population study (1981 to 1988, Canada, USA) Selected dermatitis patients	DPG Concentration : 1% Vehicle not specified Purity: no data	1670 patients patch tested between 1981 and 1988 with the screening tray and 317 of them also tested with the rubber tray. General population Reading: at 30-60 min and day 2 or day 3 Positivity: +, ++, +++	On 316 patients patch tested with the rubber tray, **4.4%** reacted to DPG.	Holness *et al.* (1997)
Selected dermatitis patients (January 1989 and March 1994, Poland)	DPG Concentration : 1%	1697 patients with suspected occupational dermatitis patch-tested	46 patients tested with DPG (19 females, 27 males) 4 positive reactions, **8.7%**:	Kiec-Swierczynska (1995)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	Vehicle: petrolatum Purity: no data	Occupational ACD diagnosed in 334 patients. 46 patients sensitive to at least 1 allergen of the standard series or who reported intolerance to rubber products patch tested with rubber series. Reading at day 2 and day 3.	- 1 in females (5.3%) - 3 in males (11.1%) Some of the patients reported intolerance to rubber products (no details), therefore this percentage may be an overestimation of the reactions to DPG.	
Selected workers with allergic reactions to thiourea compounds (rubber chemicals)(September 1985 to December 1991, Finland)	DPG Concentration : 1% Vehicle : petrolatum Purity: no data	Patch test of 423 patients with rubber chemicals series in dermatology consultation of an occupational institute.	5 patients (1%) reacted to thiourea compounds and among them 1 patient **(0.2%)** reacted to DPG (stock clerk). (Patient's occupations : stock clerk, florist, dentist, precision mechanic)	Kanerva *et al.(*1994)
Retrospective study (1978 to 1988, Spain) Selected dermatitis patients	DPG Concentration : 1% Vehicle: petrolatum Purity: no data	7000 patients suspected of occupational dermatitis. 4680 patients patch-tested. Rubber-mixes (thiuram mix, carba-mix, black-rubber-mix [or PPD-mix], mercapto-mix and naphtyl-mix) were included in this standard series. Readings at day 3 and 4.	Positive results to rubber additives: 686 13/686 patients reacted to DPG : **2.3%**	Conde-Salazar *et al* (1993)
Selected dermatitis patients	DPG Concentration : 1% Vehicle: petrolatum Purity: no data	50 patients (males= 31, females= 19) with suspected footwear dermatitis and 30 controls patch-tested with 22 allergens of a shoe series. Controls: 30 (19 male and 11 female) age- and sex-matched controls, without skin disease or systemic disease Reactions: ±, +, ++, +++, ++++ Reading at day 2 and day 3 and day 4	6 patients reacted to DPG : **12%** - 3 male patients: 12% - 3 female patients: 13% 1 control (3.3%) reacted to DPG	Saha *et al.* (1993)
Selected dermatitis patient (October 1986 to March 1988, India)	DPG Concentration : 1% Vehicle: plastibase Purity: no data	105 patients with foot dermatitis seen over a period of 18 months, diagnosed by patch test with various shoe allergens. Readings at day 2 and 4	Rubber and rubber chemicals showed positivity in 5 cases. 3 patients **(2.8%)** reacted to DPG.	Bajaj *et al.* (1988)
Workplace study on selected workers (1975 to 1980,	DPG No further information	34 agricultural workers with ACD compared with a control group of contact dermatitis patients in other occupations	Agricultural workers : **11.76 %** reacted to DPG Control group: **5.32%** reacted	Garcia-perez *et al.* (1984)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Spain)		(244) diagnosed by patch-test. Control group, 244 male patients seen during the same period, who had contact dermatitis confirmed by a standard series positive patch test.	to DPG. Difference between agricultural workers and control group highly significant.	
Retrospective study (1976 to 1980, Finland) Work place study with selected workers with known dermatitis	DPG No further information	Patients with occupational allergic contact dermatitis working in the footwear and tyre departments of a Finnish rubber factory patch-tested. 50 patients patch tested with DPG.	2 positive reactions: **4.5%**	Kilpikari (1982)
Selected dermatitis patients	DPG Concentration : 1% Vehicle: petrolatum Purity: no data	35 cases with shoe dermatitis diagnosed and patch-tested.	On the 35 cases studied, 2 reacted to DPG **(5.7%).**	Adams (1972)
Selected dermatitis patients (September 1967 to January 1970, Poland)	DPG Concentration: 1% Vehicle : Yellow paraffin Purity: no data	1205 patients with primary contact, atopic, nummular and stasis dermatitis, and unclassified eczema patch-tested with series of 43 substances. 744 patients tested with DPG. Reading at day 2 and day 4.	**9.9%** positive reactions - 11.6% in men - 8.3% in women	Rudzki et al. (1970)
Selected dermatitis patients	DPG Concentrations : 1% and 2% Vehicle not specified Purity: no data	30 patients with ACD related to wearing rubber boots patch-tested with rubber chemicals. 15 patients tested with DPG. Reading at day 2 and day 4 and day 7 Reactions: +, ++ ,+++, ++++	1 patient reacted to DPG 1% (reaction +) **(6.7%)** The same patient reacted to DPG 2% (reaction ++)	Ross (1969)

Table 12: Case reports:

Test substance,	Relevant information about the study (as applicable)	Observations	Reference
DPG Concentration: 1%	49-year-old female surgical scrub nurse with a 2-month history of hand dermatitis.	Patch-test: Positive reaction to DPG. The patient switched to rubber accelerator-free	Young *et al.* (2021)

Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Vehicle: petrolatum Purity: no data	Reading at day 4 Reactions: +, ++ or +++ ?+ : doubtful	gloves and her hand dermatitis cleared completely. One month later: depigmented patches involving the dorsal and ventral hands and wrists appeared involving a similar distribution to her ACD. It was diagnosed with contact leukoderma. After treatment with topical tacrolimus 0.1% ointment twice daily, her leukoderma showed noticeable improvement two months later.	
DPG No further information	23-year-old man with a 3-year history of hand eczema. Reported wearing rubber gloves during his work as an electrician and wearing boxing gloves at the gym. Reading at day 2, 3 and 4.	Patch-test with DPG: Positive reaction to DPG: + Patch-test with fragments of gloves: Positive reaction (+) only to the inside part of the boxing gloves. HPLC revealed the presence of DPG and N-cyclohexyl-N0 -phenyl-p-phenylenediamine inside of the boxing gloves. Minor signal to N-cyclohexyl-N0 -phenyl-p-phenylenediamine: DPG considered to be the main agent responsible for the development of the patient's hand eczema.	Corraza *et al.* (2021)
DPG No further information	(1) 55-year-old male anesthesiologist with hand eczema patch tested. (2) 45-year-old female surgeon with hand eczema patch-tested. (3) 56-year-old female surgical nurse with hand eczema (4) 59-year-old male surgeon with hand eczema Occupational exposure	(1) reaction to DPG: + (2) reaction to DPG: ++ (3) reaction to DPG: ++ (4) reaction to DPG: +	Hansen *et al.* (2020)
DPG No further information	A 55-year-old nonatopic Turkish man with a history of severe eczema on both feet and ankles and patch-tested. Readings at day 2 and day 3 Reactions: +, ++ or +++ ?+ : doubtful	Reaction to DPG: ++ (day 3). Possible current clinical relevance (a possible rubber additive in neoprene socks).	Özkaya *et al.* (2020)
DPG No further information	A 30-year-old man with hand dermatitis patch-tested Occupational exposure: medical field	Reaction to DPG: +++ Reaction to carba mix: ++	Isaac *et al.* (2019)

Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	Reading at day 3		
DPG No further information	3 women of 20, 52, and 53 years-of-age with allergic contact dermatitis, patch-tested. 53 year-old woman had dermatitis on the torso and extremities. Reactions: +, ++ or +++	53 year-old woman reacted to DPG (reaction +). The other women did not react to DPG.	Goldminz *et al.* (2018)
DPG No further information Carba mix No further information	63-year-old surgical technician with hand eczema diagnosed as occupational allergic contact dermatitis and patch-tested. Reactions: +, ++ or +++ ?+: doubtful	Patch-test: ++ positive reactions to DPG and carba mix. Two month later +++ positive reactions to all gloves from the patient's workplace containing DPG	Li *et al.* (2018)
DPG Concentration: 1% Vehicle: petrolatum Purity: no data	56-year-old Caucasian male patient, bus driver, with widespread eczema, predominantly acral and then after being treated with betamethasone microbial eczema of the feet. Diagnostic performed by patch test. Occupational exposure Reading at day 2 and day 4.	Reaction to DPG: Day 2: ++ Day 4:+++	Pacheco *et al.* (2013)
DPG No further information	A 52 year-old woman worked in a factory manufacturing rubber gas mask, who developed dermatitis on the left side of the left index finger. Patch test with rubber series of 24 compounds. Occupational exposure Reading at day 3	2 solvent used (acetone and ethanol) to verify if the reactions could be attributed to DPG irritation potential. Positive reaction to 1,3-DPG using the two different solvents. Exposure to DPG was stopped and the dermatitis gradually disappeared.	Bruze *et al.* (1994)

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Animal data:

The sensitization potential of DPG was assessed in a maximisation assay conducted according to OECD TG 406 and GLP (unpublished study report, 1995). 5 controls and 10 treated females Dunking-Hartley guinea pigs were used. The

vehicle used was paraffin oil. Intradermal induction concentration was 1% (w/w) in vehicle and the challenge topical concentration was 25% (w/w). The results of the preliminary assay are not available in the study report.

After 24 to 48 hours after removal of the dressing of the cutaneous challenge application of the test substance, **no cutaneous reactions were observed.** However, as noted in OECD TG 406, when fewer than 20 test and 10 control animals have been used, it is not possible to conclude on the sensitizing potential of the chemical. Testing additional animals of a total of at least 20 animals and 10 control is strongly recommended.

The sensitivity of guinea pigs was checked in previous studies using a positive sensitizer, Dinitro-2,4-chlorobenzene. 100% of the animals showed a positive response.

Human data:

In Europe, DPG is routinely tested alone in the rubber test series. Historically, DPG was tested in US and Canada as part of a mixture, named carba mix 3% pet. (1% DPG, 1% Zinc diethyldithiocarbamate, and 1% Zinc bis(dibutyldithiocarbamate)).

Within the framework of this CLH report, the reading criteria established by the International Contact Dermatitis Research Group (ICDRG) for the diagnostics of patch-tests is used (Johansen et al. 2015). In particular, positive allergic reactions scored as + (weak), ++ (strong), +++ (extreme) are considered.

- **Experimental induction test in human:**

One study on the induction of sensitization in human volunteers is available. 49 volunteers without known previous exposure to DPG participated to this study. For the induction phase, a series of 12 applications (0.2 g as 70% preparation in petrolatum), each of 24 hours duration was carried out during weeks 1, 2, and 3. There was a rest period during the weeks 4 and 5. For the challenge phase, a series of 4 applications (0.2 g 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. 19 of the 49 subjects displayed irritation during subsequent induction exposures. Two subjects (4%) displayed positive reactions during the 2-week challenge phase (Study summary from OECD SIDS, unpublished study from Monsanto, 1982).

These results demonstrate the sensitization potential of DPG at high concentrations**.**

- **Diagnostic patch-test data:**

More than 250 publications on DPG contact allergy are available since 1944. Only studies with a full text available are reported in the table above. Many of the studies available are retrospective and workplace studies that investigated sensitization to DPG in patients for whom there was a presumption of sensitization to the gloves used (selected patients). Indeed, the presence of DPG is reported in rubber gloves (Hansen et al. 2020, Dejonckheere, 2019, Hamnerius et al., 2014, Crepy et al., 2016). These studies were conducted in different countries worldwide. In other studies, dermatitis patients from general population were tested with rubber series containing DPG. Foot dermatitis due to the presence of DPG in shoes is also frequently mentioned. Some studies point out the irritant nature of DPG which may interfere with the interpretation of patch tests (the substance has a harmonised classification as Skin Irrit. 2). Besides, this substance is known to cause false positive reactions. Doubtful (+/-) and weakly positive (+) reactions should therefore be analyzed with caution (Geier et al. (2012), Warshaw et al. (2020)). However, strong positive reactions (++) and extreme positive reactions (+++) are unequivocal.

For the purpose of this CLH report, only ++ and +++ reactions have been considered as undoubtful sensitisation. In most of the studies, the severity of the reaction (+, ++ or +++), is not detailed. **Therefore, only studies mentioning the severity of the reaction are used for classification and presented in the table below.**

Table 13 : List of diagnostic patch-test data with reactions ++/+++ to DPG

Type of human diagnostic patch test data as described in the table 3.2 of the CLP guidance	Authors	Severity of reactions to DPG	Conclusion on frequency (high or low/moderate) in application on the table 3.2 of the CLP guidance
Unselected dermatitis patients	Dekoven *et al.* (2017)	++/+++: 0.93%	This frequency of 0.93% is considered as borderline high but **low/moderate**: threshold at 1% between high and low/moderate frequency. This conclusion is consistent with the conclusion based on Warshaw et al. (2020) that also analysed patients from the NACDG database but based on a longer period of time and therefore of a higher number of patients.
Unselected dermatitis patients	Warshaw *et al.* (2020)	++/+++: 0.2%	This frequency of 0.2% is considered as **low/moderate**: threshold at 1% between high and low/moderate frequency.
Selected dermatitis patients	Ross (1969)	++: 6.7%	This frequency is considered as **high**: threshold at 2% between high and low/moderate frequency
Selected dermatitis patients	Cao *et al.* (2010)	++: 80%	This frequency is considered as **high**: threshold at 2% between high and low/moderate frequency
Selected dermatitis patients	Uter *et al.* (2016)	++/+++: 0.64%	This frequency is considered as **low/moderate**: threshold at 2% between high and low/moderate frequency
Selected dermatitis patients	Hulstaert *et al.* (2017)	++: 5.5%	This frequency is considered as **high**: threshold at 2% between high and low/moderate frequency
Work place study with selected workers with known dermatitis due to the use of protective gloves	Geier *et al.* (2003)	++/+++: 0.07%	This frequency is considered as **low/moderate**: threshold at 1% between high and low/moderate frequency
Work place study with selected workers with known dermatitis related to protective gloves	Geier *et al.* (2012)	++/+++: 0.47%	This frequency is considered as **low/moderate**: threshold at 1% between high and low/moderate frequency
Work place study with selected workers with known exposure or dermatitis	Pontén *et al.* (2012)	++/+++: 75%	This frequency is considered as **high**: threshold at 1% between high and low/moderate frequency
Work place study with selected workers with known dermatitis to the use of rubber gloves	Baeck *et al.* (2013)	++/+++: 37%	This frequency is considered as **high**: threshold at 1% between high and low/moderate frequency
Work place study with selected workers with known dermatitis caused by rubber accelerators	Crepy *et al.* (2017)	++/+++: 56%	This frequency is considered as **high**: threshold at 1% between high and low/moderate frequency
Work place study with selected workers with known exposure	Hamnerius *et al.* (2018)	At a concentration of 1% of DPG :	These frequencies of 1.3 and 1.6 % are considered as **high** : threshold at 1% between

to gloves (rubber gloves), soaps, alcoholic hand disinfectants and hand creams		++/+++ : 1.3% At a concentration of 2% of DPG : ++/+++ : 1.6%	high and low/moderate frequency
Work place study with selected workers with known exposure to rubber gloves or dermatitis	Dejonckheere *et al.* (2019)	++/+++ : 45%	This frequency is considered as **high** : threshold at 1% between high and low/moderate frequency

In some studies (highlighted in grey in the table above), the percentage of reactions to DPG is very high. This can be explained by the selection of the test population. In these studies, the test populations had either a history of reactions to rubber gloves or to rubber accelerators. Thus, the test population represents a highly-selected group with regard to its potential to be sensitised to DPG and there may be an overestimation of reactions to DPG compared to dermatitis patients without pre-established relation to rubber/gloves. The table 3.2 of the CLP guidance indicating the frequency of occurrence in different types of populations mentions that such data is not entirely applicable to this type of highly-selected population.

The range of the frequency of reactions ++/+++ is presented below:

- Unselected dermatitis patient (Dekoven et al. (2017) and Warshaw *et al.* (2020)): 0.2 - 0.93%
- Selected dermatitis patients based on reaction to rubber boots (Ross, 1969) or to shoes (Hulstaert et al. (2017)) and to special rubber series (Uter et al. (2016)): 0.64% - 6.7%
- Highly selected dermatitis patients for their reaction to rubber gloves. Indeed, these patients were selected because they were known to have dermatitis because of chemicals present in the rubber gloves they were wearing (Cao et al. (2010)): 80%
- Selected workers with known exposure or known hand dermatitis (Hamnerius et al. 2018) or suspected glove-induced dermatitis (Geier et al. (2012 and 2003)): 0.07% - 1.6%
- Highly selected workers with known exposure or dermatitis based on reactions due to rubber gloves (Dejonckheere et al. (2019), Crepy et al. (2017), Baeck et al. (2013), Ponten et al. (2012)). . Indeed, these patients were selected because they were known to present dermatitis from wearing rubber gloves: 37–75%.

Besides, a retrospective study (2000–2016, in USA and Canada) conducted by Silverberg et al. (2021), investigated the allergens involved in the hand eczema of 1634 children (aged below 18 years) in the North American Contact Dermatitis Group (NACDG) database. 1023 patients were tested with DPG but the results were not detailed for DPG. The authors established a list of allergens with highest significance-prevalence index number in children with hand eczema. On a list of 20 allergens patch-tested, DPG was ranked 7th behind methylisothiazolinone, carba mix, thiuram mix, nickel sulfate hexahydrate, methylchloroisothiazolinone /methylisothiazolinone and formaldehyde 2%.

- **Case studies:**

A large number of publications reporting cases of skin sensitization to DPG are available and support the conclusions of the retrospective studies.

Most of these studies are to be interpreted with caution as they do not present the grade of reactions.

The majority of the publications report cases of hand eczema due to glove wearing in workers (Young et al. 2021, Corraza *et al.* (2021), Hansen *et al*. (2020), Isaac *et al.* (2019), Li *et al.* (2018)). Healthcare professionals are the most frequently reported population. In one other case study, one patient working in a factory manufacturing rubber gas mask, developed eczema on the finger (Bruze *et al.* (1994)). In general population, cases of eczema on the feet have been reported because of shoes containing DPG (Pacheco *et al.* (2013)) or socks (Özkaya *et al.* (2020)).

DPG is also part of a mixture named carba-mix mix 3% pet. (1% DPG, 1% Zinc diethyldithiocarbamate, and 1% Zinc bis(dibutyldithiocarbamate)). Studies reporting sensitization to carba mix are considered as supportive data and are presented at the end of the CLH report in Table 40 (Ito et al. (2021), Uter *et al.* (2020), Warburton *et al.* (2015 a and b), Lynde *et al.* (1982)).

- **Cross-sensitization:**

Garcia-Perez *et al.* (1984) mentioned potential cross-reactions between DPG and other related chemical structures (for example cyanamides) or pesticides derived from guanidines (dodecylguanidine). However, it must be noted that among all the available studies, including those that are very recent, this is the only study that mentions potential cross-reactions between DPG and pesticides or other structurally related substances. Therefore, these cross-reactions remain at the state of hypothesis.

Warshaw *et al*. (2020) studied the frequency of concomitant reactions to carba mix and DPG. Historically, DPG was tested in US and Canada as part of a mixture, named carba mix 3% pet. (1% DPG, 1% Zinc diethyldithiocarbamate, and 1% Zinc bis(dibutyldithiocarbamate)). They concluded that because of the structural difference between DPG and dithiocarbamates, cross-reactivity is unlikely. According to them, overlap between positive reactions to DPG and carba mix is likely due to co-sensitization from the same sources. In conclusion, a reaction to the carba mix does not mean that the person reacts to DPG, a person can react to both substances but DPG tested alone causes reactions therefore these reactions are not due to cross reactions. Nevertheless, the evaluation is only based on reactions to DPG alone. Reactions to both DPG and carba mix are used as supportive information.

Overall, the availability of many studies show that DPG can lead to sensitization in a substantial number of persons.

The induction study in human volunteers demonstrate that DPG is sensitizing in humans with high frequency of occurrence of reactions (4%) at high concentration (70% DPG). A very large database of epidemiological studies report significant frequency of sensitization to DPG. By considering only strong positive reactions (++) and extreme positive reactions (+++) to prevent the risk of false positive reactions due to DPG irritation potential, frequency of sensitization to DPG (generally tested at 1%) is reported in the range of 0.2 - 0.93% for unselected dermatitis patients, 0.64% - 6.7% for selected dermatitis patients and 0.07% - 1.6% for selected workers with known exposure or dermatitis (table 3.2 of CLP guidance). These frequencies reach much higher figures in groups selected due to suspicion of allergy to rubber in gloves or rubber additives (37–75% for the population of selected workers with known exposure or dermatitis (Dejonckheere et al. (2019), Crepy et al. (2017), Baeck et al. (2013), Ponten et al. (2012)) and 80% for the population of selected dermatitis patients (Cao et al. (2010)). The skin sensitization potential of DPG is clearly demonstrated.

Lastly, the retrospective and clinical studies show that the frequency of sensitization to DPG is relatively stable over time from the 70s to 2021. The recent studies demonstrate that DPG exposure still occurs and induces skin sensitisation in human.

10.7.2 Comparison with the CLP criteria

According to CLP, *"Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:*

(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

(b) if there are positive results from an appropriate animal test

***Sub-category 1A**: Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.*

***Sub-category 1B**: Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered."*

There are both experimental studies and human data assessing skin sensitisation properties of DPG. In the maximisation assay (Magnusson and Kligman) conducted according to OECD TG 406, DPG did not induce cutaneous reactions in the guinea pig. However, it should be noted that too few animals were tested in this study according to the revised guideline (June 2022).

A significant number of epidemiological studies performed since the 70s report positive sensitization responses to DPG in human population. As detailed above, the skin sensitization potential of DPG is clearly demonstrated based on human data and a classification for skin sensitization is justified.

Human data have been analysed to determine if they are sufficient for sub-categorisation.

Sub-categorisation is based on the frequency of occurrence of skin sensitization in humans.

Each publication was allocated to a population type as indicated in Table 3.2 of the CLP guidance. For the studies with sufficient information and detailed above, the mean reaction frequencies for each population type were compared with the criteria set in the CLP guidance.

Human diagnostic patch test data	High frequency	Low/moderate frequency
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies:		
1: all or randomly selected workers	≥ 0.4 %	< 0.4 %
2: selected workers with known exposure or dermatitis	≥ 1.0 %	< 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

* Only one or two types of information may be sufficient for sub-categorisation.

Taking into account all available studies, the number of published cases is higher than 100 cases.

As detailed above, in studies in which patients were highly-selected, in particular for a suspicion of allergy to rubber gloves, were largely above threshold defining high frequency (Dejonckheere et al. (2019), Crepy et al. (2017), Baeck et al. (2013), Ponten et al. (2012), Cao et al. (2010)). The test population therefore represents a highly-selected group with regard to its potential to be sensitized to DPG and they may represent an overestimation of reactions to DPG compared to dermatitis patients without pre-established relation to rubber/gloves.

In other studies, the frequency of occurrence was concluded as high in a workplace study on selected health-care workers (Hamnerius et al., 2018) and in selected dermatitis patients (Hulstaert et al. (2017) and Ross (1969)). Other studies were indicating a low/moderate frequency of occurrence. However, only on ++ or +++ reactions were considered, the percentage of sensitization reactions is probably underestimated.

Overall, based on the weight of evidence, the frequency of occurrence of skin sensitization to DPG is considered high.

Exposure data	Relatively low exposure (weighting)	Relatively high exposure (weighting)
Concentration / dose	< 1.0% < 500µg/cm² (score 0)	≥ 1.0% ≥ 500µg/cm² (score 2)
Repeated exposure	< once/daily (score 1)	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitizer)	<100 exposures (score 0)	≥100 exposures (score 2)

Regarding 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 Anses (2020), in general in rubber goods, the percentage of DPG is estimated to be maximum **0.23%** after vulcanization step. There is no information on the quantity of DPG in medical gloves.

Workers are exposed using rubber gloves containing DPG and general population are exposed through articles such as shoes, balloons or tyres. Based on these information, the exposure can be considered as repeated once/daily and above 100 exposures.

Adding up the scores for each category, the final score for exposure is 4. The score of 4 corresponds to a relatively low exposure.

Table 3.4 Sub-categorisation decision table

	Relatively low frequency of occurrence of skin sensitisation	Relatively high frequency of occurrence of skin sensitisation
Relatively high exposure (score 5-6)	Sub-category 1B	Category 1 or case by case evaluation
Relatively low exposure (score 1-4)	Category 1 or case by case evaluation	Sub-category 1A

Based on this table and considering human data, DPG fulfils criteria for classification Skin Sens. 1.

The frequency of occurrence of skin sensitization to DPG is considered high and the exposure is established low. Therefore, a classification sub-category 1A is warranted for DPG.

10.7.3 Conclusion on classification and labelling for skin sensitisation

DPG should be classified Skin Sens. 1A – H317 according to CLP Regulation.

10.8 Reproductive toxicity

10.8.1 Adverse effects on sexual function and fertility

Table 14: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD TG 443 (Cohorts 1A, and 1B with extension to cohort F2) GLP Rats, Sprague-Dawley Males and females Number of animals per sex per dose: - Parental generation: 24 per sex per dose - F1: 20 per sex per dose (Cohorts 1A and 1B)	DPG Purity: 98.7% Vehicle: 0.5% methylcellulose in drinking water treated by reverse osmosis Dose levels: 0, 5, 15, 25 mg/kg bw/day Administration: once daily by gavage Duration of exposure: - P males: at least 10 weeks of treatment - P females: at least 8 to 10 weeks of treatment	**General toxicity:** **Mortality (unscheduled death) and clinical observations in these animals:** P generation: No unscheduled death in males. *5 mg/kg bw/day:* 2 females euthanized on PND 1 and 2. *15 mg/kg bw/day:* 2 females euthanized on Day 24 p.c (post conception) and on PND2. *25mg/kg bw/day:* 5 females euthanized on PND1 and 1 female on Day 23 p.c.	Unpublished study report, 2021 Reliability 1 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	- Cohort 1A: both males and females: from weaning post-natal day (PND 22) until euthanasia (from Day 90 p.p., to Day 93 p.p. maximum). - Cohort 1B: In males: from weaning (PND 22) for at least 10 weeks before mating, during the mating period (up to 2 weeks), and after euthanasia of F2 pups (on PND 4). In females: from weaning (PND 22) for at least 10 weeks before mating, during the mating period (up to 2 weeks), during gestation, during lactation until PND 4 inclusive until euthanasia for females with no delivery (26 days after the last day of the mating period).	Cohort 1A: No unscheduled death. Cohort 1B: No unscheduled death in males. Control group: One female euthanized on Day 4 p.c. One female (pregnant): euthanized for no delivery on Day 25 p.c. *15 mg/kg bw/day:* 2 females euthanized on PND1. *25 mg/kg bw/day:* One female found dead on study Day 1. One female euthanized on Day 24 p.c. 4 females euthanized for dead litter on PND1. **Body weight and body weight gain:** P generation, cohort 1A and 1B: no adverse effect on body weight and body weight change. **Food consumption:** No adverse effect seen in P Generation, cohort 1A and cohort 1B during the study. **Clinical signs in termined as scheduled animals:** P generation: In all treated groups of both sex, ptyalism was observed before and/or after dosing. *25 mg/kg bw/day in females:* Dyspnea after treatment (4/18) Exophtalmos (2/18) Hunched posture before and/or after treatment (2/18) Piloerection (4/18) Clonic convulsion, locomotory difficulties, loss of balance, staggering gait and/or tonic seizures after treatment (6/18) Cohort 1 A: In all treated groups of both sex ptyalism was observed before and/or after dosing. Cohort 1B:	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		25 mg/kg bw/day in males: Abdominal breathing and dyspnea after treatment (1/20) Half-closed eyes before (1/20) and after treatment (3/20) Hunched posture, hypoactivity, lateral recumbency, piloerection and/or ventral recumbency (6/20) Clonic/tonic convulsion, loss of balance and/or staggering gait after treatment (10/20) *25 mg/kg bw/day in females:* Half-closed eyes after treatment (2/15) Hypoactivity, lateral recumbency, and/or ventral recumbency after treatment (6/15) Clonic convulsion, loss of balance and/or staggering gait after treatment (14/15) **Organ observations:** <u>P generation:</u> *From 15 mg/kg bw/day:* In males: Statistically significant increase in absolute and relative-to-body liver weights, correlated with microscopic hepatocellular hypertrophy. In females: Same trend with lower amplitude without statistical significance. Statistically significant increased absolute and relative-to-body adrenal gland weights (up to +23%). No microscopic correlates. <u>Cohort 1A:</u> *From 5 mg/kg bw/day:* Statistically significant increased absolute and relative-to-body liver weights in females treated *25 mg/kg bw/day:* Statistically significant increased relative-to-body weights in males. These effects correlated with hepatocellular hypertrophy at microscopic examination in both sex. <u>Cohort 1B:</u> Increased absolute (statistically significant at 15 mg/kg bw/day) and/or relative-to-body liver weights (statistically significant from 15 mg/kg bw/day in males) recorded in males	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		and females treated at ≥ 15 mg/kg bw/day. No microscopic correlates. **Other findings:** At 5 mg/kg bw/d, in P generation, one female with an adenocarcinoma in mammary gland. At 25 mg/kg bw/d, in cohort 1B generation, one female with an adenocarcinoma in mammary gland. **Effects on thyroid hormones (P and cohort 1A):** In P generation females: *25 mg/kg bw/day:* Statistical increase in T4 concentrations (+38%) associated with a decreased in TSH concentrations (-10% not statistically significant). Cohort 1A males: *5 and 15 mg/kg bw/day:* Statistical decrease of T4 concentrations. No effect on weight and histopathology of the thyroid was observed in any generation, males and females. **Effects on fertility and sexual function:** **Estrous cycle:** P generation: *From 5 mg/kg bw/day:* Tendency toward a lower mean number of days of metestrus (4.9 for controls, 4.0 at 5 mg/kg/day, 3.7 at 15 mg/kg/day and 3.3 at 25 mg/kg bw/day). Statistical significance achieved at 15 and 25 mg/kg bw/day Tendency toward an increase in the mean number of estrus (3.5 for controls, 3.8 at 5 mg/kg bw/d, 3.4 at 15 mg/kg bw/d and 4.3 at 25 mg/kg bw/d). Statistical significance at 25 mg/kg bw/d. Cohort 1A: *25 mg/kg bw/day:* Same trends observed without statistical significance (metestrus: 3.8 vs 4.6 in controls; estrus: 4.3 vs 4.0 in controls).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Duration of gestation: P generation: No difference in mean duration of gestation. But increased gestation length (23-24 days) noted at individual level. *5 mg/kg bw/day*: 2/22 females with a gestation period of 24 days associated with a high pup mortality rate. *25 mg/kg bw/day:* 7/24 females with 23-day gestation periods (not statistically significant) associated to a high post-implantation loss. Cohort 1B: *15 mg/kg bw/day:* 1/18 female had 23 days of gestation *25 mg/kg bw/day:* 7/19 females had 23 days of gestation. Statistically significant increase in the mean duration of gestation at (22.3 vs 21.9 in controls). **Reproduction difficulties (difficulty to deliver and reddish vaginal discharge):** P generation: *5 mg/kg bw/day:* One female presented reddish vaginal discharge associated with dead litter. *15 mg/kg bw/day*: One female had dystocia with abdomen increased in size. *25 mg/kg bw/day:* One female presented difficulty to deliver. Cohort 1B: *25 mg/kg bw/day :* One female presented difficulty to deliver. **Follicles count:** Cohort 1A:	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		From 5 mg/kg bw/day: Tendency toward a dose-related decrease in the mean number of primordial follicles (8.68 at 5 mg/kg bw/day, 6.65 at 15 mg/kg bw/day, 5.75 at 25 mg/kg bw/day vs 9.31 mg/kg/day in controls) (no statistical significance achieved).	
OECD TG No. 421 GLP Rats, Sprague-Dawley Males and females 10 animals per sex per group	DPG Purity: 98.9% Vehicle: 0.5% methylcellulose aqueous solution Dose levels: 0, 5, 15, 25 mg/kg bw/day Administration: once daily by gavage Duration of exposure: In males: - 4 weeks before pairing, - during the pairing period (up to 17 days), - until sacrifice of the females (at least 10 weeks in total). In females: - 4 weeks before pairing, - during the mating period (up to 17 days), - during gestation, -during lactation until day 4 post-partum inclusive (or until sacrifice), - until sacrifice for non-pregnant females.	**General toxicity:** Parental generation: **No mortality in any group due to parental toxicity.** **Body weight and food consumption:** *25 mg/kg bw/day:* Males had a statistically significant mean body weight gain 20% lower than that of the controls. Slightly reduced mean female body weight gain during the gestation period (-10%, not statistically significant). Statistically significant lower mean female food consumption from days 14 to 20 of gestation (29 g/animal/day vs. 35 g/animal/day, $p<0.01$). Correlated with the slight reduction in body weight gain of the females during the gestation period. Food consumption remained slightly lower during the lactation period (not statistically significant). **Clinical signs:** Lateral decubitus and mydriasis in one male (day 27 of dosing only) and one female (day 1 of lactation only), abnormal locomotion in one occasion for one female and staggering gait in one male. Salivation for all animals. **Organ weight, macroscopic and microscopic examinations** No effects on organ weights nor any treatment-related macroscopic or microscopic findings. **Effects on fertility and sexual function:** No effects on sperm motility No effects on mating, fertility, gestation or delivery	Unpublished study report, 2010 Reliability: 1
Oral testicular toxicity and male	DPG Purity: 99.9%	**General toxicity:** Some minor change in body weight during	Unpublished study report,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
fertility study No guideline, GLP Mice, albino, CD1 180 males, 130 females 25 males per dose 22 females for the group doses: 0, 4 and 16 mg/kg bw/day.	Vehicle: acetic acid in demineralised water Dose levels : 0, 0.06, 0.25, 1, 4 and 16 mg/kg bw/ day daily by gavage Duration of exposure: 8 weeks premating period for males. 11 selected males of the groups exposed to 0, 4 and 16 mg/kg/day mated with untreated females.	the premating period without dose response. **Mortality:** No female died during the study. In males: *control group:* 1 animal *0.06, 0.25, 4 and 16 mg/kg bw/day respectively:* 3 – 6 – 2 – 4 animals **Gross examination, organ weight, microscopic examination in males:** Gross examination at necropsy did not reveal any treatment related changes. No significant differences in organ weights occurred between the groups. Microscopic examination of the testes did not show any effect of treatment with DPG. **Clinical signs in parent mice:** *In all groups:* Some animals with minor piloereaction (including in control group) and low general body condition (one animal in control group also: probably due to a dosing error). **Effects on fertility and sexual function:** *0, 4 and 16 mg/kg bw/ day respectively:* Mating index: 77.3, 86.4, 86.4% Female fertility index: 59.1, 86.4, 77.3% Male fertility index: 72.7, 90.9, 81.8% Fecundity index: 76.5, 100, 89.5% **Sperm analysis:** *16 mg/kg bw/day:* Statistically significant increase in folded tails (mean incidence: 50 vs 20 in controls / 1000 cells examined). Data not available for the other treated groups.	1989 Koëter et al. (1992) Reliability 3 (not reliable) Poor reproductive performance in the controls
Study on seminal cytology, testicular histology and reproductive	DPG Purity: There were some undefined impurities in the test material. No more information	<u>Mice:</u> Few information on general toxicity: Histopathological data:	Bempong, 1983 (Bempong, Jan. 21, 1987, personnal

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
toxicity in mice and hamster. No guideline, Not GLP Mice, C57BL/6JxDBA2, males and females Syrian hamster, males No information on the number of treated animals/dose, food and water consumption, clinical signs.	available. Vehicle: 0.025% acetic acid <u>Mice:</u> <u>Male reproductive organs analysis:</u> Exposure period: up to 15 weeks <u>Reproductive study :</u> Exposure period (male only): 90 days After 7 days of exposure, the animals were mated at weekly intervals to 12-wk-old virgin untreated females. Females sacrificed after 13 days of pregnancy. Premating exposure period (males): 7 days Premating exposure period (females): no exposure Administration: continuously in drinking water Dose levels: 0, 4 or 8 mg/kg bw/day <u>Syrian hamster :</u> <u>Male reproductive organs analysis:</u> Exposure period : up to 80 days (not clearly indicated) Administration: continuously in drinking water Dose levels: 0, 4 or 8 mg/kg bw/day	*8 mg/kg bw/day:* parietal peritoneum saturated with fatty tissues. Mesentery and greater omentum showed the greatest evidence of fatty tissue accumulation compared to the control group. **<u>Effects on fertility and sexual function:</u>** *From 4 mg/kg bw/day:* Non-linear time-dependant increase in the frequency of sperm morphological abnormalities within 85 days of observation: - *In controls:* from 1.8 to 5.3% with a mean of 3.5%. - *At 4 mg/kg bw/day*: from 16.2 to 42.4%. - *At 8 mg/kg bw/day:* from 38.6 to 75.1%. Significant decrease in testicular weights after 5, 7, 9, and 15 weeks of treatment Significant decrease in sperm count, 7, 9, and 15 wk after treatment. At 8 mg/kg bw/day of DPG, significant differences in sperm counts were noted 5 weeks after treatment. Presence of germinal cells in the epididymis of treated mice. More germinal cells than spermatozoa in cytological preparation when prolongation of DPG treatment. Presence of irregularly shaped seminiferous tubules with no defined basement membrane, loss of interstitial cells, and limited numbers of spermatids and spermatozoa in the lumen of the tubules compared to controls. Significant differences between treated-groups and controls, on fertility indices after 5 weeks (number of pregnant females: 19/20; 16/20; 11/20, for each group respectively at week 5 and 20/20; 17/20; 8/20 at week 7). Differences between the two doses of DPG became evident in the 7th week. Significant reduction in the number of implants per pregnancy in mice observed at the week 12: *4 mg/kg bw/day:* 7.5 ±2.3 implants per pregnancy. *8 mg/kg bw/day:* 5.5 ±1.9 implants per pregnancy. No information on number of implants in control at week 12.	communication reported by the SIDS, 2002) Reliability : 3 (not reliable) Study design not clear. Impurities (not detailed) mentioned by the author.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Syrian hamster: No information on general toxicity **Effects on fertility and sexual function:** *In controls:* Abnormal sperm morphology ranged from 2.0 to 13.6% with a mean of 9.2%. *From 4 mg/kg bw/day:* From day 75 to the end of the experiment, steady increases in the frequency of anomalous sperm: up to 50 and 80% of abnormal sperm at 4.0 and 8.0 mg/kg bw/d, respectively. Fluctuations in the levels of sperm abnormalities in all preparations from day 30 to day 75.	

Table 15: Summary table of other studies relevant for toxicity on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Repeated Dose 90-day oral toxicity study in rodent Similar to OECD TG 408 GLP Rats F344/N 10 animals per sex, dose and strain	DPG Purity : 98.9% Dose levels: 0, 250, 500, 750, 1500, or 3000 ppm in feed Equivalent : 0, 17/17, 32/32, 49/50, 100/95, 181/184 mg/kg bw/d in male and female rats, respectively. Feeders were changed daily, 7 days per week. Duration of exposure : 13 weeks	**In F344/N rats:** **Mortality:** All animals survived except at 3000 ppm with all females and 6/10 males died. **Food consumption:** Decreased as exposure concentrations increased above 500 ppm with feed consumption 34% to 40% less than the controls in males and females that received 3000 ppm. **Body weight**: *1500 and 3000 ppm groups:* Mean body weights of male and females rats decreased compared to controls (-21 % and -48% in males and -7.5% and -14% in females) (statistical significance not mentioned). **Organ weight:** Organ weights for groups receiving 750 ppm or greater were significantly lower than those of the controls. **Changes in hematology parameters in**	NTP, 1995 Reliability 2 (reliable with restriction)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		animals receiving 1500 and 3000 ppm: Mild polycythemia observed at Day 5 in the 3000 ppm male and female rats, and to a lesser extent in the 1500 ppm females indicated by greater erythrocyte counts, hematocrit values, and hemoglobin concentrations than controls. Slightly lower reticulocyte counts at Day 5 in 3000 ppm male and female rats and 1500 ppm females. **Changes in clinical chemistry parameters primarily in the 1500 and 3000 ppm groups:** Greater alkaline phosphatase activity and bile acid concentration than controls occurred in an exposure-related manner in male and female rats. By Week 13, alkaline phosphatase activity and bile acid concentration were greater than the controls in all groups of exposed rats; these changes are consistent with cholestasis. Total protein, creatinine, cholesterol, and triglyceride concentrations in the 1,500 and 3,000 ppm groups were lower than the controls and these differences are consistent with inanition according to NTP. **Clinical signs:** *≥ 1500 ppm beginning at Week 2 :* Rats appeared thin and had ruffled fur, with discolorations of the tail, ears, and scrotum or vaginal area. Salivation, hypoactivity, convulsions and seizures observed in some male and female rats in these groups, and abnormal posture (staggering) in most males and females. Other clinical signs observed in these groups: hyperactivity, hunched posture, ptosis, ataxia, dyspnea, and bristly hair. **Gross necropsy and microscopy observations:** Female reproductive organs and reproductive parameters:	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		From 750 ppm: Overall reduction in uterus size diagnosed as hypoplasia. Occurred with greater incidence and severity in the three highest exposure groups. *Among 750 and 1500 ppm group:* Length of the estrous cycle greater than controls. Statistically significant at 750 ppm (6.00 vs 4.95 days in controls), not statistically significant at 1500 ppm (5.67 vs 4.95 days in controls with and estrous cycle longer than 12 days or unclear in 1 of 10 animals). Males reproductive organs and reproductive parameters: *1500 ppm:* Significant reduction in sperm motility in males (83.69% vs 94.76% in controls).	
Repeated Dose 90-day oral toxicity study in rodent Similar to OECD TG 408 GLP Mice, B6C3F 10 animals per sex, dose and strain	DPG Purity : 98.9% Dose levels: 0, 250, 500, 750, 1500, or 3000 ppm 1,3-diphenylguanidine in feed Equivalent : 0, 38/46, 75/93, 114/141, 231/285, 457/577 mg/kg bw/d in male and female mice, respectively Feeders were changed daily, 7 days per week. Duration of exposure : 13 weeks	**In B6C3F1 Mice :** All animals survived. **Body weight :** *1500, and 3000 ppm:* Lower mean body weights of both males and females compared to controls (not statistically significant) especially during the latter part of the study (-13.5% and –19% in males and -12% and -20% in females at 1500 and 3000 ppm respectively). **Food consumption :** Similar to control groups in all exposed groups of both sexes. **Organ weight:** Significantly lower absolute organ weights and greater relative organ weights than controls were observed for several organs in the 1500 or 3000 ppm groups. **Clinical signs:** *750, 1500, and 3000 ppm:* Thin appearance in female mice Thin appearance in male mice in the 3000 ppm group. Alopecia, abnormal posture, ptosis, and bristly hair observed in both sexes.	NTP, 1995 Reliability 2 (reliable with restriction)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Gross necropsy and microscopy observations : No treatment-related gross or microscopic lesions observed in male or female mice. **Males reproductive organs and reproductive parameters :** *3000 ppm:* Significant greater numbers of spermatid heads (20.52 vs 17.10 10^7/g testis in the control) and lower sperm motility (51.56% vs 84.84% in the control) **Female reproductive organs and reproductive parameters :** *3000 ppm:* Estrous cycle length significantly greater than controls (5.15 vs 4.30 days).	

10.8.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

1) The effects of DPG on sexual function and fertility were investigated in an Extended One Generation Reproductive Toxicity Study (EOGRTS) including a F2 generation, conducted according to OECD TG 443 (Unpublished study report, 2021). The dose levels tested were 5, 15 and 25 mg/kg bw/day. This study is considered as the key study.

In the study report, Historical Control Data are reported. However, they are constituted of only two EOGRTS studies for reproductive toxicity with only one including a F2 extension. No HCD were available for cohort 1A. The number of the studies used for HCD is not sufficient to be representative of normal biological variability. These HCD are therefore not considered relevant by DS for comparison with the study results. The concurrent control group is anyway the most relevant comparator, historical control data cannot be used to dismiss statistically significant findings.

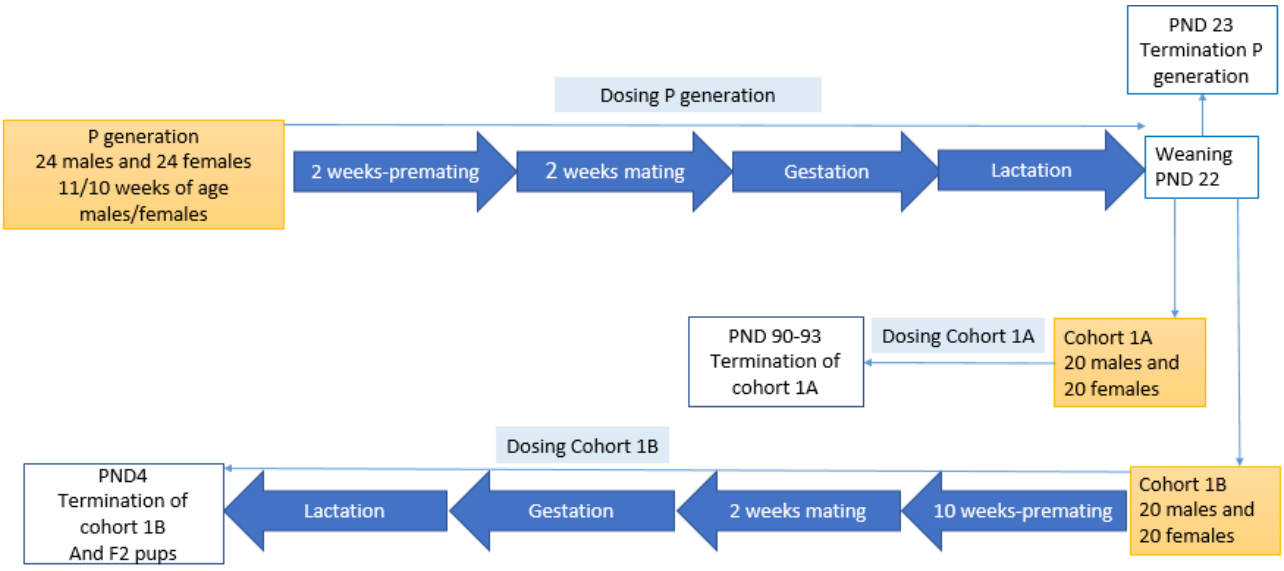


Figure 1: Study design of EOGRTS (2021)

Cohorts 1A = Reproductive/developmental toxicity testing

Cohorts 1B = Reproductive/developmental toxicity testing with extension to produce a F2 generation

Mortality

Unscheduled mortality was observed in P generation females at all doses (0, 2, 2, 6) (but not in the control group). There was no unscheduled death in males.

The mortality linked to reproductive troubles and the clinical signs observed in these females before sacrifice are detailed below**.** These females were sacrificed because of dead litter (pups born alive) and difficulties to deliver. The clinical signs were not the reason for the sacrifice.

At 5 mg/kg bw/day:

One female was prematurely euthanized on PND1 because of a dead litter. This female showed piloerection, hunched posture pallor of eyes/extremities and reddish **vaginal discharge** before sacrifice.

At 15 mg/kg bw/day:

One female was prematurely euthanized on Day 24 p.c. because of the **difficulties to deliver** associated with an abdomen increased in size (attributed to dystocia by the study author). This female showed severe clinical signs (piloerection, hunched posture, generalized pallor, **vaginal discharge**) before sacrifice.

At 25 mg/kg bw/day:

One female was prematurely euthanized on Day 23 p.c. because of the difficulties to deliver. The female showed cold to the touch, lateral recumbency, tonic seizures, clonic convulsion, dyspnea and exophthalmos before sacrifice.

Regarding cohort 1B, mortality was observed in females (3, 0, 3, 6), there was no unscheduled death in males.

Mortality linked to reproductive troubles and the clinical signs observed in these females before sacrifice are detailed below. These females were sacrificed because of dead litter, difficulties to deliver, no delivery or no pregnancy. The clinical signs were not the reasons for the sacrifice.

In the control group:

One female (pregnant) was euthanized for no delivery on Day 25 p.c.. This female did not showed difficulty to deliver. No clinical sign was observed in this female.

One female (not pregnant) was euthanized on Day 25 p.c.

At 15 mg/kg bw/day:

One female (not pregnant) was euthanized on Day 25 p.c.

At 25 mg/kg bw/day:

One female was prematurely euthanized for difficulties to deliver on Day 24 p.c. This female showed piloerection, hunched posture and abdominal breathing.

One female was prematurely euthanized for humane grounds on PND1. This female showed lateral recumbency and loss of balance.

Overall, DS notes that most of these females were sacrificed around the delivery date and/or shortly after and dead litter and/or difficulty to delivery were reported.

There were no unscheduled deaths in Cohort 1A animals that were not mated.

Body weight and food consumption

In P generation females, at 25 mg/kg bw/day, a significant increase in the mean body weight was observed at the end of the lactation period (+5.1% at PND 14 and +4.5% at PND 21). The mean body weight change was significantly increased between PND 7 to 14 (+21g vs +7g in controls). No change was noted in P generation males excepted for the 15 mg/kg bw/day group on the day 36 (+2.8%).

In cohort 1A females, at day 64, a trend toward an increased mean body weight was noted from 15 mg/kg bw/day onwards but was not dose-related (+5% at 15 mg/kg bw/day statistically significant, + 4.6% at 25 mg/kg bw/day not statistically significant). The mean body weight change was significantly increased between the days 57 to 64 at the mid dose only (+11g vs +7g in controls) and between the days 1 to 64 at the mid and high doses (+231 g and + 232g vs 216g in controls). No change was noted in cohort 1A males.

In cohort 1B females, during the premating and post-mating periods, from day 22 to day 71, a trend toward an increased mean body weight was noted from 15 mg/kg bw/day onwards but was not dose-related (<10%). The mean body weight gain was significantly increased at the high dose during the days 15 to 22 (+37g vs +31g), the days 22 to 29 (+26g vs +22g in controls), the days 43 to 50 (+17g vs +13g in controls), and from the mid dose for the days 1 to 71 (+238g, + 243g vs +218g in controls). During the pregnancy period, a significant increase in the mean body weight was observed in females from 15 mg/kg bw/day. The changes were not dose-related and the amplitude was generally lower than 10% expected for the day 20 p.c. (end of pregnancy) where the amplitude was +10.3%. During the lactation, at PND1, the mean body was also significantly increased (+10.5%) at the mid dose and at the high dose (+7.4%). At PND4, the mean body weight was also significantly increased at the high dose (+9.3%) at 15 mg/kg bw/day only. In cohort 1B males, a significant increase in the mean body weight was observed at 15 mg/kg bw/day only between the days 43 to 71 and at day 106 (<10%).

There was no change in food consumption excepted for the cohort 1B males where the food consumption was significantly increased at the mid dose during the days 22 to 29 (+10.3%), 36 to 43 (+9.1%), 43 to 50 (+9.1%), 57 to 64 (9.4%) and 99 to 106 (+9.7%).

Clinical signs

In terminated as scheduled animals several clinical signs were observed (please see annex I for detailed clinical signs).

In both generations and sexes, ptyalism was observed mainly after dosing.

Several P generation females of the high dose group displayed dyspnea, exophtalmos, hunched posture, lateral or ventral recumbency and piloerection. 6 females from the high dose group displayed clonic convulsion, locomotory difficulties, loss of balance, staggering gait and/or tonic seizures after treatment, between the days 42 to 62.

In cohort 1B, several males displayed hunched posture, lateral recumbency, piloerection and/or ventral recumbency in the high dose group and 10 males presented clonic/tonic convulsion, loss of balance and/or staggering gait after treatment between the days 90 to 108. In females, half closed eyes, lateral recumbency, and/or ventral recumbency were observed and 14 females presented clonic convulsion, loss of balance and/or staggering gait after treatment between days 94 to 100. These signs were observed mainly after dosing.

The clinical signs hypoactivity, clonic/tonic convulsion, locomotory difficulties, loss of balance, staggering gait and/or tonic seizures are suggestive of neurologic disorders and are observed in P generation females and males and females of

the cohort 1B. In P generation, these signs were observed at the end of the pregnancy period. These findings were transient and observed after dosing only (mainly not observed on next days after appearance). In cohort 1B, these signs were observed in both sexes from study day 94 after dosing for 1 to 3 days. **It has to be noted that these signs were observed in the high dose groups only in both generations.**

Effects on organs were observed in the P generation and cohort 1A:

In P generation, statistically significant increase in absolute and relative-to-body liver weights were recorded in males treated at ≥ 15 mg/kg bw/day (up to +11%; $p<0.01$) and correlated with the microscopic hepatocellular hypertrophy. A similar trend was observed in females without statistical significance (except in relative-to-body weights at 15 mg/kg bw/day, + 7%; $p<0.01$). In cohort 1A, statistically significant increase in absolute and relative-to-body liver weights were noted in females treated at ≥ 5 mg/kg/day and in relative-to-body weights in males treated at 25 mg/kg/day (up to +18%; $p<0.01$ or 0.05). This correlated with hepatocellular hypertrophy at microscopic examination. In cohort 1B, statistically significant increase in liver weights were recorded in females (absolute weight only from 15 mg/kg bw/day) and in males (absolute weight only at 15 mg/kg bw/day, relative-to-body weights from 15 mg/kg bw/day).

DS notes that two females developed an **adenocarcinoma in mammary gland,** one in the parental generation at 5 mg/kg bw/day and one in the cohort 1B at 25 mg/kg bw/day. There is no information to conclude if this effect is spontaneous or linked to the treatment. However, literature data report that, generally, mammary adenocarcinoma occurred spontaneously after 30 weeks of age (around 7.5 months) in female Sprague Dawley rats (Son et Gopinath, 2004). There are only a few case reports of mammary adenocarcinoma in Sprague Dawley females aged less than 20 weeks old (Oshi, 1995, Kuzutani 2012). One rat developed a mammary adenocarcinoma at 10 weeks (week 4 of the study) in the Oshi (1995) study and at 12 weeks old (10 days after reception of animals by the laboratory) in the Kuzutani (2012) study. In a study conducted by Chvedoff (1979), 2 of 300 (incidence: 0.67%) female rats that had nursed their offspring and 3 of 830 (0.36%) virgins had palpable mammary tumors before 6 month of age. In a study conducted by Son (2004), from 1264 Sprague Dawley females (controls of 20 carcinogenicity studies of Huntingdon, Cambridgeshire PE28 4HS, UK): only one developed a mammary adenocarcinoma (incidence: 0.08%) between week 21 to 30 of the study (29-38-week old corresponding to 7-9-month old). These studies points that the occurrence of mammary adenocarcinoma is not expected in an EOGRTS study where animals were about 4 months (parents) and 3.5 months (cohort 1B) of age.

Regarding thyroid hormones, in females of the P generation and at 25 mg/kg/day only, the mean concentration in T4 was high when compared to controls (+38%, $p<0.05$). This was not correlated with macroscopic and microspic finding. In cohort 1A males, T4 was low at 5 and 15 mg/kg bw/day (-5 and -8% respectively, no statistical significance) but not at 25 mg/kg bw/d (+3, no statistical significance). There was no dose response relationship and there were no macroscopic or microscopic test item-related findings in the thyroid glands.

Adverse effects on fertility and sexual function were recorded in parental generation and in cohorts 1A and 1B.

In the P generation, an **increase in the number of females with a gestation length of 23 to 24 days** was noted at the highest dose. However, there was no change in the mean duration of gestation (see table below). Among these females:

At 5 mg/kg bw/day, two females presented a gestation length of 24 days associated with a high pup mortality rate. One of the two females presented a total dead litter. Clinical signs such as piloerection, hunched posture pallor of eyes/extremities, reddish vaginal discharge were noted before the sacrifice. The other one had 1 live pup and 9 dead pups.

At 15 mg/kg bw/day, one female showed difficulties to deliver associated with an abdomen increased in size, **attributed to dystocia by the study author.** This female was sacrificed for difficulty to deliver on day 24 p.c..

At 25 mg/kg bw/day, there was an increase of females presenting a gestation length of 23 day when compared to the control group (7 females vs 3 in controls). Among them, one female presented difficulties to deliver in presence of clinical signs indicative of neurotoxicity (cold to the touch, lateral recumbency, tonic seizures, clonic convulsion, dyspnea and exophthalmos). Three other females had total dead litter and presented clinical signs (piloerection, hunched posture, pallor of eyes/extremities, ventral recumbency, staggering gait, exophthalmos and/or dyspnea before sacrifice).

Table 16: Duration of gestation in P females

Dose level (mg/kg bw/day)	0	5	15	25
Number of pregnant females	23 (95.8%)	22 (95.6%)	23 (95.8%)	24 (100%)
Females with 21 days of gestation	1	0	1	1

Females with 22 days of gestation	19	18	19	14
Females with 23 days of gestation	3	2	1 (dystocia)	7 (one with difficulty to deliver; 3 total litter loss)
Females with 24 days of gestation	0	2 (1 total litter loss)	0	0
Mean duration of gestation	22.1 ± 0.4	22.3 ± 0.6	22.0 ± 0.3	22.3 ± 0.6

An effect on gestation length was observed for cohort 1B. In this cohort, at 25 mg/kg bw/day, the mean duration of gestation length was statistically significantly higher than in controls (22.3 vs. 21.9 days in controls). There was 0/16, 0/20, 1/18 and 7/19 females with 23 days of gestation in control, low-, mid- and high-dose groups, respectively. No gestation period over 24 days was observed. Among these females:

At 15 mg/kg bw/day, two females presented dead litters and one of the two had clinical signs (piloerection, hunched posture, pallor, dyspnea). No adverse clinical signs before death were observed in the second female.

At 25 mg/kg/day, one female presented difficulties to deliver with clinical signs (piloerection, hunched posture and abdominal breathing). Two other females had dead litter and one of the two presented clinical signs (staggering gait and loss of balance before death).

Table 17: Duration of gestation in cohort 1B

Dose level (mg/kg bw/day)	**0**	**5**	**15**	**25**
Females with 23 days of gestation	0/16	0/20	1/18	7/19 (one with difficulty to deliver)
Mean duration of gestation	21.9 ± 0.3	21.9 ± 0.3	22.1 ± 0.2	22.3* ± 0.6

*: $p<0.05$

Overall, there is a consistent effect on the duration of gestation length among P and F1 generations that is statistically significant in F1 generation. The longer duration of gestation is often associated with dystocia, difficulty of deliver and total litter loss. This observation supports an effect on fertility.

Effects of DPG on estrous cycle were investigated.

No significant effect on the mean duration of the cycle was observed in P generation. The study report concluded that 79.2% of the females in the P generation had normal cycles in the controls. In application of OECD Guidance document n°106 for histologic evaluation of endocrine and reproductive tests in rodents (2009) part 5, only duration of the cycle was considered to establish whether a cycle is normal or not. When individual data were checked by DS, it is to be noted that only 50% of the controls in the P generation have regular estrous cyclicity based on the succession of at least 2 complete cycles during the 15 days of monitoring. The observed inappropriate sequence of phases creates uncertainties on the validity of calculation of the mean duration of cycles in the control group and on the conclusion of an absence of effect in experimental groups.

However, effects on the duration of phases of the estrous cycle were recorded (considering the number of days spent in each stage of the cycle). In P females, from 15 mg/kg bw/day, the length of the metestrus was statistically significantly lower than in controls with a dose-related effect. At 25 mg/kg bw/day, the length of the estrus was significantly increased. The same trend on the duration of phases was observed in the cohort 1A females without statistical significance (see table 19).

Table 18: Estrous cycle in P generation and cohort 1A

	P Generation females				**Cohort 1A females**			
Dose level (mg/kg bw/day)	0	5	15	25	0	5	15	25
Number of females examined	24	24	24	24	20	20	20	20

Mean percent of females cycling normally (%) – study report	79.2	95.8	91.3	95.8	100	95	100	100
Mean percent of females cycling normally (%) – DS recalculation	50	54,17	54,17	37,5	90	70	70	65
Mean percent of females with all stages (%)	100	100	100	95.8	100	95	100	90
Mean number of days of diestrus (± SD)	3.8 ± 1.9	4.0 ± 1.3	4.7 ± 2.3	4.5 ± 2.6	3.5 ± 1.7	4.3 ± 1.7	3.7 ± 1.5	4.1 ± 2.0
Mean number of days of proestrus (± SD)	2.8 ± 0.9	3.2 ± 0.7	3.3 ± 1.0	3.0 ± 1.0	3.9 ± 0.4	3.4 ± 0.9	3.7 ± 0.7	4.0 ± 0.2
Mean number of days of estrus (± SD)	3.5 ± 0.8	3.8 ± 0.6	3.4 ± 0.9	**4.3** ± 1.2**	4.0 ± 0.8	4.3 ± 0.6	4.3 ± 0.6	4.3 ± 0.4
Mean number of days of metestrus (± SD)	4.9 ± 1.5	4.0 ± 1.3	**3.7* ± 1.4**	**3.3# ± 1.7**	4.6 ± 1.5	4.1 ± 1.5	4.4 ± 1.6	3.8 ± 2.1
Mean number of cycles (± SD)	2.8 ± 0.8	3.0 ± 0.3	2.8 ± 0.5	2.8 ± 0.5	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Mean duration of cycles (days) (± SD)	4.5 ± 1.7	4.0 ± 0.1	4.4 ± 1.7	4.1 ± 0.3	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.0	4.0 ± 0.1

**: p<0.05, **: p<0.01, #: p<0.001*

SD: standard deviation

No significant effect was observed on the number of primordial follicles in P females. However, in cohort 1A, from 5 mg/kg bw/day, a non-statistically significant but dose related **decrease in the mean number of primordial follicles** was recorded. Despite a decrease of respectively 29 and 38% at the mid- and highest doses, the statistically significance was not reached, this can be explained by high standard deviation in the control. Adequate historical control would have been useful to better interpret this result but were not available. Moreover the analysis of the second ovary was not performed. It is stated in the OECD TG 151 (guidance document supporting OECD test guideline 443 on the extended one generation reproductive toxicity test) the following regarding follicle count : "*Where high dose group counts are less than 85% of control but are not statistically significantly different, it is recommended that the second ovary be processed, with an evaluation of an increased number of sections (e.g. 50 sections or approximately 5% of the ovary) to establish if the intergroup difference is statistically significant. Animals in Cohort 1B may be used if it is considered that evaluating additional ovaries could aid in clarifying the results*". In conclusion, as the decrease in the number of primordial follicle count is higher than 25% and is clearly dose-related, this points to a fertility effect, despite the lack of statistically significance.

Table 19: Follicle count in cohort 1A females

	Doses (mg/kg bw/d)			
	0	5	15	25
Primordial follicles (mean ± SD)	9.31 ± 6.09	8.68 ± 6.77	6.65 ± 3.07	5.75 ± 4.30
%/control group		-7	-29	-38
Corpora lutea (mean ± SD)	19 ± 7.36	_	_	18.6 ± 6.52
%/control group				-2

SD: standard deviation

There was no effect on sperm parameters in both generation.

In conclusion, effects on sexual function are observed in the EOGRTS. Effects on the gestation duration, including dystocia and difficulties to deliver, a decrease in follicle count, and indications of effects on the estrous cycle are recorded. It has to be noted that developmental toxicity is reported in this study and described in section 10.8.4.

DS notes that a classification Repr. 1B, FD - H360 is also proposed by the study authors of the EOGRTS.

2) In addition, a study conducted according to OECD TG 421 (Reproduction/Developmental Toxicity Screening Test) (2010) is available. The dose tested were 5, 15 and 25 mg/kg bw/day by gavage. 10 rats Sprague-Dawley/ sex/dose were used. At the high dose, suggestive signs of neurotoxicity (one male with staggering gait, one female with locomotory difficulties, one male with lateral decubitis and one male and one female with mydriasis) were occasionally

recorded. There was no effect of treatment with DPG on organ weights, macroscopic post-mortem examination and microscopic examination at any dose-level. There was no also effect on reproductive parameters. However, it can be noted that the OECD 421 guideline study is a screening assay. According to the OECD guideline, this protocol is only "*designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition*". Statistical analysis is also rather limited since only 10 animals of both sexes were included. It has to be noted that developmental toxicity is reported in this study and described in section 10.8.5.

3) In the NTP (1995) 13-week sub-chronic toxicity study, F344/N rats and B6C3F mice (n=10/sex/group) were respectively exposed in feed to doses of 0, 500, 750, and 1500 ppm and 0, 250, 750 and 3000 ppm (equivalent to 0, 17/17, 32/32, 49/50, 100/95, 181/184 mg/kg bw/day in male and female rats respectively and equivalent to 0, 38/46, 75/93, 114/141, 231/285, 457/577 mg/kg bw/d in male and female mice, respectively) (NTP, 1995). Effects of DPG on reproductive organs (weight and histology) were investigated.

The results in F344/N rats are the following:

A high mortality was reported at 3000 ppm: 4/10 males survived and there was 100% mortality for females at the end of the study. Thus, results from this group are not detailed thereafter.

At 1500 ppm, a marked decrease of the mean body weight (21% of controls for males and 14% of controls for females) and average food consumption was observed in males and females. At 750 ppm, only a slight decrease of body weight was seen (< 10%).

At 1500 ppm, from week 2, some rats presented signs possibly linked to neurological disorders as hypoactivity, convulsions and seizures. Abnormal posture characterized as staggering was noted in most males and females. Other clinical signs observed in this group included hyperactivity, hunched posture, ptosis, ataxia, dyspnea, and bristly hair.

Changes in hematology parameters were seen in rats receiving 1500 ppm. At Day 5, a mild polycythemia occurred in females. This was indicated by greater erythrocyte counts, hematocrit values, and hemoglobin concentrations than controls and would be consistent with a relative polycythemia related to dehydration and hemoconcentration. There were slightly lower reticulocyte counts at Day 5 in females.

Regarding adverse effects on fertility:

From 750 ppm, uterine hypoplasia was seen in females with greater severity and incidence compared to controls. However, detailed incidence was not available in the article. A statistically s**ignificant increase in the length of the estrous cycle** was also noted at 750 ppm. A non-significant increase of the diestrus and a non-significant decrease of the estrus were noted at 750 ppm (calculated in terms of percentages of the cycle). At 1500 ppm, the changes were lower than at 750 ppm and not statiscally significant (see table below).

Table 20: Estrous cycle in F344/N Rats

Study Parameters	0 ppm	500 ppm	750 ppm	1,500 ppm
n	10	10	10	9
Necropsy body weight (g)	204 ± 4	195 ± 3	191 ± 3**	177 ± 2**[2]
Estrous cycle length (days)	4.95 ± 0.05	5.00 ± 0.00	6.00 ± 0.33**	5.67 ± 0.44[3]
Estrous stages (% of cycle)				
Diestrus	38.2	38.2	44.5	41.8
Proestrus	14.5	19.1	16.4	15.5
Estrus	30.0	24.5	24.5	22.7
Metestrus	17.3	18.2	14.5	20.0

[1] Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
[2] n=10.
[3] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
** Significantly different (P≤0.01) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.

In the 1500 ppm dose level group, males displayed a **significant reduction in sperm motility**.

Table 21: Summary of reproductive organ toxicity and spermatogenesis in males F344/N Rats

Study Parameters	0 ppm	500 ppm	750 ppm	1,500 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	374 ± 6	358 ± 5	347 ± 4**	300 ± 7**
Left epididymis	0.480 ± 0.010	0.482 ± 0.009	0.496 ± 0.010	0.464 ± 0.008
Left cauda epididymis	0.196 ± 0.005	0.199 ± 0.005	0.198 ± 0.006	0.186 ± 0.004
Left testis	1.55 ± 0.02	1.56 ± 0.02	1.51 ± 0.03	1.48 ± 0.02
Spermatid measurements				
Spermatid heads (10^7/g testis)	11.09 ± 0.68	10.46 ± 0.29	10.91 ± 0.44	10.84 ± 0.41
Spermatid heads (10^7/testis)	17.14 ± 0.98	16.31 ± 0.40	16.50 ± 0.63	16.00 ± 0.54
Spermatid count (mean/10^{-4}mL suspension)	85.70 ± 4.90	81.55 ± 1.98	82.48 ± 3.13	80.00 ± 2.71
Epididymal spermatozoal measurements				
Motility (%)	94.76 ± 1.42	92.30 ± 1.76	87.34 ± 3.75	83.69 ± 2.77**
Concentration (10^6/g caudal epididymal tissue)	331.5 ± 33.2	259.0 ± 29.6[2]	290.5 ± 26.3	598.2 ± 139

[1] Data presented as mean ± standard error. Differences from the control group for epididymal, cauda epididymal, and testis weights, spermatid measurements, and sperm concentration are not significant by Dunn's test.
[2] n=9.
** Significantly different (P≤0.01) from the control group by Dunnett's test (necropsy body weight only) or Shirley's test.

The results in B6C3F mice are the following:

No mortality was observed at all doses. At 750 ppm and 3000 ppm, the mean body weight of both sexes was lower compared to controls (from -7% to -20%). From 750 ppm, females presented a thin appearance that was also seen in males in the 3000 ppm exposure group. There was no difference on the mean feed consumption at all doses during the study**.**

In males, at 3000 ppm, **greater number of spermatid heads and lower sperm motility** were noted.

Table 22: Reproductive **Tissue Evaluations in males B6C3F mice**

Study Parameters	0 ppm	250 ppm	750 ppm	3,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	35.9 ± 0.6	34.7 ± 0.7	33.8 ± 0.6*	29.1 ± 0.3**
Left epididymis	0.055 ± 0.002	0.061 ± 0.001	0.056 ± 0.002	0.054 ± 0.002
Left cauda epididymis	0.022 ± 0.001	0.024 ± 0.001	0.022 ± 0.001	0.021 ± 0.001
Left testis	0.123 ± 0.004	0.125 ± 0.003	0.125 ± 0.003	0.117 ± 0.003
Spermatid measurements				
Spermatid heads (10^7/g testis)	17.10 ± 0.78	17.33 ± 0.94	18.67 ± 0.81	20.52 ± 1.04*
Spermatid heads (10^7/testis)	2.09 ± 0.09	2.17 ± 0.12	2.31 ± 0.07	2.37 ± 0.08
Spermatid count (mean/10^{-4}mL suspension)	65.28 ± 2.71	67.78 ± 3.83	72.20 ± 2.24	74.18 ± 2.45
Epididymal spermatozoal measurements				
Motility (%)	84.84 ± 3.43[2]	82.86 ± 4.99	78.86 ± 8.08	51.56 ± 11.77*
Concentration (10^6/g caudal epididymal tissue)	1107 ± 234	791 ± 186	904 ± 271	676 ± 201

[1] Data presented as mean ± standard error. Differences from the control group for epididymal, cauda epididymal, and testis weights, spermatid heads per testis, spermatid count, and sperm concentration are not significant by Dunn's test.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.
** Significantly different (P≤0.01) from the control group by Dunnett's test.

In females exposed to 3000 ppm, a significant **increase in the estrous length** was noted with a non-significant but dose-related decrease of the diestrus (calculated in terms of percentages of the cycle).

Table 23: Estrous cycle in B6C3F mice

Study Parameters	0 ppm	250 ppm	750 ppm	3,000 ppm
n	10	10	10	10
Necropsy body weight (g)	29.3 ± 0.7	28.5 ± 0.6	27.4 ± 0.5*	22.9 ± 0.2**
Estrous cycle length (days)	4.30 ± 0.13	4.45 ± 0.16	4.10 ± 0.07	5.15 ± 0.27*
Estrous stages (% of cycle)				
Diestrus	33.3	26.7	30.0	28.3
Proestrus	20.8	21.7	20.0	20.0
Estrus	26.7	35.8	29.2	39.2
Metestrus	19.2	15.8	20.8	12.5

[1] Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
* Significantly different (P≤0.05) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.
** Significantly different (P≤0.01) from the control group by Dunnett's test.

In summary, in the 90-day study, consistent findings were observed in reproductive parameters. Increased length of estrous cycle, altered sperm motility and decreased sperm motility were reported in both species. F344/N rats seems more sensitive than B6C3F mice to DPG toxicity with effects reported at lower doses.

4) A non-guideline study (unpublished study report, 1989, Koëter *et al*. 1992) investigated the effects of DPG on testicular toxicity and male fertility, and the repercussions of these effects on embryogenesis. Male Albino CD1 mice were exposed by gavage to doses of 0.06, 0.25, 1, 4 and 16 mg/kg bw/day for 8 weeks. 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 12-13 selected males from the control and 16 mg/kg bw/day dose group. The remaining 11 males in the control, 4 and 16 mg/kg bw/d groups were mated with non-dosed females.

No differences were found between control and dosed groups in body weight gain during the dosing period, macroscopic observations and organ weights at necropsy**.** Some animals presented minor piloerection (including in control group) and low general body condition (one animal in control group also: probably due to a dosing error).

Sperm abnormalities were noted in the highest exposure group with a **statistically significant increase of sperm with normal head but folded tails**. No effect was reported in the other sperm parameters (see table 26). This effect is difficult to interpret since sperm parameters were not evaluated in the lower dose groups. No effect was reported in the mating index, female and male fertility index and fecundity index. However, DS notes that sperm should be drastically altered to significantly impact fertility in rodents. Regarding the overall interpretation of the study, it is noted that the values in the control group were low (below 78%). Therefore, it is not possible to conclude about the effects of DPG on fertility and sexual function from this study.

Table 24: Number of morphologically abnormal sperm cells in male albino CD1 mice

No. of animal	1,3-Diphenylguanidine (mg/kg body wt per day)	
	0	16.0
	13	12
Incidence of sperm with:		
Absent hook	24 ± 12	18 ± 20
Banana-like head	1 ± 2	1 ± 1
Amorphous head	36 ± 32	30 ± 39
Folded tail and normal head	20 ± 12	50 ± 24*
Folded tail and abnormal head	13 ± 11	8 ± 5
Twin tail	1 ± 1	1 ± 1
Total number of abnormal sperm cells	95 ± 45	108 ± 59

5) Bempong *et al*. (1983) investigated the effects of DPG on seminal cytology, testicular development and fertility of C57BL/6JxDBA2 mice (males and females) and male Syrian hamsters. Two experiments seem to have been conducted.

To study sperm morphology, 3 groups of mice or hamsters received the following concentrations of DPG *ad libitum*: 0 (solvent control), 4.0, or 8.0 mg/kg bw/d for a period up to 15 weeks (the exposure period is less clear for hamsters but they were exposed for a minimum of 80 days).
Weighting, quantitative sperm analysis and histological analysis of the testes were performed in mice.

Regarding histopathological data, in the high dose group, the parietal peritoneum was saturated with fatty tissues and the mesentery and the greater omentum showed the greatest evidence of fatty tissue accumulation compared to the control group.

In the control hybrid mice, abnormal sperm morphology ranged from 1.8 to 5.3% with a mean of 3.5%; in the control inbred hamsters, a range of 2.0 to 13.6% with a mean of 9.2% was observed. In treated mice, a nonlinear increase in the frequency of sperm abnormalities was observed. The incidence of DPG-induced abnormal sperm in mice exposed to 4 mg/kg bw/day ranged from 16.2 to 42.4% and from 38.6 to 75.1% in the 8.0 mg/kg bw/day group. In the inbred Syrian hamsters, fluctuations in the levels of DPG-induced sperm abnormalities were observed in all preparations from day 30 to day 75. From days 75 to the end of the experiment, steady increases in the frequency of anomalous sperm were observed. A significant decrease in sperm count and testes weight from week 5, and irregularly shaped seminiferous tubules in mice were observed.

In a reproductive study, 10-week-old male mice were exposed to 0 (0.025% acetic acid), 4.0 or 8.0 mg/kg bw/day DPG prepared in acetic acid at a final concentration of 0.025%. Exposure was ad libitum and the duration of exposure was 90 days. After 7 days of exposure, the animals were mated at weekly intervals to 12-wk-old virgin untreated females. On day 13 of pregnancy the female animals were sacrificed and the number of implants and frequencies of early (moles) and late fetal lethality per pregnancy were determined. The fertility index, expressed as the ratio of the number of pregnant females to the number of females mated in a specified mating group, was determined. The purity of DPG is not reported but the author indicated that some impurities were present in the test material (without further information). No females were treated in the studies.

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. See table below:

Table 25: Effects of DPG on reproduction in mice

Week	Dose (mg/kg bw/day)	Number of pregnant mice/ Total number of female mated	Number of implants		Dead foetuses per pregnancy	
			Per females	Total	Early	Late
1	0	20/20	11.8	236	0.54	0.35
	4	18/20	10.3	186	0.44	0.39
	8	12/20	9.8	118	0.67	0.33
3	0	20/19	12.4	248	0.40	0.35
	4	16/20	11.1	178	0.05	0.44
	8	14/20	10.6	148	0.64	0.43
5	0	19/20	12.2	232	0.53	0.37
	4	16/20	10.9	175	0.75	0.69
	8	11/20	9.3	103	0.91	0.73
7	0	20/20	11.3	236	0.45	0.25
	4	17/20	10.4	177	1.35	1.06
	8	8/20	9.6	77	2.13	1.88

Significant reduction in the number of implants per pregnancy in mice were also observed at week 12. At 4 mg/kg bw/day, there was 7.5 ±2.3 and at 8 mg/kg bw/day, there was 5.5 ±1.9 implants per pregnancy. However, there is no information on number of implants per pregnancy in control at week 12.

However, the reliability of the study was questioned due to the unknown purity of the test material and the presence of impurities according to the author (Bempong, Jan. 21, 1987, personnal communication reported by the SIDS, 2002). Indeed, aniline a major product of thermal decomposition of DPG could be present. Aniline present a harmonized classification including Carc. 2; H351: suspected of causing cancer Muta. 2; H341: suspected of causing genetic defects. STOT RE 1; H372**: Causes damage to organs through prolonged or repeated exposure. The nature of the impurities is not known. Moreover, there are no data on general toxicity and the number of animals is not reported. Besides, the protocol is not clearly described.

10.8.3 Comparison with the CLP criteria

For classification on sexual fertility and reproduction the CLP criteria have been followed.

Adverse effects on sexual function and fertility are defined as "*any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems*".

Known human reproductive toxicant: "*the classification of a substance in this Category 1A is largely based on evidence from humans*"

As there is no human study available, a classification of DPG in category 1A is not warranted.

Presumed human reproductive toxicant: "*the classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."*

Suspected human reproductive toxicant: "*substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."*

During the TC C&L discussion in 1997, a classification in category 2 for fertility was agreed for DPG. A well-conducted EOGRTS and a screening reproductive toxicity study are now available since then.

The OECD TG 421 (Reproduction/Developmental Toxicity Screening Test) study does not highlight effects on sexual function and fertility. The dose tested were the same as those in the EOGRTS (5, 15 and 25 mg/kg bw/day). However, it can be noted that the OECD 421 guideline study is a screening assay. According to the OECD guideline, this protocol is only "*designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition*". Only 10 animals of both sexes were included. Therefore, the statistical analysis is limited. Thus, the sensitivity of this study is clearly lower than for the EOGRTS.

Effects on reproductive function were observed in the key EOGRTS (2021) in Sprague Dawley rats in parental generation and F1 generation as well as in the NTP 90-day studies in mice and rats.

In relation to effects observed in females, effects on gestation length were observed in both generation of the EOGRTS. In the parental generation, a tendency toward an increase in the number of dams with an increased gestation length was noted (3-4-1-7 females with a gestation duration of 23-24 days in the control group and 5, 15 or 25 mg/kg bw/day group, respectively). Statistically significance was not reached in the mean duration of gestation. In cohort 1B, the mean duration of gestation length was statistically significantly higher than in controls at the high dose. In both generation, effects on gestation duration were associated in some females with **dystocia** and **difficulties to deliver**. Moreover, it is noted that 0-2-2-6 P0 females (for control, low, mid and high dose, respectively) and 3-0-3-6 F1 females (for control, low, mid and high dose, respectively) were sacrificed around the delivery date and shortly after. Dystocia, dead litter and/or difficulty to deliver were reported in the treated groups. This mortality suggests a direct effect on fertility.

A non-statistically significant dose-related **decrease in the number of primordial follicles** was also reported in the cohort 1A of the EOGRTS. The decrease was higher than -25% from 15 mg/kg bw/d but the standard deviation of the different values were very high. The analysis of the second ovary was not performed. As the decrease of the number of primordial follicle count is higher than 25% and is clearly dose-related, this points to a fertility effect, despite the lack of statistically significance.

Regarding general systemic toxicity in the EOGRTS, clinical signs related to neurotoxicity were reported in both generations at 25 mg/kg bw/day. Some treated females were sacrificed but most of them occurred around the delivery date and shortly after, with signs of dystocia, difficulties to delivery and/or dead litter. Body weight was not affected. **The effects observed on reproductive function in females in this study are not considered secondary to general toxicity as these effects also occurred at lower dose levels without overt toxicity (5 and 15 mg/kg bw/day).**

Although not fully consistent, effects on the estrous cycle were reported in both the EOGRTS and the 90-day NTP toxicity studies. In the P generation from the EOGRTS, from 15 mg/kg bw/day, a significant dose-related decrease in the mean number of days of metestrus ($p<0.05$ for mid dose, $p<0.001$ for high dose) was observed, compared to controls. An increase of the mean number of days of estrus at 25 mg/kg bw/day ($p<0.01$) was also noted. The mean duration of cycles was not significantly affected. However, an inappropriate sequence of phases in many control females was noted by the DS and creates uncertainties on the validity of calculation of the mean duration of cycles in the control group and on the possibility to conclude on an absence of effect in experimental groups in the P generation. Estrous cycle disturbance was also noted in the 90-day repeated dose NTP studies. The length of estrous cycle was prolonged from 750 ppm in F344/N Rats and at 3000 ppm in B6C3F mice. A non-significant increase of the diestrus and a non-significant decrease of the estrus were noted at 750 ppm in rats (calculated in terms of percentages of the cycle). In mice, a non-significant decrease but dose-related of the diestrus was observed at 3000 ppm.

Differences between the phases affected in the estrous cycle can be explained by the differences in species and strains used, and the different study methodology used. Nevertheless, effects on the estrous cycle are observed in two studies and two different strains and species.

Regarding systemic toxicity in the 90-day study in rats, overt toxicity was reported at 3000 ppm. All females and 6 males died. No mortality occurred in other dose groups. At 1500 ppm, there are some decreased body weight (14% of controls for females), clinical signs including neurological disorders and changes in haematological parameters. At 750 ppm, only a slight decrease of body weight was seen (< 10%). Organ weights for groups receiving 750 ppm or greater were significantly lower than those of the controls.

Regarding systemic toxicity in the 90-day study in mice, no mortality was observed. At 750 ppm and 3000 ppm, the mean body weight of females was lower compared to controls (from -12 to -20%). Effects on estrous length occurred at 3000 ppm in presence of a decreased in BW of 22% compared to controls. No clear conclusion can be drawn. However, similar findings are observed in rats and cannot be considered as secondary to overt toxicity in the EOGRTS and in the 90-day rat study.

Besides in the NTP study in female rats, uterine hypoplasia was seen with greater severity and incidence in the three highest dose groups (from 750 ppm equivalent to about 50 mg/kg bw/day). This effect was attributed by the NTP to a lower feed consumption and poor body conditions. However, the DS note that the food consumption was decreased of only 9% and the body weight of <10%.

From the EOGRTS, DS notes the observation of 2 adenocarcinoma in the mammary gland (1 in the parental generation at 5 mg/kg bw/day and 1 in the 1B generation at 25 mg/kg bw/day) taking into account the rarity of this pathology in rodent younger than 30 weeks of age. These findings provides supportive indications toward an action of DPG on female sexual function.

Overall, regarding female fertility and taking into account the EOGRTS and the NTP studies, there is evidence that DPG causes disruption of the estrous cycle. An increase in gestation length was also observed in the EOGRTS associated with dystocia, difficulties to deliver and dead litters, as well as a biologically but not statistically significant decrease in the number of primordial follicles in cohort 1A.

In relation to effects observed in males, no effects on reproductive parameters are reported in the EOGRTS and in the OECD 421 study. However, effects on male reproductive parameters in F344/N rats and B6C3F mice were observed in the NTP study.

In rats, significant **reduction in sperm motility** was observed in the 1500 ppm dose level group (equivalent to about 100 mg/kg bw/day). In mice, at 3000 ppm (equivalent to 457 mg/kg bw/d), a **greater number of spermatid heads and lower sperm motility** were observed. However, it is difficult to conclude whether these effects are due to the reprotoxicity of the test substance or to the decrease of body weight observed in these two species tested. At 1500 ppm, a marked decrease of the mean body weight (-21%) and of average food consumption (-14%) was observed in male rats. In mice, the mean body weights of males was lower than those of the control groups especially during the latter

part of the study and reached -19% at 3000 ppm. In male rats, changes in hematology parameters were seen at 1500 ppm. At 1500 ppm, some male rats presented signs possibly linked to neurological disorders as hypoactivity, convulsions and seizures. Abnormal posture characterized as staggering was noted in most males. Other clinical signs observed in this group included hyperactivity, hunched posture, ptosis, ataxia, dyspnea, and bristly hair.

Similar effects on sperm parameters were reported in other studies of low reliability. **Sperm abnormality as sperm with normal head but folded tails** was observed in albino CD1 mice treated with 16 mg/kg bw/day of DPG for 8 weeks (unpublished study report, 1989, Koëter et al. 1992). Other sperm parameters were not affected nor fertility performance. DS notes that the reproductive indices were already low in the control group. No significant general toxicity was observed in this study.

Finally, the study conducted by Bempong et al. (1983) in male and female C57BL/6JxDBA2 mice and male Syrian hamsters showed a time and dose-dependent increase in the **sperm abnormalities** (not statistically significant), as well as a **decrease in sperm count** (statistically significant) **and testes weight** (statistically significant) **and irregularly shaped seminiferous tubules** in mice from 4 mg/kg bw/day exposed for 15 weeks. There was a dose-dependent **decrease of the fertility index and the number of implants per pregnant** female mice (statistically significant from the 5th week). This study is not considered as reliable due to methodological deficiencies (no information on the number of animals, on purity and few information on general toxicity).

The absence of effects on the sperm in the EOGRTS and the OECD 421 study may be explained by the differences in dose levels, protocol of administration (gavage versus diet) and species tested (rats, mice or hamsters). In particular, in the NTP study, the effects on the sperm occurred in rats exposed to doses equals to higher than 100 mg/kg bw/day via the diet although the EOGRTS and the OECD 421 study used doses up to 25 mg/kg bw/day by gavage. Sperm alterations were also reported in mice and hamsters.

Overall, the studies available show adverse effects on sexual function and fertility of females such as effects on the estrous cycle in different studies in different strains and species, longer duration of gestation, dystocia and difficulties to deliver as well as a decrease in the number of primordial follicle in the EOGRTS. In this key study, it is possible to separate these effects from other toxic effects as they appear below the dose causing general toxicity. In the NTP study these effects on cyclicity occurred from 750 ppm (about 50 mg/kg bw/day) where general toxicity was low in rats (limited decrease of body weight (< 10%)) and from 1500 ppm in mice but were associated to a decrease of body weight of 13%. Therefore, these effects cannot be considered as secondary consequences to general toxicity in rats.

Effects on sperm parameters such as sperm abnormalities and decrease of sperm motility are reported in different repeated dose toxicity studies (but not in the EOGRTS) in different species. These effects occurred at high dose in presence of some general toxicity and are considered as supportive evidence.

Based on the data, there is clear evidence of adverse effects on sexual function and fertility and classification as Repr. 1B (H360F) is proposed.

10.8.4 Adverse effects in development

Table 26 Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD TG 443 (Cohorts 1A, and 1B with extension to cohort F2) GLP Rats, Sprague-	DPG Purity: 98.7% Vehicle: 0.5% methylcellulose in drinking water treated by reverse osmosis Dose levels: 0, 5, 15, 25	General toxicity and effects on sexual function and fertility are presented in table 14 of section 10.8.1. **<u>Effects on development of the offspring:</u>** **Post-implantations loss:** <u>P generation:</u> *25 mg/kg bw/day :* Statistically significant increase in the mean percent	Unpublished study report, 2021

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Dawley Males and females Number of animals per sex per dose: - Parental generation: 24 per sex per dose - F1: 20 per sex per dose (Cohorts 1A and 1B)	mg/kg bw/day Administration: once daily by gavage Duration of exposure: - P males: at least 10 weeks of treatment - P females: at least 8 to 10 weeks of treatment - Cohort 1A: both males and females: from weaning post-natal day (PND 22) until euthanasia (from Day 90 p.p., to Day 93 p.p. maximum). - Cohort 1B: In males: from weaning (PND 22) for at least 10 weeks before mating, during the mating period (up to 2 weeks), and after euthanasia of F2 pups (on PND 4). In females: from weaning (PND 22) for at least 10 weeks before mating, during the mating period (up to 2 weeks), during gestation, during lactation until PND 4 inclusive until euthanasia for females with no delivery (26 days after the last day of the mating period).	of post-implantation loss (27.1% vs 15.6% in controls). In cohort 1B: *From 5 mg/kg bw/day*: Non-statistically significant but dose-related increase in the mean percent of post-implantation loss (18.6% at 5 mg/kg bw/d, 22.1% at 15 mg/kg bw/d, 24.6% at 25 mg/kg bw/d vs 13.6% in controls). **Dead litters (pups born alive):** P generation: *5 mg/kg bw/day:* 1/24 female presented reddish vaginal discharge associated with dead litter. *15 mg/kg bw/day*: 1/24 female presented dead litter. *25 mg/kg bw/day:* 3/24 females presented dead litters. Cohort 1B: *15 mg/kg bw/day :* 2/20 females had dead litter. *25 mg/kg bw/day :* 2/21 females had dead litters. **Pups survival and clinical signs:** F1 pups: At PND 1: Dose-related tendency towards lower number of viable F1 pups: down to 9.3 live pups at 25 mg/kg bw/d vs. 12.0 for controls (not statistically significant). Live birth index: 95.1%, 87.2%, 87.9% and 72.0% for controls, low, mid and high dose respectively (not statistically significant). Between PND 1 to 4: Mortality of F1 pups: dose-related statistically significant increased: 14 pups (4.9%) for controls, 35 pups (12.5%) at 5 mg/kg bw/day, 47 pups (16.2%) at 15 mg/kg bw/day, 82 (32.3%) at 25 mg/kg bw/day. Number of litter affected statistically	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		significant at 25 mg/kg bw/day. PND 4: Decreased viability index before culling: 94.8% for controls, 90.1% at 5 mg/kg bw/day, 85.0% at 15 mg/kg bw/day, 74.4% at 25 mg/kg bw/day (not statistically significant). Clinical signs: From the low dose, some pups at PND, presented findings as scab on nose, emaciated appearance, generalized pallor, absence of milk in stomach, hematoma on head and cold of the touch. <u>F2 pups:</u> *From 15 mg/kg bw/day:* On PND1: Decrease in the live birth index (not statistically significant): 100% for controls, 84.1% at 15 mg/kg bw/day, 67.5% at 25 mg/kg bw/day. Between PDN 1 and 4: Dose-related statistically significant increase in mortality of F2 pups: 2 pups (1.0%) for controls, 8 (3.4%) pups at 5 mg/kg bw/day, 56 pups (24.2%) at 15 mg/kg bw/day, 73 (37.4%) at 25 mg/kg bw/day. On PND4: Tendency toward a decreased viability index F2 pups on PND4 (not statistically significant): 99.0% for controls, 97.4% at 5 mg/kg bw/day, 80.6% at 15 mg/kg bw/day, 56.6% at 25 mg/kg bw/day. *25 mg/kg bw/day:* Statistically significant decrease in the mean number of live F2 pups at PND 1 compared to controls (7.8 vs 11.4 in controls) Clinical signs: At PND1 and during lactation: Increase in pups with finding from 15 mg/kg/ bw/day. Some F2 pups in test groups presented clinical signs as cold to the touch, emaciated appearance and hypoactivity. In F2 found dead pups: Dose related increase in autolysis from the low dose. Some pups presented absence of milk in the stomach. **Offspring body weight :**	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Lactating F1 pups: *25 mg/kg bw/day:* Body weight lower at PND 1 only (males: 7.7g vs 8.4g for controls and females: 7.3g vs 7.8g for controls). Statistically significant in males. Body weights return to control values thereafter. Cohort 1A: no adverse effect on body weight and body weight change. Cohort 1B: no adverse effect on body weight and body weight change during premating, post-mating, pregnancy and lactation periods. Lactating F2 pups: body weight lower at PND 1 (males: 7.3g vs 8.1g for controls and females: 7.2g vs 7.7g in controls). Statistically significant in males. At PND 4, there is a tendency toward a return to control values and differences were not statistically significant. **Immunology findings (cohort 1A):** *15 mg/kg bw/day:* In males: when compared with controls, statistically significant increase of NK cells both in terms of relative (4.8 vs. 3.4%) and absolute counts (11397 cells/mg of spleen compared to 7001 cells/mg of spleen) and, in terms of relative counts, a decrease of T cells (36.6 vs. 42.5%). *25 mg/kg bw/day:* In males: no significant difference was observed. In females: when compared with controls, statistically significant decrease of NK cells, both in terms of relative (2.6 vs. 3.8% of splenocytes) and absolute counts (5879 vs. 10713 cells/mg of spleen).	
OECD TG No. 421 GLP Rats, Sprague-Dawley Males and females 10 animals per sex per group	DPG Purity: 98.9% Vehicle: 0.5% methylcellulose aqueous solution Dose levels: 0, 5, 15, 25 mg/kg bw/day Administration: once daily by gavage Duration of exposure: In males:	General toxicity and effects on sexual function and fertility are presented in table 14 of section 10.8.1. **Effects on development of the offspring:** **Unscheduled death of dams due to dead litters:** *Control* One female sacrificed following the death of its litter on day 3 or 4 of lactation *5 mg/kg bw/day:* One female sacrificed following the death of its litter on day 3 or 4 of lactation. *25 mg/kg bw/day:*	Unpublished study report, 2010

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	- 4 weeks before pairing, - during the pairing period (up to 17 days), - until sacrifice of the females (at least 10 weeks in total). In females: - 4 weeks before pairing, - during the mating period (up to 17 days), - during gestation, -during lactation until day 4 post-partum inclusive (or until sacrifice), - until sacrifice for non-pregnant females.	One female sacrificed following the death of its litter on day 3 or 4 of lactation. No relevant clinical signs, macroscopic or microscopic findings observed in these 3 females. **Body weight and clinicals signs in F1 pups:** *At 5 and 15 mg/kg/day:* No effect on body weight. *From 15 mg/kg bw/day :* Increased incidence of hypoactivity (12 pups from one litter at 15 mg/kg/day and three pups from one litter at 25 mg/kg/day; all three pups noted cold to the touch, vs. none in control pups). *25 mg/kg bw/day:* Not statistically significant decreased body weight gain during the lactation period compared to controls (-29% and -31%, for males and females respectively) and lower mean body weights on day 5 of lactation (-14% and -15%, for males and females respectively).	
Oral testicular toxicity and male fertility study No guideline, GLP Mice, albino, CD1 180 males, 130 females 25 males per dose 22 females for the group doses: 0, 4 and 16 mg/kg bw/day.	DPG Purity: 99.9% Vehicle: acetic acid in demineralised water Dose levels : 0, 0.06, 0.25, 1, 4 and 16 mg/kg bw/ day daily by gavage Duration of exposure: 8 weeks premating period for males. 11 selected males of the groups exposed to 0, 4 and 16 mg/kg/day mated with untreated females.	General toxicity and adverse effects on fertility and sexual function developed in table 14, in section 10.8.1 **Effects on development:** **Implantation disturbances:** Some increase observed in dosed groups in the numbers of early and late resorptions and dead fetuses, but according to the author, all values relating to post implantation loss were low in all groups when compared to historical control values for this strain of mice (data not available). **Gross necropsy:** Litter data did not reveal any treatment related effect.	Unpublished study report, 1989 Koëter et al. (1992) Reliability 3 (not reliable) Poor reproductive performance in the controls
Range-finding teratogenicity study GLP Rats, Sprague Dawley; females 5 animals/group	DPG Purity not specified Vehicle : aqueous methylcellulose 0.5% Dose levels : 0, 10, 50, 100, 150 and 200 mg/kg bw/day Administration : daily by	**General toxicity in dams:** **Mortality:** *At 100, 150 and 200 mg/kg bw/day :* Excessive maternal toxicity: all animals died (except one in the 100 mg/kg/day group). **Clinical signs:**	Unpublished study report, 1985 Reliability : 2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	gavage Exposure: day 6 to 15 of gestation	*At 50 mg/kg bw/day:* Lethargic behaviour and ataxia: 4 animals Prostrate behaviour and tachypnea: 1 animal Dried red material around the nose: 2 animals Salivation: 1 animal **Body weight and body weight gain:** *At 50 mg/kg bw/day:* Body weight gain less than the control (31 vs 57g). Following treatment, body weight gain greater than control (64 vs 57g). Mean body weight lower than the control on gestation days 9, 12, 16 and 20. *At 100 mg/kg bw/day:* Body weight loss during gestation period (-5). Following treatment, body weight gain less than the control (31 vs 57g). Mean body weight lower than control on gestation days 9, 12, 16 and 20. **Effects on development of the offspring**: No adverse effect on intrauterine survival at 10 and 50 mg/kg bw/day.	
Teratology study in rats EPA Health Effects Test Guidelines 560/6-82-001 GLP Rats, Sprague Dawley; females 25 animals/ groups	DPG Purity : not specified Vehicle: aqueous methylcellulose 0.5% Dose levels: 0, 5, 25, 50 mg/kg bw/day Administration: daily by gavage Exposure: day 6 to 15 of gestation.	**<u>General toxicity in dams:</u>** No unscheduled mortality *<u>25 mg/kg bw/day :</u>* **Clinical signs:** Lethargic behaviour, Salivation prior to dosing, Hair loss in the pelvic and abdominal areas, Brown material around the nose **Body weight:** Slight (not statistically significant) decrease of the mean body weight gain during the treatment and then very slight increase in body weight gain following treatment. Mean body weight comparable to controls <u>50 mg/kg bw/day:</u>	Unpublished study report, 1986 Reliability : 2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Clinical signs: Hair loss in the pelvic, abdominal, thoracic, urogenital, inguinal, dorsal back and tail areas. Lethargy and tachnypea, decrease of limb tone and protrastion and ataxy in all animals. Salivation and piloerection Chronic convulsions seen as single incidence. Lacrimation, clear nasal discharge, dried red material around the nose, red urogenital discharge and yellow urogenital matting observed as single incidence. **Body weight:** Statistically significant decrease of maternal body weights (-10.5% on GD20) and body weight gains (-25% for GD0-20). No information on corrected body weight. **Effects on the development of the offspring:** *5 mg/kg bw/day:* One female with 12 early resorptions *25 mg/kg bw/day* 3 fetuses from one dam with bent ribs. *50 mg/kg bw/day:* Statistically significant decrease in mean fetal body weight at 50 mg/kg bw/d (-12%) One female with 5 late resorptions. Slight increase in foetuses with reduced ossification and increase in bent ribs (5.1%).	
Not guideline, not GLP Mice, ICR-JCL, 19/20 females /dose (0, 0.25, 1.0, 4.0 mg/kg bw/day) 7 females / dose (10.0 mg/kg bw/d)	DPG No information on purity Vehicle: carboxymethylcellulose Doses: 0.25, 1.0, 4.0, or 10.0 mg/kg bw/day (actual ingested) Administration: once daily by gavage Exposure: days 0 to 18 of gestation	**General toxicity in dams:** No maternal toxicity effects. **Effects on development of the offspring:** • No significant differences in the percentage of dead foetuses, early or late in gestation, average litter size, sex ratio, and pup body weight between the exposed mice and the controls. • Significant retard in ossification of the talus seen in the fetuses of mothers treated with 4.0 mg/kg bw/day. • Malformations such as open eyelids or polydactyly seen sporadically in different	Yasuda et al. (1980) Reliability: 2 (no information on the age and weight of mice at the beginning of the study)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		groups without dose-response.	

10.8.5 Short summary and overall relevance of the provided information on adverse effects on development

1) The effects of DPG on development were investigated in an Extended One Generation Toxicity Study (EOGRTS) including extension with a F2 generation, conducted according to OECD TG 443 (Unpublished study report, 2021). The dose levels tested were 0, 5, 15 and 25 mg/kg bw/day. This study is considered as the key study.

In dams, unscheduled deaths were reported in 0, 2, 2, 6 females of the P generation (for 0, 5, 15, 25 mg/kg bw/day, respectively) and 3, 0, 3, 6 females of the cohort 1B.

In this study, **effects on the development of the offspring** were characterized by death of the developing organisms.

Post-implantation loss was reported in both parental and F1 generation. Post-implantation loss was calculated as [(Number of implantation sites - Number of live pups) / Number of implantation sites)] x 100. The number of stillborn was not reported. It is therefore supposed that the number of live pups at birth is equal to the number of delivered pups. In P generation, the increase in post-implantation loss was statistically significant at 25 mg/kg bw/day. In cohort 1B, a dose-related trend in the increase in post-implantation loss was observed at ≥ 5 mg/kg bw/day but was not statistically significant at any dose.

Table 27: Post-implantation **loss in P generation and cohort 1B females**

	P generation females				**Cohort 1B females**			
Dose levels (mg/kg/day)	**0**	**5**	**15**	**25**	**0**	**5**	**15**	**25**
Number of females which delivered with liveborn	23	22	22	23	17	20	19	19
Mean number of implantation sites (± SD)	14.9 ± 2.6	15.3 ± 2.6	15.3 ± 2.7	14.8 ± 3.3	13.1 ± 2.4	14.3 ± 2.0	**15.7** ± 2.2**	13.5 ± 2.9
Mean percent of post-implantation loss (% ± SD) [(Nb of implantation sites – Nb of live pups) / Nb of implantation sites)] x 100	15.6 ± 14.0	16.5 ± 10.4	13.3 ± 13.3	**27.1* ± 21.0**	13.6 ± 13.7	18.6 ± 12.8	22.1 ± 12.1	24.6 ± 24.5

*Statistical significance: *: p<0.05, **: p<0.01.*

Euthanasia of females was decided based on dead litter found postnatally in 0, 1, 1, 3 females of the P generation and 0, 0, 2, 2 females of the cohort 1B. Details are presented below. For these females, the clinical signs were not the reason for the sacrifice.

In P generation:

At 5 mg/kg bw/day, one female was prematurely euthanized on PND1 because of a dead litter. This female showed piloerection, hunched posture pallor of eyes/extremities and reddish vaginal discharge before sacrifice.

At 15 mg/kg bw/day, one female was prematurely euthanized on PND2 because of a total dead litter, this female showed no clinical sign before sacrifice.

At 25mg/kg bw/day, three females were prematurely euthanized on PND1 because of a total dead litter. The females showed piloerection, hunched posture, pallor of eyes/extremities, ventral recumbency, staggering gait, exophthalmos and/or dyspnea before sacrifice.

In Cohort 1B:

At 15 mg/kg bw/day, one female was prematurely euthanized because a dead litter on PND1. No adverse clinical signs were observed before death. Another female was prematurely euthanized because of dead litter on PND1. This female showed piloerection, hunched posture, pallor and dyspnea.

At 25 mg/kg bw/day, one female was prematurely euthanized for a dead litter on PND1. No adverse clinical signs were observed before death. Another female was prematurely euthanized for a total dead litter on PND1. This female showed before death, staggering gait and loss of balance.

Clinical signs were observed in terminated as well as scheduled animals mainly at the highest dose. Neurological signs were not observed at doses below 25 mg/kg bw/day.These signs are detailed in the section 10.8.2.

Effects on liver and thyroid hormones are presented in the section 10.8.2. Effects on liver were mainly observed from 15 mg/kg bw/day.

Females presenting postnatally a **dead litter (pups born alive)** were observed at all doses in P generation and from 15 mg/kg bw/day in the cohort 1B (detailed above).

Besides**,** in F1 pups, a dose-related tendency towards lower number of viable pups and **live birth index** ([Number of live pups on Day 1 p.p. / Number of delivered pups] x 100) was highlighted from 5 mg/kg bw/day. In F2 pups the same tendency was observed from 15 mg/kg bw/day. The study report did not explicit the statistical test used regarding the live birth index. A statistical analysis was conducted by DS. The normality tests showed that the data do not follow a Gaussian distribution. Thus, a Kruskall-Wallis test was conducted followed by Dunn's multiple comparisons test (GraphPad Prism). When the statistical analysis was reassessed by DS, the decrease of the live birth index was statistically significant in F2 pups from 15 mg/kg bw/day (*p<0.01* at 15 mg/kg bw/day and *p<0.0001* at 25 mg/kg bw/day).

At 25 mg/kg bw/day, a statistically significant lower mean number of live pups at PND 1 was noted in F2 pups. Moreover, a dose-related increase of mortality of F1 and F2 lactating pups (dead, missing and/or cannibalized pups) during PND 1 to 4 was seen with statistical significance in F1 pups at all doses and from 15 mg/kg bw/day in F2 pups. As a consequence, a trend toward a decreased pups **viability index** on PND 4 ([Number of surviving pups before culling on Day 4 p.p. / Number of live pups on Day 1 p.p.] x 100), was observed in both generations. The study report did not explicit the statistical test used regarding the viability index. A statistical analysis was conducted by DS. The normality tests show that the data do not follow a Gaussian distribution; and the hypothesis of residual homogeneity was rejected by the Bartlett's test. Thus, a Kruskall-Wallis test was conducted followed by Dunn's multiple comparisons test (GraphPad Prism). When the statistical analysis was reassessed by DS, the decrease of the viability index was statistically significant in F1 pups ($p<0.05$) at the high dose and in F2 pups (*p< 0.05* at 15 mg/kg bw/day*; p<0.0001* at 25 mg/kg bw/day) from the mid dose. These increases in pup mortalities led to significant decrease of mean litter size at PND 1 (F2 pups only) PND 4 (F1 and F2 pups) at the high dose.

At 25 mg/kg/day, on PND1, body weights of F1 and F2 pups were decreased compared to controls with statistical significance in males (F1 pups: 7.7g vs 8.4g for males and 7.3g vs 7.8g for females; F2 pups: 7.3g vs 8.1g for males and 7.7g vs 7.2g for females). The body weight returns to control values thereafter. The study design do not allow to discriminate if these effects are due to lactation or in utero exposure. Clinical signs were observed in some pups of both generation, mainly from 15 mg/kg bw/day in F1 pups and F2 pups terminated at scheduled. An absence of milk in the stomach, hypoactivity, emaciated appearance, dehydration and autolysis were noted in these pups. In F1 and F2 pups found dead, absence of milk in the stomach and autolysis were observed from 5 mg/kg bw/day.

Table 28: delivery data in P generation and cohort 1B females

	F1 pups				**F2 pups**			
(mg/kg bw/day)	**0**	**5**	**15**	**25**	**0**	**5**	**15**	**25**
Number of litters	23	22	22	23	17	20	19	19
Number of pups delivered (total)	288	281	291	254	194	234	231	195
Mean litter size at birth (± SD)	12.5 ± 2.9	12.8 ± 2.5	13.2 ± 3.0	11.0 ± 4.1	11.4 ± 2.8	11.7 ± 2.7	12.2 ± 2.2	10.3 ± 4.1

Mean litter size Day 1 p.p. (± SD)	12.0 ± 3.4	11.8 ± 4.0	11.5 ± 3.2	9.3 ± 4.0	11.4 ± 2.8	11.4 ± 2.6	10.8 ± 2.8	**7.8** ± 4.7**
Mean litter size Day 4 p.p. (± SD)	11.9 ± 3.3	11.7 ± 2.4	11.6 ± 3.4	**8.8* ± 4.2**	11.3 ± 2.8	11.3 ± 2.6	10.3 ± 2.6	**6.7# ± 4.4**
Number of entire dead litter	0	2	1	**5***	0	0	2	4
Litter with Dead, Missing and/or Cannibalized pups, Nb. (%)								
Days 1-4 p.p.	5 (21.7)	8 (36.4)	10 (45.5)	**14* (60.9)**	2 (11.8)	2 (10.0)	13 (68.4)	17 (89.5)
Days 1-21 *p.p.*	6 (26.0)	8 (36.4)	10 (45.5)	**15* (65.2)**	/	/	/	/
Dead, Missing and/or Cannibalized pups, Nb. (%)								
Days 1-4 *p.p.*	14 (4.9)	**35** (12.5)**	**47# (16.2)**	**82# (32.3)**	2 (1.0)	8 (3.4)	**56# (24.2)**	**73# (37.4)**
Days 5-7 *p.p.*	2 (0.7)	0 (0.0)	0 (0.0)	1 (0.4)	/	/	/	/
Days 8-14 *p.p.*	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	/	/	/	/
Days 15-21 *p.p.*	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	/	/	/	/
Live birth index (% ± SD)	95.1 ± 11.8	87.2 ± 29.7	87.9 ± 19.0	**72.0 ± 35.4**	100.0 ± 0.0	97.8 ± 9.8	**84.1 ± 25.2 *(p<0.01)*[1]**	**67.5 ± 33.7 *(p<0.0001)*[1]**
Viability index (% ± SD). Live pups PND4/PND1 Difference from control in %	94.8 ± 11.8	90.1 ± 23.2 -9%	85.0 ± 26.6 -10%	**74.4 ± 34.7 -22% *(p<0.05)*[1]**	99.0 ± 2.8	97.4 ± 9.8 -2%	**80.6 ± 25.3 -19% *(p<0.05)*[1]**	**56.6 ± 32.8 -43% *(p<0.0001)*[1]**
Lactation index (% ± SD). Live pups PND21/PND4	98.5 ± 4.0	100.0 ± 0.0	99.5 ± 2.2	99.4 ± 2.4	/	/	/	/
pups with absence of milk in stomach, pups/litter	2/1	0/0	2/1	9/6	0/0	0/0	8/4	23/8
pups with autolysis, pups/litter	4/3	13/5	13/7	15/6	0/0	7/1	27/10	22/11

*Statistical significance: *: p<0.05, **: p<0.01; #: p<0.001 according to study report*
1: statistical analysis conducted by DS

Regarding immunotoxicity, effects in cohort 1A animals were observed from 15 mg/kg bw/day. In males, at 15 mg/kg bw/day, a statistically significant increase of NK cells, both in terms of relative (4.8 vs. 3.4%) and absolute counts (11397 cells/mg of spleen compared to 7001 cells/mg of spleen) were observed as well as a decrease of T cells (36.6 vs. 42.5%) in term of relative count. These effects were not found at the high dose in males. In females, at 25 mg/kg bw/day, a statistically significant decrease of NK cells, both in terms of relative (2.6 vs. 3.8% of splenocytes) and absolute counts (5879 vs. 10713 cells/mg of spleen) were observed.
This gives indications that cell mediated immunity may be affected by DPG.

DS notes that a classification Repr. 1B, H360 is also proposed by the study authors of the EOGRTS.

2) A study conducted according to OECD TG 421 (Reproduction/Developmental Toxicity Screening Test) is available (Unpublished Study report, 2010). The doses tested were 0, 5, 15 and 25 mg/kg bw/day. 10 rats Sprague-Dawley/sex/dose were used. Two females at 5 and 25 mg/kg bw/day and one from control group presented dead litters. Male and female pups from the group treated at 25 mg/kg bw/day had lower mean body weight gain over the lactation

period (body weight: -14% and -15%, for males and females respectively on lactation day 5; not statistically significant). Other developmental parameters were not affected. In particular, effects on pups viability as those reported in the EOGRTS were not observed in this study. However, the OECD 421 protocol is only "*designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition*". Statistical analysis is also limited since only 10 animals of both sexes were included.

3) A non-guideline study (unpublished study report, 1989, Koëter et al. 1992) investigated the effects of DPG on testicular toxicity and male fertility, and the repercussions of these effects on embryogenesis. Male Albino CD1 mice were exposed by gavage to doses of 0.06, 0.25, 1, 4 and 16 mg/kg bw/day for 8 weeks. No females were treated. 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 12-13 selected males from the control and 16 mg/kg bw/day dose group. The remaining 11 males in the control, 4 and 16 mg/kg bw/d groups were mated with non-dosed females (within 14 days after the 8-week dosing period).

In this study, some increase were observed in dosed groups in the numbers of early and late resorptions and dead foetuses. According to the author, all values relating to post-implantation loss were low in all groups when compared to historical control values for this strain of mice. However, historical controls were not available. Litter data did not reveal any treatment-related effect.

4) In a range finding teratogenicity study, 5 Sprague-Dawley female rats per group were exposed daily by gavage to doses of 10, 50, 100, 150 and 200 mg/kg/day of DPG in 0.5% of aqueous methylcellulose during the days 6 to 15 of gestation (Unpublished study report, 1985). Gross necropsies were performed on animals which died and uterine examinations were performed on gestation day 20.

All animals in the 150 and 200 mg/kg/day group and 4 animals at 100 mg/kg/day died between gestation day 7 and 11. This was attributed to overt toxicity. No mortality was observed in the other groups. In the 50 mg/kg bw/day group and the surviving animal of the 100 mg/kg/day group, clinicals signs as lethargy, ataxy and dried red material around the nose were seen. A body weight loss was observed in the 100 mg/kg bw/day during the gestation period (-5). Following treatment, the body weight gain was less than the control (31 vs 57g). Mean body weight were lower than the control on the gestation days 9, 12, 16 and 20. In the 50 mg/kg bw/day, the body weight gain was less than the control (31 vs 57g). Following treatment, the body weight gain was greater than the control (64 vs 57g). Mean body weight were lower than the control on the gestation days 9, 12, 16 and 20. To be noted, no statistical analysis was carried out in this study. The necropsy examinations of the animals that died showed congestion of the liver, kidneys, lungs, stomach and intestines, haemorrhagic intestines with loss of epithelium, enlarged adrenal glands, and meningeal or basal haemorrhage of the brain No gross internal morphological changes were observed at the time of the uterine examination in the 10 and 50 mg/kg/day treated groups.

No foetoxicity was seen in the 10 and 50 mg/kg bw/day groups. However, the aim of this study was to select doses for the main study and not conclude on the reproductive toxicity of DPG. The number of animals is therefore low and no statistical analysis have been conducted.

5) In the prenatal developmental toxicity study conducted according to EPA Health Effects Test Guidelines 560/6-82-001, 25 Sprague-Dawley female rats per group were exposed daily by gavage to doses of 0, 5, 25 and 50 mg/kg bw/day of DPG in 0.5% of aqueous methylcellulose during the days 6 to 15 of gestation (Unpublished study report, 1986). On gestation day 20, cesarian sections were performed on all surviving females and fetal evaluation of morphology was performed.

No unscheduled mortality was observed in the dams during the study. At 50 mg/kg bw/day, females presented a significant decrease of maternal body weight (-10.5% on GD20) and body weight gains (-25% for GD0-20). There was no information on corrected body weight to uterus weight to know if these effects were consecutive to foetal death. Clinical signs were observed such as chronic convulsions, lethargy and tachnypea, decrease of limb tone, prostration, ataxy, lacrimation, hair loss, clear nasal discharge, dried red material around the nose, red urogenital discharge and yellow urogenital matting. At 25 mg/kg bw/day, lethargic behaviour was also observed once in different animals. No clinical signs were observed in the 5 mg/kg bw/day group.

One female in the 5 mg/kg bw/day group had twelve early resorptions. This effect was not seen in the 25 mg/kg bw/day group. At 50 mg/kg bw/day, one female that had 5 late resorptions. In the 50 mg/kg bw/day group, foetoxicity was observed as a significant decrease in mean fetal body weight (-12%) and a slight increase in foetuses with reduced ossification and an increase in bent ribs (5.1%). These effects may be secondary to severe maternal toxicity at this dose level. At 25 mg/kg bw/d, 3 fetuses from one dam, had bent ribs. According to the author of the study, this finding was

in the range of the historical data. However, historical data were based on one single study and no detailed information was presented. No foetoxicity was seen at 5 mg/kg bw/day.

6) In a study conducted by Yasuda et al (1980), pregnant mice ICR-JCL were exposed by gavage to 0, 0.25, 1, 4 and 10 mg/kg bw/day of DPG in 0.5% of carboxymethylcellulose during the days 0 to 18 of pregnancy (Yasuda et al. 1980). Mice were killed on day 18 of pregnancy. Uterine examination was performed for site of implantation and fetal death. No difference of body weight and no abnormalities (no further information available) were observed in treated mothers. Some abnormalities were noted in the foetuses such as open eyelids, but this effect was also seen in controls and without dose response. Ossification in the talus was significantly retarded in fetus in the 4 mg/kg bw/day group (incidence of ossified talus: 10.4% in controls, 4% in 0.25 mg/kg bw/day, 3.4% in 1 mg/kg bw/day, 2.5% in 4 mg/kg bw/day (*p<0.05*), 5.3% in 10 mg/kg bw/day).

10.8.6 Short summary and overall relevance of the provided information on effects on or via lactation

There is no study conducted with DPG that provide specific information on effects on or via lactation.

10.8.7 Comparison with the CLP criteria

For classification on adverse effects on development of the offspring the CLP guidance (2017) was followed.

"Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency. "

Known human reproductive toxicant: "*the classification of a substance in this Category 1A is largely based on evidence from humans*"

As there is no human study available, a classification of DPG in category 1A is not warranted.

Presumed human reproductive toxicant: "*the classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."*

Suspected human reproductive toxicant: *"substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."*

Effects on development of the offspring were mainly observed as **death of the developing organism**. These effects were primarily seen in the key EOGRTS (2021) in Sprague Dawley rats in both F1 and F2 pups.

In P generation, a statistically significant increase of post-implantation losses was noted at 25 mg/kg bw/day ($p<0.05$). In cohort 1B females, post-implantation loss was not statistically significant increased but was dose-related from 5 mg/kg bw/d.

Effects on peri / post-natal viability were consistently observed in both generations and were mainly characterized by:

- at pup level: there was an increase of the mean number of dead, missing and/or cannibalized pups between PND1-4 from 5 mg/kg bw/day ($p<0.01$ to $p<0.001$) and from 15 mg/kg bw/day ($p<0.001$) in F2 pups. This effect is clearly dose related. There was a statistically significant decrease of mean number of live pups on PND1 at 25 mg/kg bw/day ($p<0.001$) in F2.

- at litter level: statistically significant decrease of mean litter size on PND1 ($p<0.01$) and PND4 ($p<0.001$) in F2 pups and only on PND4 in F1 pups at 25 mg/kg bw/day ($p<0.05$). There was an increase of entire dead litters between PND1-4 ($p<0.05$) in F1 pups.
- statistically significant decrease of live birth index: when the statistical analysis was recalculated by DS, the decrease was statistically significant in F2 from 15 mg/kg bw/day ($p<0.01$ and $p<0.0001$).
- statistically significant decrease of viability index: when the statistical analysis was recalculated by DS, the decrease was statistically significant in both generations in F1 ($p<0.05$) at 25 mg/kg bw/day and F2 ($p<0.05$ and $p<0.0001$) from 15 mg/kg bw/day.
- Indications of immunotoxicity effects were recorded in cohort 1A in males at 15 mg/kg bw/day with a significant increase of the relative and absolute count of NK cells and a significant decrease of the relative count of T cells; and in females at 25 mg/kg bw/day, with a significant decrease of the relative and absolute count of NK.

Effects were observed in both generations and are considered as clear evidence of developmental effects of DPG.

The main findings related to general systemic toxicity in dams in the EOGRTS are clinical signs related to neurotoxicity that were reported in both generations at 25 mg/kg bw/day. It can be noted that the increase in the mean number of dead, missing and/or cannibalized pups between PND1-4 in both generations appear below the dose of 25 mg/kg bw/day causing maternal toxicity. Besides, when the statistical data were reassessed by DS, the viability index of F2 pups was significantly decreased from 15 mg/kg bw/day. Therefore, developmental effects cannot be non-specific effects secondary to general toxicity. In the study conducted according to OECD TG 421, two females at 5 and 25 mg/kg bw/day and one from control group presented dead litters. Even if no clear effects on pups were reported in this study, this points to a developmental effect considering that similar findings were reported in the EOGRTS. DS notes that the OECD 421 study is a screening test and so provide limited information compared to the EOGRTS.

In the prenatal toxicity study conducted according EPA Health Effects Test Guidelines 560/6-82-001 (Unpublished study report, 1986), a slight increase of post implantation losses was observed at 5 mg/kg bw/day due to early resorptions was observed in one female at 5 mg/kg bw/day and one female at 50 mg/kg bw/day had 5 late resorptions. Even if no clear effects on foetus were reported in this study, this points to a developmental effect considering that similar findings were reported in the EOGRTS. DS notes that peri/post-natal mortality as that reported in the EOGRTS cannot be evidenced in a teratogenicity study.

Overall, the available data provide clear evidence of a severe adverse effect on the development of the offspring, characterised as fetal and/or pup mortality. The effects are primarily found in the EOGRTS but consistent indications can also be found from the other studies. Moreover, effects on the development such as total litter death, mortality of pups during PND 1 to 4 or the decrease of viability index in F2 pups appear below the dose causing maternal toxicity characterised mainly as clinical signs of neurotoxicity. There is no mechanistic evidence to indicate that the observed effects are not relevant for humans.

Therefore DPG warrants to be classified as Repr. 1B, H360D.

10.8.8 Adverse effects on or via lactation

10.8.9 Comparison with the CLP criteria

There is no specific data with DPG that provide information on effects on or via lactation. Thus, no classification can be proposed for this endpoint.

10.8.10 Conclusion on classification and labelling for reproductive toxicity

Reproductive toxicity has been primarily reported in an EOGRTS (2021) according to OECD TG 443 in rats. Effects on fertility were characterized by several reproductive troubles:

- effects on gestation length,
- difficulties to deliver and dystocia, and
- alteration of the estrous cycle.
- Indication decreased number of primordial follicles. The 90-day NTP study also reported change of the estrous cycle length in rats and mice. .

Effects on sperm parameters are reported in presence of general toxicity in different repeated dose toxicity studies in different species, such as sperm abnormalities and decrease of sperm motility and are considered as supportive evidence (NTP, 1995; Koëter et al. 1992; Bempong et al. (1983).

Effects on development were identified in the EOGRTS (2021) as postimplantation losses and pups mortality (dead litters and increase of mortality between days 1 to 4 p.p.). These effects were found in other supportive studies: presence of dead litters in an OECD TG 421 study (2010), resorptions in the teratology study (Unpublished study report, 1986).

Based on these animal data, a classification as Repr. 1B, H360FD is proposed.

10.9 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.10 Specific target organ toxicity-repeated exposure

Table 29: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
OECD TG 443 (Cohorts 1A, and 1B with extension to cohort F2) GLP Rats, Sprague-Dawley Males and females Number of animals per sex per dose: - Parental generation: 24 per sex per dose - F1: 20 per sex per dose (Cohorts 1A and 1B)	DPG Purity: 98.7% Vehicle: 0.5% methylcellulose in drinking water treated by reverse osmosis Dose levels: 0, 5, 15, 25 mg/kg bw/day Administration: once daily by gavage Duration of exposure: - P males: at least 10 weeks of treatment - P females: at least 8 to 10 weeks of treatment - Cohort 1A: both males and females: from weaning post-natal day (PND 22) until euthanasia (from Day 90 p.p., to Day 93 p.p.	Detailed in the section 10.8 Reproductive toxicity **Mortality (unscheduled death) and clinical observations in these animals:** <u>P generation:</u> No unscheduled death in males. Mortality in females: 0, 2, 2, 6 <u>Cohort 1A:</u> No unscheduled deaths. <u>Cohort 1B:</u> No unscheduled death in males. Mortality in females: 3, 0, 3, 6 **Regarding neurotoxicity:** **P Generation:** *25 mg/kg bw/day:* At the end of the pregnancy period (Study Day 42-62), series of clinical signs suggestive of neurologic disorders in 8/18 surviving **females** (**clonic convulsion, locomotory difficulties, loss of balance, staggering gait and/or tonic seizures**). Disorders transient and observed after dosing only. **Cohort 1B:** *25 mg/kg bw/day:* In **both sexes**, series of clinical signs suggestive of neurologic disorders also observed in 10 males and 14 females (**clonic/tonic convulsion,**	Unpublished study report, 2021 Reliability: 1 Key study

	maximum). - Cohort 1B: In males: from weaning (PND 22) for at least 10 weeks before mating, during the mating period (up to 2 weeks), and after euthanasia of F2 pups (on PND 4). In females: from weaning (PND 22) for at least 10 weeks before mating, during the mating period (up to 2 weeks), during gestation, during lactation until PND 4 inclusive until euthanasia for females with no delivery (26 days after the last day of the mating period).	**loss of balance and/or staggering gait**) mainly from Study Day 94-100 for females and days 98-108 for males. **Signs of neurotoxicity appear from 25 mg/kg bw/day: STOT RE category 2 (P generation females: threshold between 13 and 130 mg/kg bw/day for STOT RE category 2 for approximatively 70 days of exposure).** **Cohort 1B males and females: threshold between 8 and 80 mg/kg bw/day for STOT RE category 2 for approximately 110 days of direct exposure)).**	
OECD TG 421 GLP Rats, Sprague-Dawley Males and females 10 animals per sex per group	DPG Purity: 98.9% Vehicle: 0.5% methylcellulose aqueous solution Dose levels: 0, 5, 15, 25 mg/kg bw/day Administration: once daily by gavage Duration of exposure: In males: - 4 weeks before pairing, - during the pairing period (up to 17 days), - until sacrifice of the females (at least 10 weeks in total).	Detailed in the section 10.8 Reproductive toxicity **Mortality:** No unscheduled mortality in males. Females sacrificed: 1, 1, 0, 1 No relevant clinical signs, macroscopic or microscopic findings observed in these 3 females. **Regarding neurotoxicity:** *At 25 mg/kg bw/day:* Clinical signs suggestive of neurologic disorders: **lateral decubitus and mydriasis** in one male (day 26 of dosing only) and one female (day 1 of lactation) **and abnormal locomotion** in one occasion and **staggering gait** in one male. **Signs of neurotoxicity appear from 25 mg/kg bw/day: STOT RE category 2 (threshold between 13 and 130 mg/kg bw/day (approximatively 70 days of exposure).**	Unpublished study report, 2010 Reliability: 1

	In females: - 4 weeks before pairing, - during the mating period (up to 17 days), - during gestation, -during lactation until day 4 post-partum inclusive (or until sacrifice), - until sacrifice for non-pregnant females.		
Repeated Dose 90-day oral toxicity study in rodent Similar to OECD TG 408 GLP Rats, F344/N and mice, B6C3F 10 animals per sex, dose and strain	DPG Purity : 98.9% Dose levels: 0, 250, 500, 750, 1500, or 3000 ppm DPG in feed Equivalent to: 0, 17/17, 32/32, 49/50, 100/95, 181/184 mg/kg bw/d in male and female rats, respectively. 0, 38/46, 75/93, 114/141, 231/285, 457/577 mg/kg bw/d in male and female mice, respectively Feeders were changed daily, 7 days per week. Duration of exposure : 13 weeks	Detailed in the section 10.8 Reproductive toxicity **Mortality :** **Rats F344/N:** 6 males and all females died in the 3000 ppm group. No mortality at lower doses. **Mice B6C3F:** No mortality **<u>Regarding neurotoxicity:</u>** **Rats F344/N:** Clinical signs of toxicity noted primarily in rats *at ≥ 1500 ppm* (**100/95 mg/kg bw/day** for respectively males and females) beginning at Week 2 : **Hypoactivity, convulsions and seizures** observed in some male and female rats in these groups, and **abnormal posture (staggering)** in most males and females. Other clinical signs observed in these groups: **hyperactivity, ataxia.** **Mice B6C3F:** *At 3000 ppm, (457/577 mg/kg bw/day* for respectively males and females)**: abnormal posture.** **Signs of neurotoxicity appear from 100/95 mg/kg bw/day for respectively males and females rats: STOT RE category 2 (threshold between 10 and 100 mg/kg bw/day).** **No classification warranted based on mice results.**	NTP, 1995 Reliability 2 (reliable with restriction)
Repeated Dose 28-day oral toxicity study in rodent	DPG Purity: 99.9% Vehicle : corn oil	**<u>Mortality:</u>** During the administration period : Mortality rate: 0, 0, 0, and 10% for males and 0,	Unpublished study report, 2000 Reliability : 2

OECD TG 407 GLP Crj:CD(SD)IGS [SPF] rats 5 animals/sex for the doses 10 and 30 mg/kg bw/day 10 animals/sex for the control group and for the 90 mg/kg bw/day group.	Doses: 0, 10, 30 and 90 mg/kg bw/day. Repeated oral administration conducted for 4 weeks (28 days) Recovery period of 2 weeks	0, 0, and 70% for females. No death observed during recovery period. **Body weight** *90 mg/kg bw/day:* In males: statistically significant decrease in body weight during the treatment period (-13% to -18%), with weight gain during the 4th week of dosing. At the beginning of the recovery period, body weight significantly low, but a progressive increase in body weight was observed thereafter, so that weight gain values were significantly high during the recovery period. In females, statistically significant decrease in body weight during the first week of the dosing period (-10%). **Food consumption:** *90 mg/kg bw/day:* In males: significantly low food consumption observed (-25%) during the treatment period then significantly high values noted during the recovery period. In females, food consumption significantly lower at weeks 1, 2 and 3 (-26, -17 and -25%). **Regarding neurotoxicity:** In males: *90 mg/kg bw/day group*: In both sexes : staggering gait, decreased spontaneous motor activity and startle response Symptoms not observed during the recovery period. **Blood chemistry:** In males, significantly low values noted for blood glucose in the 30 and 90 mg/kg groups, and significantly high values in blood urea nitrogen, total bilirubin, A/G ratio, ALT and alkaline phosphatase in the 90 mg/kg group. In females, significantly high values noted for total cholesterol, neutral fats and ALT in the 30 mg/kg group. Significantly high values also noted for total protein in the 10 mg/kg group, and for chloride in the 10 and 30 mg/kg groups, but according to the study author, the changes did not correspond to the dose. **Urinalysis** *90 mg/kg bw/day:* In males: At the end of the administration period, significant increase in urine volume and	(reliable with restrictions) Study report in Japanese with tables in english

		significantly low values in specific gravity. An increase in ketone bodies and negative protein was also observed in that same group. **Organs observations:** *90 mg/kg bw/day:* At the end of the treatment period in males, statistically significant decrease of weights of brain (absolute and relative), heart (absolute and relative), liver (absolute), kidney (absolute), spleen (absolute), adrenals (relative), testes (relative) and epididymis (relative). At the end of the recovery period, in the high dose group, significantly high values noted for the weight of the heart (relative) and the brain (absolute) in males and for the absolute and relative weight of the adrenals in females. **Histopathological examination:** No change observed in the brain (including the brain stem), spinal cord and sciatic nerve *30mg/kg bw/day:* Brown liver observed in females Decrease in fatty changes in males (5, 3, 1 and 0 in males, 4, 5, 3 and 1 females respectively). *90 mg/kg bw/day:* Brown liver observed in both sexes Red eardrums observed in both sexes Observation in kidney: hydropic changes in renal collecting tubules in both sexes. Increase of females with immature uterus: (0, 0, 0, 6). Decrease in fatty changes in both sexes (5, 3, 1 and 0 in males, 4, 5, 3 and 1 females respectively). **Signs of neurotoxicity appear at 90 mg/kg bw/day: STOT RE category 2 (threshold between 30 and 300 mg/kg bw/day) for 28 days of exposure.**	
Repeated Dose 2-week oral toxicity study in rodent No guideline followed Not GLP Range finding	1,3-diphenylguanidine Purity: 98.9% Vehicle : 0.5% methylcelluose aqueous solution Doses : 0, 30, 60 and 75 mg/kg	**<u>Mortality:</u>** *0 and 30 mg/kg bw/day:* No unscheduled death. *60 mg/kg bw/day:* 2 males and one female out of six animals survived until scheduled sacrifice. *75 mg/kg bw/day:*	Unpublished study report, 2010b Reliability: 3 (not GLP, no guideline, low number of animals)

study (previous to unpublished OECD 421 study report, 2010) Rats, Sprague-Dawley 3 animals/sex/dose	bw/day Exposure : 2 weeks Administration : daily by gavage	Only one male survived until scheduled sacrifice. One female was prematurely sacrificed on day 13 because of body weight loss and clinical signs of locomotory difficulties and soiled urogenital and mouth regions. Lateral recumbency had been observed from day 6, with loss of balance, mydriasis, hypoactivity and half-closed eyes. **Body weight:** Decreased body weight in females from 30 mg/kg bw/day and in males from 60 mg/kg bw/day. **Food consumption:** Decreased food consumption in in females from 30 mg/kg bw/day and in males from 60 mg/kg bw/day. **Regarding neurotoxicity:** *At 30 mg/kg bw/day:* One female had staggering gait and loss of balance on day 2 only and one male had loss of balance on day 9 only. *At 60 mg/kg bw/day:* Clinical signs were observed until mid- to late-week 2; one surviving male had no further clinical signs from day 12 while the other surviving male still had staggering gait on day 14. *At 75 mg/kg bw/day:* The one surviving male still had several clinical signs on day 14 (lateral recumbency, hypersensitivity to touch and noise, locomotory difficulties). **Signs of neurotoxicity appear from 30 mg/kg bw/day: STOT RE category 1 (threshold at 60 mg/kg bw/day for an exposure of 14 days).**	
Range-finding teratology study (previous to Unpublished study report, 1986) GLP Rats, Sprague Dawley females 5 animals/group	1,3-diphenylguanidine Purity not specified Vehicle : aqueous methylcellulose 0.5% Dose levels : 0, 10, 50, 100, 150 and 200 mg/kg bw/day Administration :	Detailed in the section 10.8 Reproductive toxicity **Mortality:** All animals exposed at doses ≥100 mg/kg died except one in the 100 mg/kg/day group. **Regarding neurotoxicity:** Lethargic behaviour (5 animals at 100 mg/kg bw/day, 5 animals at 150 mg/kg bw/day, 3 animals at 200 mg/kg bw/day) ataxia (5 animals at 100 mg/kg bw/day, 5 animals at 150 mg/kg bw/day, 5 animals at 200 mg/kg bw/day). Convulsions noted once in one animal in the 100	Unpublished study report, 1985 Reliability: 2

	daily by gavage Exposure: day 6 to 15 of gestation	mg/kg bw/day on gestation day 8. Clinical signs of toxicity in the 50 mg/kg bw/day group without causing death: lethargic behaviour and ataxia occurred in four animals. **<u>Organs observation:</u>** **In males:** Brain relative weight significantly increased in all groups dosed with DPG *At 500 ppm:* Decrease of the absolute weight of the heart, liver and spleen were observed in the dose group. *From 800 ppm:* Significant decrease of the absolute weight of the heart, liver, kidneys and spleen. *From 1500 ppm*: Significant decrease of the absolute weight of lung and brain. The relative weights of the ardrenals were also increased. **In females:** *At 500 ppm:* Slightly but statistically significantly decrease in absolute weight of liver. Statistically significant increase in relative brain weight. *At 800 ppm:* Slightly but statistically significantly decrease in absolute weight of brain, lungs. *At 1500 ppm*: Significant reductions of absolute weight of heart and liver and slight reductions for adrenals, kidneys, lungs and spleen were observe. Statistically significant increase in relative brain weight. *At 3000 ppm:* Absolute weights of the brain, heart and spleen were significantly reduced whilst kidneys and lungs were slightly reduced. **Signs of neurotoxicity appear from 50 mg/kg bw/day (in one animal): STOT RE category 1 (threshold at 100 mg/kg bw/ day for 10 days of exposure).**	
Teratology study in rats EPA Health Effects Test Guidelines 560/6-82-001	1,3-diphenylguanidine Vehicle : aqueous methylcellulose 0.5% Purity not	<u>Detailed in the section 10.8 Reproductive toxicity</u> No unscheduled mortality **<u>Regarding neurotoxicity:</u>** *25 mg/kg bw/day:*	Unpublished study report, 1986 Reliability: 1

GLP Rats, Sprague Dawley females 25 animals/groups	specified Dose levels: 0, 5, 25, 50 mg/kg bw/day Administration: daily by gavage Exposure: day 6 to 15 of gestation	Lethargic behaviour (in one animal) *50 mg/kg bw/day:* Lethargy and tachypnea, decrease of limb tone and prostration and ataxy in all animals. Chronic convulsions seen as single incidence in one animal. **Signs of neurotoxicity appear from 25 mg/kg bw/day (in one animal), signs are more severe at 50 mg/kg bw/day: STOT RE category 1 (threshold at 100 mg/kg bw/ day for 10 days of exposure).**	
Repeated Dose 2-week oral toxicity study in rodent No guideline followed Range finding study (previous to OECD TG 408) Not GLP Rats, Sprague-Dawley 5 animals/sex/dose	1,3-diphenylguanidine Doses: 300, 500, 800, 1500 and 3000 ppm (approximatively 36, 56, 73, 119 or 200 mg/kg bw/day) Exposure: 2 weeks Administration: continuously in diet	**Mortality:** *3000 ppm (200 mg/kg bw/day):* 5 premature decedents (no information on sex). **Body weight:** Dose related reductions in body weight gain observed. Animals in the 3000 ppm dose group showed an actual reduction in body weight over the course of the 2 week dosing period. **Food consumption:** Dose related reductions in food consumption were observed. **Regarding neurotoxicity:** *From 800 ppm (73 mg/kg bw/day) :* Reduced body tone *3000 ppm (200 mg/kg bw/day) :* Ataxia **Signs of neurotoxicity appear from 73 mg/kg bw/day: STOT RE category 1 (threshold at 60 mg/kg bw/day for an exposure of 14 days).**	Unpublished study report, 1980a Reliability: 4 (report not available and not enough information, not GLP, do not follow a guideline)
Repeated Dose 90-Day Oral Toxicity Study OECD guideline 408 GLP Rats, Sprague-Dawley 15 animals/sex/dose	1,3-diphenylguanidine Doses: 50, 150, 500 ppm (approximatively 4, 11, 37 mg/kg/day, nominal) Exposure: 90 days Administration: continuously in diet	**Mortality:** *At 500 ppm:* 1male (week 4 at 500 ppm) and 1 female (control) **Body weight:** *At 500 ppm:* Decreased in males (-15% for the majority of the dosing period) and females. More severe at the beginning of treatment **Food consumption:** *At 500 ppm:*	Unpublished study report, 1982 Cited by the SIDS, 2002 Reliability: 4 (Secondary literature)

		-14% in males and -12% in females **Urinalysis:** *At 500 ppm:* Reduction in urine volume in males, slight aciduria in females **Clinical signs:** no effects **Clinical chemistry:** *At 500 ppm:* Increased Alanine aminotransferase, alkaline phosphatase, sodium levels in males, increase alkaline phosphatase, decreased chloride, total protein and calcium levels in females at weeks 6 but not week 13. **Haematology:** *At 500 ppm*: Slight increase white blood cells in male and females at week 6 and 1. According the study author, large degree of intergroup variation suggests that it is of little biological significance. **Histopathological examinations**: No effects.	

10.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Several studies, in majority conducted in rats, report signs of neurotoxicity.

In the EOGRTS, Sprague-Dawley rats were exposed to DPG at dose levels of 5, 15 and 25 mg/kg bw/day. The study is detailed in the part 10.8 Reproductive toxicity (Unpublished study report, 2021).

In the P generation, at the highest dose tested, several females (terminated as scheduled) presented signs of neurotoxicity as clonic convulsion, locomotory difficulties, loss of balance, staggering gait and/or tonic seizures, hypoactivity. **These signs appeared at the end of the pregnancy period, in 8 out of 18 surviving females (study days 42-62). The signs were transient and observed after dosing only for a few days and then not observed anymore in the following days.**

Among the females sacrificed prematurely, clinical signs linked to neurotoxicity were observed at the high dose:

- 2 females were sacrificed for humane ground at PND1 and presented tonic seizures and tonic convulsion.
- 3 females were prematurely euthanized at PND1 (Study Days 43, 46 and 44) because of dead litter and presented staggering gait before sacrifice.
- One female was prematurely euthanized on Day 23 p.c. (Study Day 42) because of the difficulties to deliver and presented tonic seizures and clonic convulsion before sacrifice.

There was no unscheduled death in P generation males and no sign of neurotoxicity was recorded.

In cohort 1B, at 25 mg/kg bw/day, signs of neurotoxicity were seen in both sexes as clonic/tonic convulsion, loss of balance and/or staggering gait. The signs appeared from Study Day 94 after dosing for 1 to 3 days. There were no unscheduled death in male rats. Clinical signs of neurotoxicity in Cohort 1B generation are detailed in the tables below.

Table 30: Clinical Signs in cohort 1B males (Number of Animals Affected per group)

Dose level (mg/kg bw/day)	0	5	15	25
Number of males	20	20	20	20
Clonic/tonic convulsion, loss of balance and/or staggering gait (after treatment)	0	0	0	10 (days 90-108)
Hypoactiviy	0	0	0	6 (days 69-113)

Table 31: Clinical Signs in Terminated as Scheduled cohort 1B Females (Number of Animals Affected per group)

Dose level (mg/kg bw/day)	0	5	15	25
Number of terminated as scheduled females	17	20	17	15
Clonic convulsion, loss of balance and/or staggering gait (after treatment)	0	0	0	14 (days 94-100)
Hypoactiviy	0	0	0	6 (days 95-100)

Among the females sacrificed prematurely, clinical signs linked to neurotoxicity were observed at the high dose:

- One female was prematurely euthanized for humane grounds on PND1 (Study Day 97) and presented hypoactivity and clonic convulsion.
- One female was prematurely euthanized for humane grounds on PND1 (Study Day 96) and presented loss of balance.
- One female was prematurely euthanized for dead litter on PND1 (Study Day 97) and presented staggering gait and loss of balance before death.

In summary, signs of neurotoxicity occurred in animals exposed at the top dose of 25 mg/kg bw/day in the EOGRTS study. In addition, it was observed occasionally in some dams that were prematurely sacrificed at all dose levels.

In the OECD TG 421 study, Sprague-Dawley rats were exposed to doses 5, 15, 25 mg/kg bw/day to DPG. The study is detailed in the part 10.8 Reproductive toxicity (Unpublished study report, 2010). At 25 mg/kg bw/day, mydriasis was seen in one male at day 26 of dosing only and in one female at day 1 of lactation. Abnormal locomotion was seen in one occasion and staggering gait in one male. This suggest neurotoxic effects of DPG.

A NTP study conducted according to a protocol similar with OECD TG 408 is available. Rats, F344/N and mice, B6C3F were used (NTP, 1995). Animals were exposed during 13 weeks to dose levels of 0, 250, 500, 750, 1500, or 3000 ppm of DPG in feed (equivalent to 0, 17/17, 32/32, 49/50, 100/95, 181/184 mg/kg bw/day in males and females rats respectively and 0, 38/46, 75/93, 114/141, 231/285, 457/577 mg/kg bw/d in male and female mice, respectively). In the study conducted with rats, six males and all females in the 3,000 ppm groups died or were killed moribund before the end of the 13-week study. **Clinical signs related to neurotoxicity were recorded in the two higher exposure groups beginning at week 2.** Hypoactivity, convulsions and seizures were observed in some male and female rats in these groups, and abnormal posture as staggering was noted in most males and females. In mice, abnormal posture and ptosis were observed in mice in the higher exposure group, without mortality in this group. DS notes that there was no numerical data on the incidence of these effects in the publication.

A **4-week study** conducted according OECD TG 407 and GLP compliant is available. Crj:CD(SD)IGS [SPF] rats were exposed to 0, 10, 30 and 90 mg/kg of DPG in corn oil for 4 weeks followed by a recovery period of 14 days (Unpublished study report, 2000). Five animals per sex were used for the doses 10 and 30 mg/kg bw/day and 10 animals per sex were used in the control group and for the 90 mg/kg bw/day group.

The mortality rate in the 0, 10, 30 and 90 mg/kg groups was 0, 0, 0, and 10% for males and 0, 0, 0, and 70% for females.

In males, at the high dose, significantly low body weights were noted during the dosing period (-13% to -18%), with weight gain during the fourth week of dosing. At the beginning of the recovery period, body weight was significantly low, but a progressive increase in body weight was observed thereafter, so that weight gain values were significantly high during the recovery period. In females, at the high dose, significantly low body weights were noted during the first week of the dosing period (-10%), and a trend towards low values continued with two animals during the recovery period, but progressive weight gain was noted.

At the high dose, in males, significantly low food consumption was observed (-25%) during the treatment period then significantly high values were noted during the recovery period. In females, at the high dose, food consumption was significantly lower at weeks 1, 2 and 3 (-26, -17 and -25%).

Clinical signs of neurotoxicity were observed at 90 mg/kg bw/day in both sexes and included staggering gait, decreased spontaneous motor activity and startle response. These symptoms were not observed during the recovery period. The details are presented below:

Table 32: Clinical signs of neurotoxicity in males

		Days of experiment														Total (1 to 14)
Finding of clinical observation	Dose level (mg/kg bw/d)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Staggering gait	90	0	0	1	1	2	0	0	0	1	0	0	1	3	1	5
Decrease spontaneous motor activity		2	1	0	0	2	0	1	0	0	0	0	0	0	3	6
Startle response		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		Days of experiment														Total (1 to 28)
Finding of clinical observation	Dose level (mg/kg bw/d)	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Staggering gait	90	1	0	1	2	0	3	2	0	1	1	2	0	0	1	7
Decrease spontaneous motor activity		0	0	0	0	0	0	1	1	2	0	0	0	0	0	6
Startle response		0	0	0	1	1	0	1	0	0	0	0	0	0	0	2

Table 33: Clinical signs of neurotoxicity in females

		Days of experiment														Total (1 to 14)
Finding of clinical observation	Dose level (mg/kg bw/d)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Staggering gait	90	0	0	1	2	3	0	1	2	3	4	2	2	4	1	9
Decrease spontaneous motor activity		0	0	0	2	1	0	1	0	0	0	0	2	0	0	4
Startle		0	0	0	0	0	0	0	0	1	0	0	0	1	0	0

response																

		Days of experiment														Total (1 to 28)
Finding of clinical observation	Dose level (mg/kg bw/d)	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Staggering gait	90	2	2	0	1	3	2	3	2	1	3	1	0	0	0	10
Decrease spontaneous motor activity		0	0	0	0	0	0	0	1	0	0	0	0	0	0	5
Startle response		1	1	0	0	0	0	0	1	0	1	0	1	0	0	7

Regarding haematological data, at the end of the administration period, in males, significantly high values were noted for mean corpuscular hemoglobin concentration (MCHC) in the mid and high dose groups. Significantly low values were noted for mean corpuscular volume (MCV) in the 90 mg/kg group but there were no significant changes in the hematocrit values, hemoglobin or red blood cell count. Among females, significantly high values were noted for platelets in the 30 mg/kg group. At the end of the recovery period, no significant difference was noted.

Regarding blood chemistry, in males, significantly low values were noted for blood glucose in the 30 and 90 mg/kg groups, and significantly high values in blood urea nitrogen, total bilirubin, A/G ratio, ALT and alkaline phosphatase in the 90 mg/kg group. In females, significantly high values were noted for total cholesterol, neutral fats and ALT in the 30 mg/kg group. Significantly high values were also noted for total protein in the 10 mg/kg group, and for chloride in the 10 and 30 mg/kg groups, but according to the study author, the changes did not correspond to the dose.

At the end of the administration period, males displayed a significant increase in urine volume and significantly low values in specific gravity were noted in the 90 mg/kg group. An increase in ketone bodies and negative protein was also observed in that same group.

Among males in the 90 mg/kg group, at the end of the treatment period, a statistically significant decrease of the weights of the brain (absolute and relative), heart (absolute and relative), liver (absolute), kidney (absolute), spleen (absolute), adrenals (relative), testes (relative) and epididymis (relative). At the end of the recovery period, in the high dose group, significantly high values were noted for the weight of the heart (relative) and the brain (absolute) in males and for the absolute and relative weight of the adrenals in females.

Regarding histopathological data, no change were noted in the brain (including the brain stem), spinal cord and sciatic nerve.

An increase of animals presenting brown liver in animals (From 30 mg/kg bw/day for females and 90 mg/kg bw/day for males) and red eardrum (at 90 mg/kg bw/day in both sexes) but no histological correlation was found. A significant decrease in fatty changes was noted in liver in males at 30 and 90 mg/kg bw/day (number of animals: 5 in controls, 3 at 10 mg/kg bw/day, 1 at 30 mg/kg bw/day and 0 at 90 mg/kg bw/day). In kidneys, hydrophic changes in collecting tubules were observed in both sexes at 90 mg/kg bw/day (significant in males). An increase of females with immature uterus was noted at the high dose (0, 0, 0, 6). However, the statistical analysis is unclear in this study and there are inconsistencies between the results descriptions and the tables.

The following studies are considered as supportive evidence, as the effects reported are consistent with those observed in chronic and subchronic studies but are of lower relevance for STOT RE classification (range-finding studies with limited number of animals or studies with shorter duration of exposure). These studies are dose-range finding studies or teratogenicity studies.

In the range-finding for the OECD 421 study, Sprague-Dawley rats (3/sex/group) were exposed to doses of 30, 60 and 75 mg/kg bw/day of DPG in a 0.5% methylcellulose aqueous solution for 2 weeks (Unpublished study report, 2010b). In each dose group, unscheduled mortality was the following: 0/6, 0/6, 3/6, 4/6.

At the high dose, body weight loss (between -8 g and -25 g) and little body weight gain were observed in animals. At the mid dose, the 3 remaining animals had occasional weight loss but generally gained weight until the end of the study.

Of these 3 surviving animals, two had an overall body weight gain (+46 g or +4 g) while the third had an overall body weight loss (-11 g). In this group, the body weight loss was between -8g and -34g. At 30 mg/kg bw/day, no difference in mean body weight or body weight gain was noted in males. The females gained little weight or lost weight throughout the study resulting in a small overall body weight gain (-74% when compared with the controls).

Regarding food consumption, the groups treated at 60 or 75 mg/kg/day had moderately to markedly reduced food consumption when compared with the controls from day 1 to day 11, after which the remaining animals consumed similar, or only slightly lower, amounts of food to the controls. There were no effects of treatment with the test item on mean male food consumption at 30 mg/kg/day but the mean female food consumption was lower than that of the controls throughout the study.

Regarding neurotoxicity, at 30 mg/kg bw/day, one female had a staggering gait and a loss of balance which was observed on day 2 only and one male presented a loss of balance on day 9 only. At 60 mg/kg bw/day, only 3 animals survived; the clinical signs were observed until mid- to late-week 2. One surviving male had no further clinical signs from day 12, the other surviving male still presented piloerection, staggering gait and salivation on day 14. The one surviving female displayed no important clinical signs from day 13. At 75 mg/kg bw/day, an important mortality was observed (only 1 male survived until scheduled sacrifice, the other animals died between days 2 and 13). The surviving male presented several clinical signs on day 14 (lateral recumbency, hypersensitivity to touch and noise, locomotory difficulties and salivation).

The **range-finding study conducted prior to the main teratology study in rats** (unpublished study report, 1986) displayed consistent results with the main teratology study (unpublished study report, 1985). Five female Sprague Dawley were exposed to doses of 10, 50, 100, 150 and 200 mg/kg bw/day daily during the days 6 to 15 of gestation. The study is detailed in the part 10.8 Reproductive toxicity. All animals died in the 150 and 200 mg/kg/day dose groups and only one animal survived in the 100 mg/kg/day group. Signs of neurotoxicity appeared from 50 mg/kg bw/day and were characterised by lethargic behaviour and ataxia in four animals, prostrate behaviour and tachypnea in one animal primarily during the initial dosing days (gestation days 6-9).

In the **main teratology study in rats**, Sprague-Dawley rats were exposed to doses of 5, 25 and 50 mg/kg bw/day (Unpublished study report, 1986). The study is detailed in the part 10.8 Reproductive toxicity. No unscheduled mortality was observed in this study. At 25 mg/kg bw/day, lethargic behavior was noted once in one animals. At 50 mg/kg bw/day, all animals were lethargic and has tachypnea and decreased limbtone and with one exception all animals were prostrate and ataxic. Clonic convulsions, lacrimation, clear nasal discharge, dried red material around the nose, red urogenital discharge and yellow urogenital matting were observed as single evidence. The details are presented in the table below.

In the remaining **2-week study** (previous to the OECD TG 408, unpublished study report, 1982), 5 Sprague-Dawley rats/sex were exposed to doses of 300, 500, 800, 1500 and 3000 ppm (approximatively 36, 56, 73, 119 or 200 mg/kg bw/day) continuously in diet (Unpublished study report, 1980a). No abnormalities were detected in the groups receiving 0, 300 and 500 ppm DPG. Dose-related reductions in body weight gain were observed. Animals in the 3000 ppm dose group showed an actual reduction in body weight over the course of the 2-week dosing period. Dose-related reductions in food consumption were observed. At 3000 ppm, 5 animals died during the second week of dosing. Signs of neurotoxicity were observed from 800 ppm (73 mg/kg bw/day) as reduced body tone. Regarding effects on organs, in males, decrease of the absolute weight of the heart, liver and spleen were observed in the 500 ppm dose group. From 800 ppm, significant decrease of the absolute weight of the heart, liver, kidneys and spleen. From 1500 ppm, significant decrease of the absolute weight of lung and brain. Brain relative weight was significantly increased in all groups dosed with DPG. The relative weights of the adrenals in animals receiving 1500 ppm were also increased. In females, the absolute weight of the brain, lungs (at 800 ppm) and liver (at 500 ppm) were slightly but statistically significantly decreased. At 1500 ppm, significant reductions of the heart and liver and slight reductions for adrenals, kidneys, lungs and spleen were observed. At 3000 ppm, absolute weights of the brain, heart and spleen were significantly reduced whilst kidneys and lungs were slightly reduced in animals receiving 3000 ppm DPG. Relative organ weight analysis showed a statistically significant increase in brain weights for animals receiving 500 and 1500 ppm DPG.

In the last repeated-dose toxicity study conducted according to OECD TG 408, Sprague-Dawley rats were exposed to doses of 50, 150, 500 ppm (approximatively 4, 11, 37 mg/kg) of DPG continuously in diet for 13 weeks. No effects related to neurotoxicity were observed in the study.

10.10.2 Comparison with the CLP criteria

For classification of substances as specific target organ toxicants following repeated exposure, the CLP regulation was followed.

Category 1: *Substances classified in category 1 are substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations*

Category 2: *Substances classified in category 2 are substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.*

In exceptional cases human evidence can also be used to place a substance in Category 2.

Guidance values indicated in the tables 3.9.2 and 3.9.3 have been applied.

Only studies performed by oral route are available with DPG, thus only guidance values relevant for this route of exposure is presented here.

Route of exposure	Units	Guidance values for STOT RE 1 (applicable for 90-day studies)	Guidance values for STOT RE 2 (applicable for 90-day studies)
Oral (rat)	mg/kg body weight/day	C ≤ 10	10 < C ≤ 100

Several studies performed with DPG report signs of neurotoxicity in rats after repeated exposure, mainly characterized by locomotion and posture anomalies, convulsion and lethargy. In most studies, except for the EOGRTS, these effects appeared at doses lower than the one inducing mortality.

The 90-day study performed by the NTP (1995) clinical signs of neurotoxicity were reported in rats at doses complying with a classification STOT RE 2 and in mice at a dose higher than the thresholds indicated in the CLP guidance for category 2. This suggests that rats might be more sensitive to DPG than mice regarding neurotoxicity.

Table 34: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Reference	Study	LOAEL for neurotoxicity (mg/kg bw/day)	STOT RE 2 threshold adapted to study duration	Classification supported by the study
Unpublished study report, 2021	OECD TG 443 study	25	P generation females: Threshold between 13 and 130 mg/kg bw/day for STOT RE category 2 (approximately 70 days) Cohort 1B males and females: Threshold between 8 and 80 mg/kg bw/day for STOT RE category 2 (approximately 110 days)	STOT RE category 2
Unpublished study report, 2010	OECD TG 421 study	25	Threshold between 13 and 130 mg/kg bw/day for STOT RE category 2 (approximately 90	STOT RE category 2

			days for males and females)	
NTP, 1995	90-day oral toxicity study in rodent Similar to OECD TG 408	Rats : 100/95 for respectively males and females Mice : 457/577 for respectively males and females	Threshold between 10 and 100 mg/kg bw/day for STOT RE category 2 (90 days)	STOT RE category 2
Unpublished study report, 2000	OECD TG 407 study	90	Threshold between 30 and 300 mg/kg bw/day for STOT RE category 2 (28 days)	STOT RE category 2
Unpublished study report, 2010b	Two-weeks range-finding study for the OECD 421 study	30	Threshold between 60 and 600mg/kg bw/day for STOT RE category 2 (14 days)	STOT RE category 1
Unpublished study report, 1980a	Two-weeks range-finding study	73	Threshold at between 60 and 600 mg/kg bw/day for STOT RE category 2 (14 days)	STOT RE category 2
Unpublished study report, 1985	Range-finding study for teratogenicity study	50	Threshold between 90 and 900 mg/kg bw/day for STOT RE category 2 (10 days)	STOT RE category 1
Unpublished study report, 1986	Teratology study in rats EPA Health Effects Test Guidelines 560/6-82-001	25	Threshold between 90 and 900 mg/kg bw/day for STOT RE category 2 (10 days)	STOT RE category 1

In the EOGRTS (2021), OECD TG 421 study (2005) and 90-day NTP study (1995), the doses leading to neurotoxicity are between the cut-off of category 2 (13-130 mg/kg bw/day for P generation of the EOGRTS and animals of the OECD 422, 8 and 80 mg/kg bw/day for EOGRTS cohort 1B and 10-100 mg/kg bw/day for NTP study). In the 28 day study performed according to OECD TG 407, the effective dose for neurotoxicity also corresponds to a category 2.

The remaining studies display an exposure of two weeks or shorter. The effective doses in these studies lead to a classification as category 1.

According to CLP guidance, "*Haber's rule is used to adjust the standard guidance values, which are for studies of 90-day duration, for studies of longer or shorter durations. It should be used cautiously with due consideration of the nature of the substance in question and the resulting value produced".*

It is also stated: "*one particular problem to note is that when adjusting the guidance value for very short study durations this can lead to very high guidance values which are not appropriate […] To address this problem a pragmatic approach is proposed. For studies with exposure durations shorter than 9 days (i.e 10% of the 90 days to which the default general guidance value applies) the guidance value used should be no greater than 10 times the default guidance value. For example, the effects in an oral range-finding study of 9 days or less should be compared with a guidance value of 1000 mg/kg bw/day for STOT-RE Category 2. Expert judgement is needed for the establishment of equivalent guidance values because one needs to know about the limitations of the applicability of the proportionality*".

Moreover, the two 2-week dose range-finding studies (Unpublished study report, 2010b and Unpublished study report, 1980a), do not follow an OECD guideline and are not GLP compliant. More weight is therefore given to available studies that follow a guideline are GLP and with a 90-day exposure.

In the teratology study in rats (Unpublished study report, 1986) conducted according to EPA Health Effects Test Guidelines 560/6-82-001 and GLP compliant, the exposure duration of animals to the substance is of 10 days during pregnancy. The effective dose reported for neurotoxicity is 25 mg/kg bw/day. For an exposure duration of 10 days, the guidance value of 100 mg/kg bw/day should be used, leading to STOT-RE Category 1. However, in these studies, the exposure duration is very short, therefore they are less reliable than studies with 90-day exposure. These studies could suggest that rats might be more sensitive to the substance than mice and also more sensitive during pregnancy than non-gravid animal. However, this window of exposure is also included in the EOGRTS in which neurotoxicity signs occurred at doses complying with a classification STOT RE 2.

Considering the criteria set in the CLP guidance, it is more appropriate to use subchronic toxicity studies to conclude on the category for STOT RE classification. Adequate subchronic studies are available and all point to a classification as STOT RE category 2.

Therefore, a classification of DPG as STOT RE category 2 is proposed with the target organ: nervous system.

10.10.3 Conclusion on classification and labelling for STOT RE

DPG warrants to be classified as STOT RE category 2, H373 (nervous system).

10.11 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 35: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
According to OECD TG 111 (Hydrolysis as function of pH) Test substance : DPG Purity: 99.4%	<u>After 5 days at 50°C:</u> At pH 4: less than 10% hydrolysis, equivalent to half-life greater than 1 year at 25°C. At pH 7: less than 10% hydrolysis, equivalent to half-life greater than 1 year at 25°C. At pH 9: less than 10% hydrolysis, equivalent to half-life greater than 1 year at 25°C. <u>After 7 days at 37°C:</u> At pH 1.2: no overall loss of test item over the incubation period. Under the physiologically relevant condition of pH 1.2, 37.0 ± 0.5°C, the test item was determined to be stable over the period of 7 days.	Reliability 1 GLP study Please see annex I for more details	Unpublished study report, 2015a
Investigation of the hydrolytic properties of DPG (0.3 g/L or 0.3 wt.% in water) in relation to the pH value at 80°C.	At pH 3.5: no recognizable hydrolysis of the original substance took place over a period of 500	3 (not reliable) GLP	Wohlfahrt, R. & Niebergall

Method	Results	Remarks	Reference
Test material: DPG Purity: no data	hours. At pH 7: only 18.1% of DPG was hydrolyzed after 1000 hours. At pH 10.5: t1/2 was about 168 hours. 1,3-diphenylurea and aniline were identified as hydrolysis products by IR and UV spectroscopy. 1,3-diphenylurea was further hydrolyzed to aniline in both the acidic and alkaline environments. Under the tested conditions, DPG is stable in water.	compliance not specified	, H. 1984a Wohlfahrt , R. & Niebergall , H. 1984b Wohlfahrt , R. & Niebergall , H. 1985 SIDS, 2002
OECD TG 301D (Ready Biodegradability: Closed Bottle Test) (OECD, 1992; EU, 1992; ISO test guideline, 1994) Duration of test : 28 days Test substance : DPG Purity: 99,4% Initial concentration : 2 mg/L Reference substance : Sodium acetate anhydrous Inoculum: River water, nearest plant treating domestic wastewater biologically was 3 km upstream.	<u>% Degradation of test substance:</u> 86 after 14 days (%degradation O2 consumption) (based on ThOD-NH3) 85 after 28 days (%degradation O2 consumption) (based on ThOD-NO3) Over 60% biodegradation was achieved in a period of 8 days immediately following the attainment of 10% biodegradation.	Reliability 1 GLP Key study Deviations indicated in the study report : -ammonium chloride was omitted from the medium to prevent oxygen consumption due to nitrification (omission does not result in nitrogen limitation as shown by the biodegradation of the reference compound) -river water instead of an effluent /extract /mixture was used as inoculum. Please see annex I for	Unpublished study report, 2015b

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td></td><td></td><td>more details</td><td></td></tr>
<tr><td>Equivalent to OECD TG 301D (Ready Biodegradability: Closed Bottle Test)

Test duration: 28 days
Test substance: DPG

Purity: no data

Experiment 1:
Closed bottle test
Non-adapted sludge
Concentrations: 0.8, 2.4, 8.0, 24 mg/L DPG solutions
Measured after 5, 10 and 20 days

Experiment 2:
Closed bottle test
Adapted sludge (aerated for 14 d in contact with DPG)
0.8, 2.4, 8.0, 24 mg/L DPG solutions
Measured after 5, 10 and 20 days</td><td><u>Experiment 1 :</u>
No degradation was observed within 20 days at any concentration based on measure of O2 consumption.

<u>Experiment 2 :</u>
Results based on % degradation depending on time:

<table><tr><td></td><td colspan="3">Time (days)</td></tr><tr><td>Concentration (mg/L)</td><td>5</td><td>10</td><td>20</td></tr><tr><td>0.8</td><td>0</td><td>62</td><td>74</td></tr><tr><td>2.4</td><td>0</td><td>66</td><td>76</td></tr><tr><td>8.0</td><td>16</td><td>>LOQ</td><td>>LOQ</td></tr><tr><td>24</td><td>>LOQ</td><td>>LOQ</td><td>>LOQ</td></tr></table>
>LOQ = all available oxygen used. No measurement possible

75% of degradation after 28 days.

Degradation products were not measured.</td><td>Reliability: 3

GLP compliance not specified

Deviations: adapted sludge in experiment 2

No more information on the origin of the inoculum</td><td>Bayer, 1988
SIDS, 2002</td></tr>
<tr><td>Equivalent to OECD TG 301 B (Ready Biodegradability: CO2 Evolution Test)

Test duration: not clearly specified, probably 28 to 35 days

Inoculum: Acclimated mixture of raw sewage, soil and activated sludge.

Test substance: DPG

Purity: no data
Test concentrations: 20-21 mg/L</td><td><u>Experimentation repeated thrice:</u>

(1)
DPG: 21 mg/L
ThOD: 71%

(2)
DPG: 20 mg/L
ThOD: 68%

(3)
DPG : 20 mg/L
ThOD: 55%

Mean :
ThOD : 65%

No information on the ten-day time window</td><td>Reliability: 3

GLP compliance not specified

Deviations: flasks were agitated

No information on the acclimation meaning. According to the study author, significant problems reported with the acclimation of the inoculum.</td><td>Unpublished study report, 1979a</td></tr>
<tr><td>Biological degradation of DPG through micro-organisms originated from river or seawater of</td><td>DPG was degraded in the river water by 18% and in the seawater by 9%</td><td>Reliability 4 (Study poorly</td><td>Kondo et al. (1988)</td></tr>
</table>

Method	Results	Remarks	Reference
uncontaminated area (COD < 3 ppm). No guideline followed GLP compliance not specified Test substance: DPG Purity: no data Test concentration: 5 ml 0.2% peptone solution (pH 7; set in seawater with 3% NaCl) mixed with 4.9 ml river or sea water as micro-organisms source and 0.1 ml of a presumably aqueous DPG solution (dissolved in water, acetone or DMSO; final concentration 20 mg DPG/L). Solution incubated for 3 days in the dark at 30°C Elimination determined by analyzing the substance concentration at defined time intervals; the growth of the micro-organisms was determined by the increase of turbidity (optical density at 610 nm).	and consequently considered by the authors of the study as sparingly to moderately degradable.	documented) Not applicable for CLP purposes	
OECD Guideline 301 C (Ready Biodegradability: Modified MITI Test (I)) Sludge concentration: 30 mg/L non-adapted activated sludge Test substance: DPG Purity: no data on purity Substance concentration: 100 mg/L Test duration: 28 days	No degradation after 14 days (O2 consumption)	Reliability: 4 (Secondary literature, no information on test conditions)	MITI, 1981 Cited by SIDS, 2002
Equivalent to EU Method C.5 (Degradation: Biochemical Oxygen Demand (BOD)) Not GLP Test substance : DPG No data on purity	BOD5: 2.3 % (referred to TOD); no further information available	Reliability : 4 (Secondary literature, no information on test conditions)	Wilson et al. (1960) Cited by SIDS, 2002
Protocol equivalent to OECD TG 309 GLP compliance not specified Test substance: DPG No data on purity Substance concentration: 1 mg/L Duration of test: 14 days Sterile control with autoclaved river water.	Primary degradation (parental substance) complete within 14 days. <u>% Degradation of test substance:</u> 3 after 3 days 78 after 7 days 100 after 14 days	Reliability 3 No mineralisation was determined in this study No information on degradation	Unpublished study report, 1980b

Method	Results	Remarks	Reference
Positive control of 4 ml of 2 mg/ml quinoline int o buffered river water. Incubation temperature: 21-25°C.		products Concentration tested too high in comparison to the recommendati ons set in OECD TG 309, thus not possible to use the results to estimate the first order degradation constant and half-life. Not applicable for CLP criteria	
(Q)SAR Test material : DPG	Half Life: = 0.125 days, 1.504 hours	2 (reliable with restrictions) weight of evidence	US EPA (2009a)

11.1.1 Ready biodegradability

The biodegradation potential of 1,3-diphenylguanidine was studied in accordance of OECD TG 301D (Ready Biodegradability: Closed Bottle Test) in a study that is GLP compliant (Unpublished study report, 2015b). The validity criteria were met and this study is considered as a key study. The test concentration of DPG was 2 mg/L. Microorganisms present in river water were exposed to DPG under aerobic conditions for a period of at least 28 days. The nearest plant treating domestic wastewater biologically was 3 km upstream. Sodium acetate anhydrous was used as the reference substance. The percentage of degradation of DPG was calculated with the theoretical oxygen demand ($ThOD_{NH3}$). The biodegradation of DPG was over 60% after 8 days and 85% after 28 days. The test substance therefore fulfilled the 10-day time window criterion for ready biodegradable substances. Moreover, a complete recovery of 1,3-diphenylguanidine-nitrogen as nitrate-nitrogen demonstrates ultimate biodegradation of the test substance. Hence, the study demonstrates that DPG is readily biodegradable.

Other studies are available but are of lower reliability or were not considered acceptable due to missing information on the reporting. The study reports were not accessible and not enough details on the studies were provided in the secondary reports mentioning these studies. These studies are described below:

In a study equivalent to OECD TG 301D, two experiments were conducted with concentrations of 0.8, 2.4, 8 and 24 mg/L of DPG (Bayer, 1988, SIDS, 2002). BOD (Biological oxygen demand) was measured after 5, 10 and 20 days. In the first experiment, non-adapted sludge (activated sludge, domestic) was used as inoculums. No degradation was observed within 20 days at any concentration. The second experiment was conducted with adapted activated sludge (domestic) as inoculums. The sludge was adapted by combining inoculums with DPG and aerating for 14 days. After 5 days, no degradation was observed at 0.8 or 2.4 mg/L. At 8 mg/L, 16% degradation was obtained. Within 10 days, DPG at concentrations of 0.8 and 2.4 mg/L was degraded by 62 and 66% respectively, and within 20 days by 74 and 76%, respectively. According to these results, DPG should be considered as not readily biodegradable, but ready biodegradable in presence of adapted inoculum.

A study conducted according to a protocol similar to OECD TG 301 B (Ready Biodegradability: CO2 Evolution Test) was available (Unpublished study report, 1979a). In this study, an acclimated inoculum composed of a mixture of raw sewage, soil and activated sludge was used. The meaning of acclimated inoculum is not detailed in the report. There is no information whether this term refers to an acclimation to the experimental conditions or an adaptation to the test substance. The test concentrations of DPG were 20-21 mg/L and the flasks were agitated during the test. It should be noted that the test duration is not clearly indicated in the study but is probably of 28 or 35 days. The test was repeated thrice. The percentage of degradation of DPG was measured by CO_2 production. The mean level of biodegradation was 65% and the maximum 71%. Moreover, for some of test conditions, especially those where significant biodegradation of DPG occurs, significant problems were encountered in the pre-acclimation of the bacterial inoculum. It revealed excessive background organic carbon levels in some of the test media. As a consequence, a significant unknown carbon source would also contribute to the CO_2 production during the test. As a result, CO_2 evolution during the test could not be only due to the test item degradation. Nonetheless according to these results, DPG should be considered as biodegradable in presence of adapted inoculum.

Kondo *et al.* (1988) investigated the biological degradation of DPG through micro-organisms originated from river or seawater of uncontaminated area (COD < 3ppm). The test solution was composed of 5mL 0.2% peptone solution (pH 7; set in seawater with 3% NaCl) mixed with 4.9 mL river or sea water as micro-organisms source and 0.1 mL of a aqueous DPG solution (dissolved in water, acetone or DMSO; final concentration 20 mg DPG/L). The test solution was incubated for 3 days in the dark at 30°C and the elimination was determined by analyzing the substance concentration at defined time intervals. The growth of the micro-organisms was determined by the increase in turbidity (optical density at 610 nm). DPG was degraded in the river water by 18% and in the seawater by 9% after 3 days. The authors of the publication concluded the test substance as sparingly to moderately degradable. However, the short duration of the test (only 3 days) should be noted, that renders the study not similar to ready tests nor simulation tests. In addition, the study is poorly reported and no guideline was followed. Therefore, this study cannot be used for classification purposes, but it does provide information that a (bio)degradation was observed after 3 days under environmental conditions.

An OECD Guideline 301 C (Ready Biodegradability: Modified MITI Test (I)) was available (MITI, 1981). The test substance was tested at a concentration of 100 mg/L. Biodegradation was assessed based on the measure of O_2 consumption. No degradation was observed with a non-adapted activated sludge within 28 days. Nevertheless, it should be noted the poor reliability of this study due to the lack of available information that do not allow to assess the test validity.

Overall, the key study conducted according to OECD TG 301D demonstrates that 85% of DPG was biodegraded at day 28, and over 60% biodegradation was achieved after approximately 10 days (Unpublished study report, 2015). The 10-day time window criterion for ready biodegradable substances is therefore fulfilled. Moreover, considering the low quality of the data of other studies available compared with the good quality of the recent study, it can be concluded that DPG is readily biodegradable.

11.1.2 BOD_5/COD

One study conducted according to a protocol equivalent to EU Method C.5 (Degradation: Biochemical Oxygen Demand) is available but was judged not reliable for classification assessment (Wilson et al. (1960), SIDS, 2002). In this study, the BOD5 was established at 2.3 %.

11.1.3 Hydrolysis

Hydrolysis was tested according to the OECD TG 111 for different pH-values (4, 7 and 9) at 50 °C for 5 days and at 37°C for 7 days at pH 1.2 (Unpublished study report, 2015a). The study is GLP compliant. No hydrolysis took place at 50°C at pH 4, 7 and 9 and neither at 37°C at pH 1.2, indicating that DPG is hydrolytically stable in water at environmental pH. The estimated half-life of the substance is greater than one year at 25°C at pH 4, 7 and 9. No degradation product has been investigated in this study.

Other supportive studies (Wohlfahrt, R. & Niebergall, H.1984 and 1985), 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). In an acidic environment (pH 3.5) no recognizable hydrolysis of the original substance took place over a period of 500 hours. In the neutral range (pH 7) only 18.1% of DPG was hydrolyzed after 1000 hours. In the alkaline range (pH 10.5) the half-life was about 168 hours. 1,3-diphenylurea and aniline were identified as hydrolysis products by IR and UV spectroscopy. 1,3-diphenylurea was further hydrolyzed to aniline in both the acidic and alkaline environments. These additional studies do not investigate the potential hydrolysis of DPG under environmental conditions and therefore cannot be used for classification purpose.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

One study of low reliability is available (Unpublished study report, 1980b). The primary degradation of DPG at a concentration of 1 mg/L in filtered river water was assessed in a study conducted with a protocol equivalent to OECD TG 309. However, the test concentration (1 mg/L) exceed the maximum test concentration mentioned in the guideline (0.1 mg/L). A sterile control of autoclaved river water was used. A biodegradability test control composed of quinoline into the buffered river water was used. Only primary degradation of DPG is measured by HPLC-UV method.

The study showed a total loss of the parent substance within 14 days of exposure to non-adapted river water at pH 7.5. After a lag period of several days, DPG was degraded at first week of incubation and disappeared within 2 weeks. The sterile control was not degraded during this period. Quinoline was not degraded in 3 days but disappeared within a week. Identification of potential degradation products and estimation of ultimate biodegradation of DPG were not performed.

11.1.4.4 Photochemical degradation

Based on the data on photochemical degradation in the air obtained by QSAR, DPG is considered to rapidly degrade in the atmosphere via photo oxidation process.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.3 Environmental fate and other relevant information

A calculation method was proposed by France (as eMSCA) during the substance evaluation, for the Henry's law constant using the values of water solubility 325 mg/L, the vapour pressure of 3.7 x 10^{-10} Pa and molecular mass of 211.2, g/mol which gives a value of 2.4 x 10^{-10} $Pa.m^3.mol-1$ (Anses, 2020). This value indicates an extremely low volatility in aqueous solvent.

An OECD TG 106 (Adsorption-Desorption using a batch equilibrium method) study was conducted on 5 soils with different physico-chemical characteristics. Log Koc ranged from 2.5 to 3.13, indicating that DPG does not bind strongly on soil (Unpublished study report, 2015c).

11.4 Bioaccumulation

Table 36: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Log Kow QSAR Epi Suite	2.89	Reliability 2 (reliable with restrictions)	Epi Suite KOWWIN
Log Kow (OECD TG 107)	Log Kow = 2.42 at pH11	Reliability 3 (not applicable to CLP criteria) Method not adapted for ionised or/and surface active substance	Unpublished study report, 2012

Method	Results	Remarks	Reference
		pH not representative of environmental pH	
OECD TG 117	Log Kow > 6.2 at pH 9.3 log Kow = 4 at pH 7.01	Reliability : 3 Method not adapted for ionised or/and surface active substance	Unpublished study report, 2010
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)	Reliability 2 (reliable with restrictions)	BCFwin Program
OECD 305 C (Bioaccumulation: Test for the Degree of Bioconcentration in Fish) *Cyprinus carpio* (freshwater) Exposure: aqueous flow-through system Test substance : DPG Purity: no data Exposure : 42 days Exposure concentrations : 0.1 and 0.01 mg/L Total uptake duration: 42 days	BCF determined as below the limit of detection. BCF < 2 for a exposure concentration of 0.1 mg/L BCF < 20 for a exposure concentration of 0.01 mg/L	Reliability 4 (secondary literature, no sufficient information for assessment) GLP compliance not specified No information on concentrations being nominal or measured Study poorly documented	MITI, 1992cited by SIDS, 2002

11.4.1 Estimated bioaccumulation

A Bioconcentration Factor was estimated to be 37.73 L/kg wet wt by the BCFBAF software using the Arnot-Gobas method. The BCF is therefore considered low but this method model estimates steady-state BCF (L/kg) values for non-ionic organic chemicals. However, the BCF estimated is far below the cut-off of 500 indicated in the CLP guidance (2017).

11.4.2 Measured partition coefficient and bioaccumulation test data

Several values of Kow are available. According to CLP guidance (2017), generally, the highest valid value should take precedence.

However, DPG is considered as a slightly surface-active substance. Besides, its pKa was established to be 10.1 at 20°C by potentiometric method (Anses, 2020). Consequently, at an environmental pH (5-9), the substance is in cationic form and thus has a very high affinity for organic matter and other matrix having a high cation exchange capacity.

It is mentioned in the CLP guidance (2017) that "*for substances like strong acids and bases, substances which react with the eluent, or surface-active substances, a QSAR estimated value of Kow or an estimate based on individual n-octanol and water solubilities should be provided instead of an analytical determination of Kow. Measurements should be taken on ionisable substances in their non-ionised form (free acid or free base) only by using an appropriate buffer with pH below pK for free acid or above the pK for free base*".

In the study conducted according to OECD TG 107, the pH used was 11 which follows the guideline as this value is a unit above the pKa of the substance. However, the OECD 107 method for the determination of partition coefficient is not adapted for surface active substance.

Regarding the study conducted according to OECD TG 117, as stated in the CLP guidance, this method is not adapted for surface active substance. Moreover, the pH used, 9.3 and 7.1, were below the pKa of the substance and thus the test is not compliant with the guideline.

On the basis of these data, the QSAR estimated value should be selected for classification. The log Kow was estimated using KOWWIN v1.68., a predictive model of EPI Suite. DPG has a molecular weight of 211.27 g/mol which is within the training set molecular weights (18.02-719.02 g/mol) and the validation molecular weights (27.03-991.15 g/mol). Log Kow estimated by KOWWIN is 2.89. This value is below the cut-off of 4 indicated in the CLP guidance (2017).

In a study conducted according to OECD TG 305 (Bioaccumulation: Test for the Degree of Bioconcentration in Fish), freshwater fish *Cyprinus carpio* were exposed for 6 weeks in a flow-through system to concentrations of 0.1 or 0.01 mg/L of DPG. The study is reported by SIDS (2002). There is no data regarding the GLP compliance or purity and there is few data to assess the validity and the reliability of the results. The BCF was estimated < 2 and < 20 at exposure concentration of 0.1 and 0.01 mg/L, respectively. This study is used as a supportive data, as there is not sufficient available information to assess the reliability of these results.

Overall, based on available data, it can be concluded that DPG is not expected to bioaccumulate in aquatic organisms.

11.5 Acute aquatic hazard

Table 37: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Examination of water and wastewater and acute toxicity tests with fish US EPA Ecological Research series 660/3-75009 GLP Static Concentrations (nominal) : 0, 7.5, 14, 24, 42, 75 mg/L 10 fish per vessel 1 replicate by concentration Mean length: 29.9 mm Mean weight: 0.72 g Temperature: 22°C (± 1.0°C) pH 8.2 Standard substance (positive control) : antimycin A	*Lepomis macrochirus*	DPG Purity: > 99%	LC50 (96 h): 9.6 mg/L based on: mortality (95% CI: 7.4 - 12 mg/L)	Reliability 2 (reliable with restrictions) No detail on analytical methods Please see annex I for more details	Unpublished study report, 1979b
Examination of Water and Wastewater and acute toxicity tests with fish US EPA Ecological Research series 660/3-75009 GLP Static Concentrations (nominal) : 0, 1, 5.6, 10 mg/L 10 fish per vessel 1 replicate by concentration Mean length: 23.3 mm Mean weight : 0.20 g Temperature : 22°C (± 1.0°C) pH 8.2 Standard substance (positive control) : antimycin A	*Pimephales promelas*	DPG Purity: 99% active ingredient	LC50 (96 h): 4.2 mg/L based on: mortality (95% CI: 3.2 - 5.6)	Reliability 2 (reliable with restriction) No detail on analytical methods Please see annex I for more details	Unpublished study report, 1979c

Method	Species	Test material	Results	Remarks	Reference
Examination of Water and Wastewater and acute toxicity tests with fish US EPA Ecological Research series 660/3-75009 GLP Static Concentrations (nominal) 0, 3.2, 5.6, 10, 18, 32 mg/l 10 fish per vessel 1 replicate by concentration Mean length: 29.3 mm Mean weight : 0.26 g Temperature : 12°C (± 1.0°C) pH 8.2 Standard substance (positive control) : antimycin A	*Oncorhynchus mykiss* (previous name: *Salmo gairdneri*)	DPG Purity: > 99%	LC50 (96 h): 11 mg/L based on: mortality (95% CI: 9.2 - 13 mg/l)	Reliability 2 (reliable with restrictions) No detail on analytical methods Please see annex I for more details	Unpublished study report, 1979d
Acute toxicity test in fish Japanese Industrial Standard test (JIS K 0102 -1986 -71) Static or semi-static	*Oryzias latipes*	DPG Purity: no data	LC50 (48h) = 10 mg/L	Reliability 4 (not sufficient information for assessment and test duration not adapted for classification criteria)	MITI (1992) Cited by SIDS, 2002
Examination of Water and Wastewater and acute toxicity tests with fish US EPA Ecological Research series 660/3-75009 GLP Static Concentrations (nominal) 0, 3.2, 5.6, 10, 18, 32 mg/L 10 daphnia per concentration level 2 replicates by concentration	*Daphnia Magna*	DPG Purity: > 99%	LC50 (48 h): 17 mg/L, based on mortality	Reliability 2 (reliable with restrictions) No detail on analytical methods Please see annex I for more details	Unpublished study report, 1979e

Method	Species	Test material	Results	Remarks	Reference
Temperature : 20°C (±1°C) pH 7.8					
Acute toxicity test in aquatic invertebrates Method: UBA-Verfahrensvorschlag "Bestimmung der Schwimmunfaehigkeit beimWasserfloh "Daphnia magna" (EC0, EC50, EC100; statisches System) (May,1984) GLP Static concentrations (nominal): 0, 1.4, 2.8, 5.5, 11, 22, 44, 88, 177 mg/L Temperature : 20.5 -20.9°C pH 7.8-8.3	*Daphnia Magna*	DPG Purity: 73.8 %	EC50 (24 h): 62.4 (measurement (geometric mean) based on mobility EC50 (24 h): 73.6 mg/L based on mobility EC0 : 22 mg/L EC100 : 177 mg/L	Reliability 3 (not reliable)	Unpublished study report, 1984 Cited by SIDS, 2002
Acute toxicity test in algae Static method US EPA, 1971, Algae assay procedure : bottle test GLP Static Concentrations (nominal) : 0, 0.3, 0.6, 1.0, 3.2, 5.6 mg/l Temperature : reported as 24 +/-1°C (no measurements) pH 7.3-7.6 Initial cell density: 20 000 cells/ml Illumination approximately 3800 lux 3 replicates per concentration	*Raphidocelis subcapitata* (previous names: *Pseudokirchneriella subcapitata, Selenastrum capricornutum*)	DPG Purity: No data	ErC50 (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)	Reliability 2 (reliable with restrictions) Please see annex I for more details	Unpublished study report (1979f)
Acute toxicity test in algae Method: other: cell multiplication inhibition test according to DIN 38412, part 9 GLP Static Concentration (nominal) : 0, 0.01, 0.032,	*Scenedesmus subspicatus* (new name: *Desmodesmus subspicatus*)	DPG Purity: no data	EC50 (72 h): 2.6 mg/L based on biomass ErC50 (72 h): 7.5 mg/L based on growth rate EC10 (72 h): 0.013 mg/L based on biomass	Reliability 2 (reliable with restrictions) Please see annex I for more details	Unpublished study report, 1990a GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, BUA (1992)

Method	Species	Test material	Results	Remarks	Reference
0.1, 0.32, 1.0, 3.2, 10, 32, 100 mg/L			ErC10 (72 h): 2.1 mg/L based on growth rate		Cited in SIDS, 2002

GDCh: German Chemical Society

Regarding acute toxicity, in all the studies available for each trophic level, concentrations are expressed in nominal concentrations and no measurement were made. However, in a study compliant with OECD TG 210 (Fish, Early-life Stage Toxicity Test) presented in the section 11.6.1, the concentration of the substance was monitored. The measured concentration was between 80 and 100% of the nominal concentration. Based on these results, the substance is expected to be stable on a minimum of 3 days. The substance is soluble in water (475 mg/L at pH 7 and 325 mg/L at pH 11). The substance was tested at concentrations below the water solubility and presents a low hydrophobicity potential (log Kow of 2.89, log Koc of 2.5 to 3.13).

Thus, it seems reasonable to use nominal concentrations to assess the substance for the purpose of classification for acute aquatic toxicity.

11.5.1 Acute (short-term) toxicity to fish

5 studies assessing the acute toxicity of DPG in 4 species of fish are available.

A study conducted in the fish species *Lepomis macrochirus* according to the guidelines *US EPA Ecological Research series 660/3-75009* and *standard methods for examination of Water and Wastewater and method of acute toxicity tests with fish, macroinvertebrate and amphibians*, in static conditions, is available (Unpublished study report, 1979b). The study is GLP compliant. After 96h of exposure, the LC50 was **9.6 mg/L** (IC95% = 7.4 – 12 mg/L; nominal concentration).

A study conducted in the fish species *Pimephales promelas* according to the guidelines *US EPA Ecological Research series 660/3-75009* and *standard methods for examination of Water and Wastewater and method of acute toxicity tests with fish, macroinvertebrate and amphibian*s, in static conditions, is available (Unpublished study report, 1979c). The study is GLP compliant. After 96h of exposure, the LC50 was **4.2 mg/L** (IC95% = 3.2 - 5.6 mg/L; nominal concentration).

A study conducted in the fish species *Oncorhynchus mykiss* (previous name: Salmo gairdneri) according to the guidelines *US EPA Ecological Research series 660/3-75009* and *standard methods for examination of Water and Wastewater and method of acute toxicity tests with fish, macroinvertebrate and amphibian*s, in static conditions, is available (Unpublished study report, 1979d). The study is GLP compliant. After 96h of exposure, the LC50 was **11 mg/L** (IC95% = 9.2 – 13 mg/L; nominal concentration).

One other study available was of lower quality and was not considered acceptable due to missing information. The acute toxicity of DPG was assessed in the fish species *Oryzia latipes* according to the Japanese Industrial Standard test JIS K 0102-1986-71 in static or semi-static conditions (MITI (1992)). After 48h of exposure, the LC50 was 10 mg/L.

Overall, based on the data available, the most sensitive fish species to DPG is *Pimephales promelas*. The lower LC50, 96h is 4.2 mg/L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

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

The acute toxicity of DPG was assessed in *Daphnia magna* according to the guidelines *US EPA Ecological Research series 660/3-75009* and *standard methods for examination of Water and Wastewater and method of acute toxicity tests with fish, macroinvertebrate and amphibians*, in static conditions (Unpublished study report, 1979e). After 48h of exposure, the EC50 was 17 mg/L based on mortality (IC95% = 14 – 21 mg/L; nominal concentration). This study is considered as the key study for classification.

Another study was available and assessed the acute toxicity of DPG on *Daphnia magna* in static conditions according to the guideline UBA-Verfahrensvorschlag "*Bestimmung der Schwimmunfaehigkeit beimWasserfloh "Daphnia magna*" (EC0, EC50, EC100; statisches System) (May, 1984) (Unpublished study report, 1984; cited by the SIDS 2002). After 24h of exposure, the EC50 was 73.6 mg/L based on mobility (IC95% = 61.4 - 88.4 mg/L). It should be noted that the

result of reference substance potassium dichromate shows that strain of *Daphnia magna* used for performing the test is not sensitive enough. A low purity of the test substance was also identified as a limit of this study.

According to these data, EC50, 48h is considered to be 17 mg/L (nominal concentration) for aquatic invertebrates.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

2 studies assessed the toxicity of DPG to algae.

The toxicity of DPG on the algae *Pseudokirchnerella subcapitata* was assessed in static conditions according to EPA guideline (Static method US EPA, 1971, Algae assay procedure: bottle test) (unpublished study report, 1979e). After 96h of exposure, the ErC50 was 1.4-1.7 mg/L (nominal concentration).

The SIDS (2002) cited an unpublished study (unpublished study report, 1990) that assessed in GLP compliance the toxicity of DPG on the algae *Scenedesus subspicatus* according to guideline cell multiplication inhibition test, DIN 38412, part 9. After 72h of exposure, the ErC50 and ErC10 were 7.5 mg/L (nominal concentration), and 2.1 mg/L (nominal concentration) respectively based on the growth rate.

According to studies results, the lowest EC50 is 1.4 mg/L. The algae *Pseudokirchnerella subcapitata* is the most sensitive species tested.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

There are no tests for other aquatic organisms available.

11.6 Long-term aquatic hazard

Table 38: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD Guideline 210 (Fish, Early-life Stage Toxicity Test) GLP Semi-static Temperature: 25°C pH: 7.4-8.5 (min-max) Concentration (nominal) : 0; 0.041; 0.13; 0.41; 1.3; 4.1 mg/L Test on embryo and larvae	*Pimephales promelas*	DPG Purity: 99.14%	Based on number hatched: NOEC (34d): 1.3 mg/L (nominal concentration) Based on larval mortality: NOEC (34d): 1.3 mg/L (nominal concentration) Based on weight: NOEC (34d): 1.3 mg/L (nominal concentration) Based on length: NOEC (34d): 1.3 mg/L (nominal concentration)	Reliability : 1 Please see annex I for more details	Unpublished study report, 2014
OECD Guideline 202 part 2 (Daphnia magna Reproduction Test) GLP Semi-static Temperature : 19.7-	*Daphnia magna*	DPG Purity: no data	NOEC (21 d): 0.6 mg/L based on reproduction LOEC (21 d): 1.9 mg/L based on reproduction	Reliability : 2 (reliable with restrictions) Please see annex I for	Unpublished study report, 1990b Cited by SIDS, 2002

Method	Species	Test material	Results	Remarks	Reference
21.8 °C pH : 7.7-8.8 Concentrations not indicated (probably up to 60 mg/L, nominal)				more details	
Static method US EPA, 1971, Algae assay procedure : bottle test GLP Static Concentrations (nominal) : 0, 0.3, 0.6, 1.0, 3.2, 5.6 mg/L Temperature : reported as 24 +/-1°C (no measurements) pH 7.3-7.6 Initial cell density: 20 000 cells/mL Illumination approximately 3800 lux 3 replicates per concentration	*Raphidocelis subcapitata* (previous names: *Pseudokirchneriella subcapitata, Selenastrum capricornutum*)	DPG Purity: no data	EC50 (96 h): 1.4 — 1.7 mg/L based on growth (no. of cells or chlorophyll a) NOEC (96 h) estimated by the DS as 0.3 mg/L based on growth (no. of cells or chlorophyll a) (not defined from study report as the statistics are not presented)	Reliability : 2 (reliable with restrictions) Please see annex I for more details	Unpublished study report (1979f)
Method: other: cell multiplication inhibition test according to DIN 38412, part 9 GLP Static Concentrations (nominal) : 0, 0.01, 0.032, 0.1, 0.32, 1.0, 3.2, 10, 32, 100 mg/L	*Scenedesmus subspicatus* (new name: *Desmodesmus subspicatus*)	DPG Purity: no data	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	Reliability : 2 (reliable with restrictions)	Unpublished study report, 1990a GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, BUA (1992) Cited in SIDS, 2002

11.6.1 Chronic toxicity to fish

One reliable study assessed the long term toxicity of DPG to embryo and fish larvae according to OECD TG 210 (Fish, Early-life Stage Toxicity Test) (Unpublished study report, 2014). The study is GLP compliant.
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 in semi-static conditions. The concentrations of the test substance were monitored for the 1.3 mg/L concentration group. The measured concentrations ranged from 80 to 100% of the nominal concentrations. However, the analysis of the fresh media at day 0 and of the old media at day 2 for the concentration of 1.3 mg/L, showed a concentration of 2.2 mg/L which was considered as anomalous and possibly due to a dilution error on day 0. As no toxicity was observed in this test group, it was not considered to impact the integrity of the results. Hatching and larval mortality rates, larval length and larval weight were recorded. All fish were considered dead at day 9 with the concentration of 4.1 mg/L. No statistically 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.

11.6.2 Chronic toxicity to aquatic invertebrates

One study assessed the long term toxicity of DPG to aquatic invertebrates according to the OECD TG 202 part 2 (unpublished study report, 1990; cited by SIDS, 2002). The study is GLP compliant. Daphnia magna were exposed to DPG in semi-static conditions for 21 days. The concentrations are not clearly indicated but are probably up to 60 mg/L (nominal concentrations). DPG concentration was monitored by HPLC analysis. Results are presented for the concentrations of 0.6 and 1.9 mg/L. At the nominal concentration of 0.6 mg/l, the measured concentration was between 0.5 and 0.58 mg/L. At the nominal concentration of 1.9 mg/L, the measured concentration was between 1.7 and 1.8 mg/L. Consequently, it is possible to use the nominal concentrations.

Regarding the results, no adult mortality was observed up to 1.9 mg/L. At concentrations of 0.6 and 1.9 mg/L, a decrease of reproduction rate by 4.1 and 19.8 %, respectively, was observed. All adults died within 7 days from 6 mg/L onwards. The NOEC and LOEC were 0.6 mg/L (nominal), and 1.9 mg/L (nominal) respectively, based on reproduction.

11.6.3 Chronic toxicity to algae or other aquatic plants

Two studies assessed the toxicity of DPG to algae.

The toxicity of DPG on the algae *Pseudokirchnerella subcapitata* was assessed in static conditions according to EPA guideline (Static method US EPA, 1971, Algae assay procedure: bottle test) (unpublished study report, 1979e). The results of the test were reported as 24, 48, 72 and 96 hour EC_{50}, as the concentration of the test material estimated to cause a 50% decrease of in vivo chlorophyll a in exposed cultures as compared to the control at specified times. Cell numbers in exposed and control cultures were also determined after 96 hours of exposure. A 96 hour EC_{50} was also calculated as the concentration of the test material estimated to cause a 50% decrease of cell numbers in exposed cultures as compared to the control. The concentrations were expressed as nominal concentrations and no measurement were made.

In the OECD TG 210 study (Fish, Early-life Stage Toxicity Test) presented above, the concentration of the substance was monitored. The measured concentration was between 80 and 100% of the nominal concentration. Based on these results, the substance is expected to be stable on a minimum of 3 days. Moreover, the substance is soluble in water (475mg/L at pH 7). The substance was tested at concentrations below the water solubility and present a low hydrophobicity potential (log Kow of 2.89, log Koc of 2.5 to 3.13). Based on these considerations, the concentration is not expected to change during an exposure period of 96h. Thus, it seems reasonable to use nominal concentrations in this study.

After 96h of exposure, the ErC50 was 1.4-1.7 mg/L (based on chlorophyll a and cell number decreases). The NOEC was not defined. It is indicated that a statistical analysis (student's t-test) was performed in this study. However, the statistical significance of the results is not provided. The results are provided in the table below:

Table 39: Results of a 96h exposure of *Raphidocelis subcapitata* (previous names: *Pseudokirchneriella subcapitata, Selenastrum capricornutum*) to DPG.

Nominal concentration (mg/L)	pH	Percentage change

			Chlorophyll a				Cell number
	0h	96h	24h	48h	72h	96h	96h
Control	7.4	7.6	--	--	--	--	--
Solvent control	7.4	7.6	+4	+3	0	+4	+1
0.3	7.4	7.6	+6	-5	-10	-11	-4
0.6	7.4	7.6	+4	-26	-31	-36	-31
1	7.4	7.5	+2	-31	-46	-57	-57
3.2	7.4	7.4	-6	-47	-56	-62	-65
5.6	7.4	7.3	-10	-55	-69	-81	-75

Based on the results, is it considered that the LOEC is included between 0.3 and 0.6 mg/L. Therefore the NOEC is ≤ 0.3 mg/L.The SIDS (2002) cited an unpublished study (unpublished study report, 1990) that assessed, in GLP compliance, the toxicity of DPG on the algae *Scenedesus subspicatus* according to the guideline cell multiplication inhibition test, DIN 38412, part 9. After 72h of exposure, the ErC50 and ErC10 were 7.5 mg/L (nominal concentration), and 2.1 mg/L (nominal concentration) respectively based on the growth rate. An EC10 (72 h) of 0.013 mg/L based on biomass was established. It is indicated in the SIDS evaluation: "the concentration effect curve for biomass was sigmoid in shape, flattening out from 1 mg/l DPG and lower resulting in an abnormally low determination of EbC10. Examination of the log cell number against time leads to the conclusion that significant reduction in cell number compared to the control does not occur below 1 mg/l. Indeed, linear extrapolation of the concentration effect graph for biomass leads to a NOEC of 0.3 mg/l instead of 0.013 mg/l for the S. subspicatus study."

However, based on the graphs available, uncertainties remain on the appropriate conclusion for biomass.

The graphs are presented below:

Figure 2: concentration effect curve for biomass

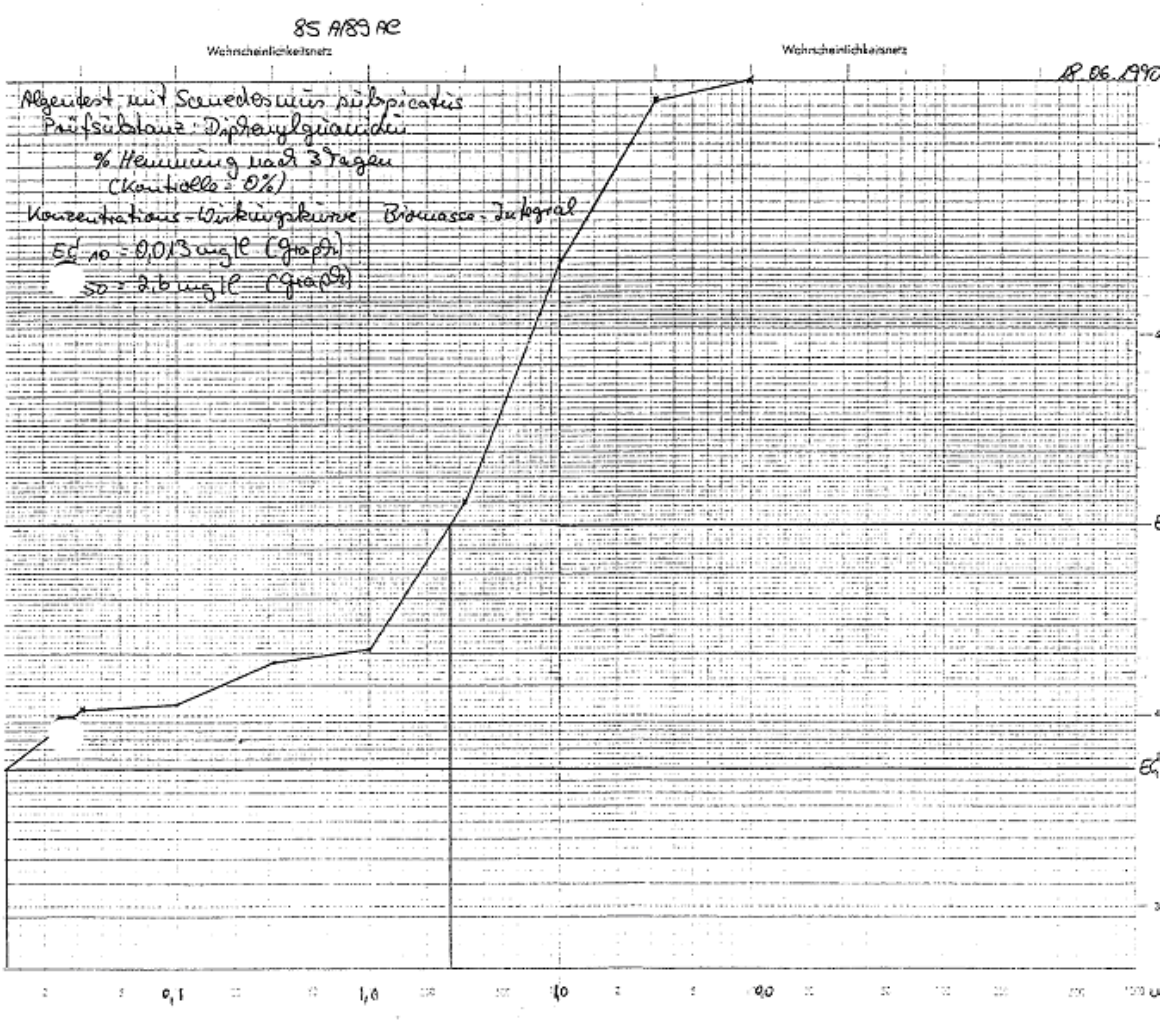
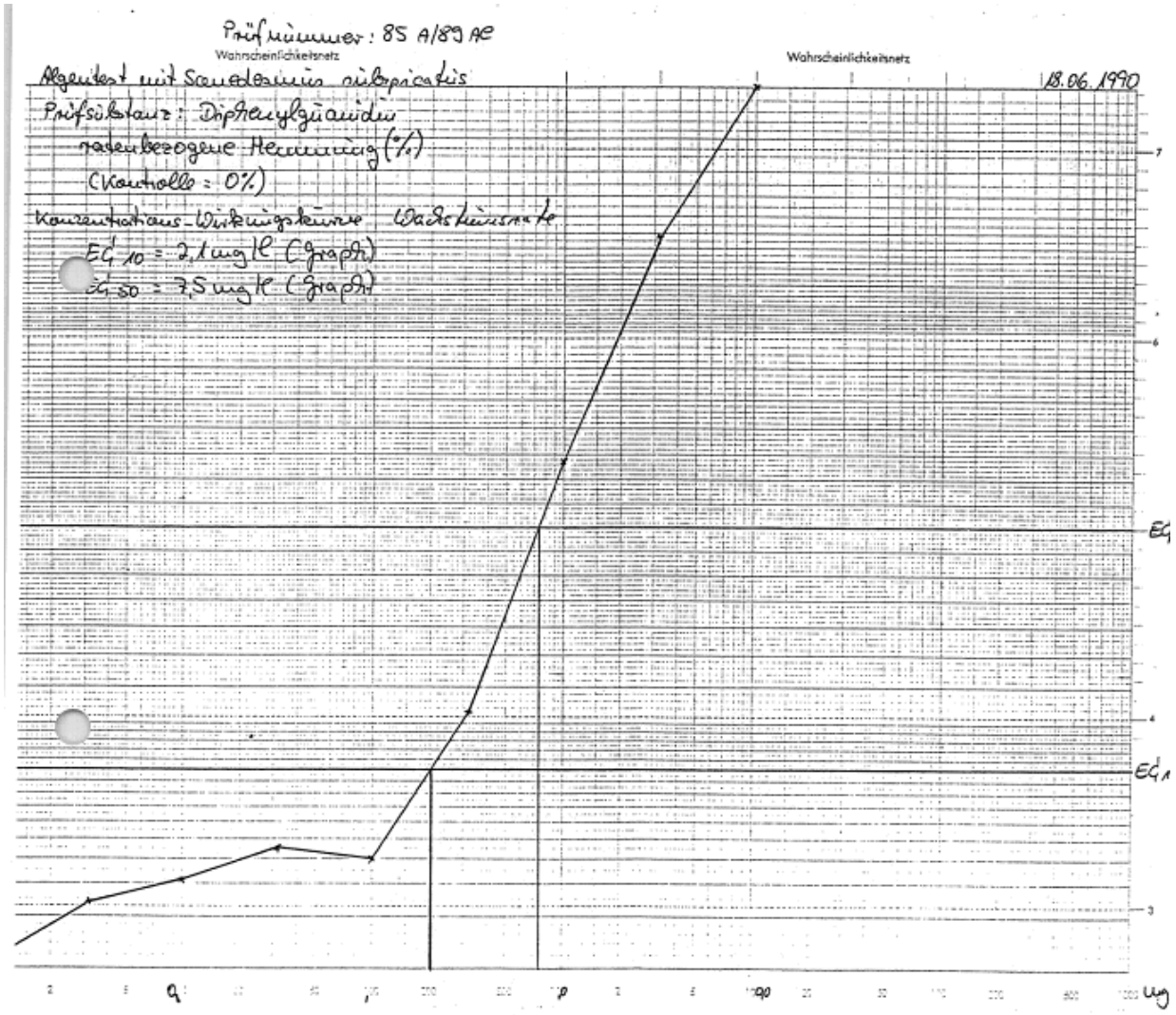


Figure 3: concentration effect curve for cell number



The DS agrees with the conclusions of the SIDS. It should be noted that the NOEC of 0.3 mg/L determined by the SIDS is consistent with the NOEC estimated in the study conducted on Pseudokirchnerella subcapitata described above. Therefore, considering the overall information, the DS considered a NOEC of 0.3 mg/L for algae or other aquatic plants based on data on Raphidocelis subcapitata.

11.6.4 Chronic toxicity to other aquatic organisms

There are no tests for other aquatic organisms available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

	CLP criteria for classification as Acute Tox. 1	1,3-diphenylguanidine	Conclusion
Acute toxicity data	**96 hr LC50 (for fish)** ≤ 1 mg/L and/or	**Fish:** *Pimephales promelas* 96 hr LC50 = 4.2 mg/L (nominal)	No classification

	48 hr EC50 (for crustacea) ≤ 1 mg/L and/or	**Invertebrates:** *Daphnia magna* 48 hr EC50 = 17 mg/L (nominal)	No classification
	72 or 96 hr ErC50 (for algae or other aquatic plants) ≤ 1 mg/L.	**Algae:** *Pseudokirchnerella subcapitata* 96 hr EC50 = 1.4 - 1.7 mg/L (nominal)	No classification

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Classification for long-term aquatic hazard takes into account acute aquatic toxicity and environmental fate data as degradability and bioaccumulation data.

	CLP criteria	1,3-diphenylguanidine	Conclusion
Rapid Degradation	Half-life hydrolysis < 16 days	Hydrolytically stable	Rapidly degradable
	Readily biodegradable in a 28-day test for ready biodegradability	OECD TG 301 D : -86 after 14 days (% degradation O2 consumption) (based on ThOD-NH3) -85 after 28 days (% degradation O2 consumption) (based on ThOD-NO3) 10-day time window criterion fulfilled	
Bioaccumulation	BCF ≥ 500	BCF =37.73 L/kg wet wt	Not bioaccumulative (low potential for bioconcentration in the aquatic environment)
	Log Kow ≥ 4	Log Kow = 2.89	
Rapidly degradable substances for which there are adequate chronic toxicity data available	**Category Chronic 1:** Chronic NOEC or ECx (for fish) ≤ 0.01 mg/L and/or Chronic NOEC or ECx (for crustacea) ≤ 0.01 mg/l and/or Chronic NOEC or ECx (for algae or other aquatic plants) ≤ 0,01 mg/l **Category Chronic 2:** Chronic NOEC or ECx (for fish) ≤ 0.1 mg/L and/or	**Fish:** *Pimephales promelas* 35 d-NOEC = 1.3 mg/L (nominal concentration) **Invertebrates:** *Daphnia magna* 21 d-NOEC = 0.6 mg/L nominal) **Algae:** *Raphidocelis subcapitata* 96 hr-NOEC estimated at 0.3 mg/L (nominal)	Aquatic chronic 3, H412 (based on invertebrates and algae)

	Chronic NOEC or ECx (for crustacea) ≤ 0.1 mg/L and/or Chronic NOEC or ECx (for algae or other aquatic plants) ≤ 0.1 mg/L **Category Chronic 3:** Chronic NOEC or ECx (for fish) ≤1 mg/l and/or Chronic NOEC or ECx (for crustacea) ≤1 mg/L and/or Chronic NOEC or ECx (for algae or other aquatic plants) ≤1 mg/L.		

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Acute aquatic hazard: Data are available for all three trophic levels (fish, crustacae and algae). All reliable short-term toxicity values are > 1 mg/L. Therefore, no acute aquatic classification is proposed. Algae is the most sensitive species.

Chronic aquatic hazard: 1,3-diphenylguanidine has an harmonised classification as Aquatic chronic 2. This classification was adopted during the TC C&L discussion in 1997. The precise basis for this previous classification is not available. A well-conducted OECD TG 301D is now available since then.

The OECD 301D test shows the substance is rapidly degradable and has a low potential for bioaccumulation in the aquatic environment. Chronic toxicity data is available for all three trophic levels. The most sensitive long-term toxicity value is 96 hr-NOEC= 0.3 mg/L (*Raphidocelis subcapitata*). The other study conducted in *Daphnia magna* also displays a 21d-NOEC lower than 1 mg/L. These studies present limits and deviations but on the basis of data available, the classification as aquatic chronic 3 is justified (instead of category 2).

Based on the criteria given in Table 4.1.0(b)(ii) of the CLP Regulation, the harmonised classification of 1,3-diphenylguanidine should be modified for this endpoint to **aquatic chronic 3, H412.**

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

14 ADDITIONAL INFORMATION ON SKIN SENSITIZATION:

Table 40: Summary table of other studies relevant for skin sensitisation with carba mix only

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Retrospective study (April 2015 to March 2019, Japan).	Carba mix (DPG 0.083 mg, ZDEC 0.083	5865 patients patch-tested with the Japanese baseline series of 24 allergens. 4965 patients patch-tested	% positive (95% CI): 5.9 (5.3-6.6) - 5.3 % reactions + - 0.6 % reactions ++/+++ - Women, % positive (95%	Ito et al. (2021)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	mg, ZDBC 0.083 mg) Vehicle not specified	with carba-mix. Reading at days 3 or 4 and 7 Reactions : +, ++ or +++ ?+ : doubtful	CI) : 4.9 (4.3-5.7) - Men, % positive (95% CI) : 9.4 (7.8 -11.3)	
Retrospective study (2015-2018, Austria, Germany, and Switzerland)	Carba mix 3% (1% DPG, 1% ZDEC, 1% ZDBC) Vehicle not specified	51 914 patients patch tested with the European baseline series (EBS) of contact allergens analysed. 18 796 patients patch tested with carba mix.	388 (2.06%) positive to carba mix. - 2.27% reactions + - 0.35% reactions +/++ - women: % positive reactions (95% CI) : 1.97% (1.74-2.22%) - men: % positive reactions (95% CI) : 4% (3.53-4.52%)	Uter *et al.* (2020)
Retrospective study (2009–2012, Austria; Switzerland; Germany; Denmark; Spain; Finland; Italy; Lithuania; Netherlands; Poland; Slovenia)	Carba mix 3% (1% DPG, 1% ZDEC, 1% ZDBC) Vehicle: petrolatum or true test	Readings at day 3 and 5 Reactions : +, ++ or +++ ?+ : doubtful	16 744 patients tested with carba mix in pet. - 2.29% positive reactions - 0.83% reactions : + ? - 0.5% reactions : ++/+++ - 1.8% reactions : + 2436 patients tested with carba mix in TRUE test - 0.48% positive reactions - 1.69% reactions : + ? - 0.16% reactions : ++/+++ - 0.37% reactions : +	Warburton *et al.* (2015 a)
Retrospective study (1996–2012, UK)	Carba mix 3% (1% DPG, 1% ZDEC, 1% ZDBC) Vehicle: petrolatum	Cases of occupational ACD reported between 1996 and 2012.	Between 1996 and 2012, 219 cases of ACD due to carba mix identified. Relative rates for ACD associated with exposure to carba mix and its ingredients showed an increasing incidence, with an average annual percentage increase of 10.1% between 1996 and 2012 (95%CI: 6.1–14.2). These data show a falling reported incidence of occupational ACD attributed to rubber chemicals, but within this a significant rise attributable to the constituents of the carba mix.	Warburton *et al.* (2015 b)
Retrospective study (1972-1981,	Carba mix 3% (1% DPG, 1% ZDEC, 1%	4190 eczema patients patch-tested to 23 screening test substances	Total during the time study : 4190 patients tested with carba mix (1492 males and 2698 females)	Lynde *et al.* (1982)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Canada)	ZDBC) Vehicle not specified		- 6.1% positive reactions - 5.6% in male patients - 6.4% in female patients	

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16 ANNEXES

Please see separate documents for:

- Non-confidential Annex I with the details of the studies relevant for classification (RI 1 and 2)
- Confidential Annex II containing the list of confidential references.