Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Dimethyl propylphosphonate

EC Number: 242-555-3
CAS Number: 18755-43-6

CLH-O-0000007021-89-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 September 2021

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: Dimethyl propylphosphonate

EC Number: 242-555-3

CAS Number: 18755-43-6

Index Number: -

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIMETHYL PROPYLPHOSPHONATE

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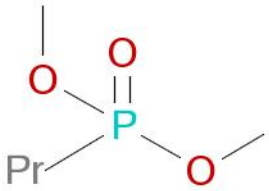
1 IDENTITY OF THE SUBSTANCE

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)	Dimethyl propylphosphonate.
Other names (usual name, trade name, abbreviation)	Phosphonic acid, P-propyl-, dimethyl ester; DMPP.
ISO common name (if available and appropriate)	Not applicable.
EC number (if available and appropriate)	242-555-3
EC name (if available and appropriate)	Dimethyl propylphosphonate.
CAS number (if available)	18755-43-6
Other identity code (if available)	Not applicable.
Molecular formula	C5H13O3P
Structural formula	
SMILES notation (if available)	CCCP(=O)(OC)OC
Molecular weight or molecular weight range	152.13
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable.
Degree of purity (%) (if relevant for the entry in Annex VI)	Not applicable.

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)
Dimethyl propylphosphonate	Mono-constituent substance	None.	Eye Irrit. 2; H319 Repr. 1B; H360

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling

No impurities relevant for classification.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

No additives relevant for classification.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	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	No current Annex VI entry										
Dossier submitters proposal	TBD	Dimethyl propylphosphonate	242-555-3	18755-43-6	Muta. 1B Repr. 1B	H340 H360FD	GHS08 Dgr	H340 H360FD	-	-	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	Dimethyl propylphosphonate	242-555-3	18755-43-6	Muta. 1B Repr. IB	H340 H360FD	GHS08 Dgr	H340 H360FD	-	-	-

Table 6: 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	Hazard class not assessed in this dossier	No
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	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Harmonised classification proposed	Yes
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	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIMETHYL PROPYLPHOSPHONATE

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification and labelling for dimethyl propylphosphonate and it was not previously discussed by the Technical Committee for Classification and Labelling under Directive 67/548/EEC.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

In accordance with article 36 (1) of CLP, justification for action is not required for substances which fulfil the classification criteria for carcinogenicity, germ cell mutagenicity or reproductive toxicity.

5 IDENTIFIED USES

According to the REACH Registration dossiers, dimethyl propylphosphonate is used in rigid foam, foam granules, rebounded PUR and CASE (coatings, adhesives, sealants and elastomers) applications by industrial and professional workers. It is also incorporated into articles which may be used by consumers (ECHA, 2020).

6 DATA SOURCES

Data for dimethyl propylphosphonate are taken from:

- Publically disseminated REACH registration dossier (ECHA, 2020).
- Unpublished study reports provided by the registrants for the mutagenicity and reproductive toxicity endpoints.
- Publically available literature.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid.	ECHA, 2020.	Measured.
Melting/freezing point	- 60 °C.	ECHA, 2020.	Measured at 101.3 kPa.
Boiling point	199 °C.	ECHA, 2020.	Measured. Pressure not reported.
Relative density	1.0202 g/ml.	ECHA, 2020.	Measured at 22 °C.
Vapour pressure	2.2 x 10^{-4} Pa.	ECHA, 2020.	Calculated from measured values at 20 °C (OECD 104).
Surface tension	No data.		
Water solubility	> 90 % at 15°C.	ECHA, 2020.	Measured.
Partition coefficient n-octanol/water	Log Kow (Pow) 0.5	ECHA, 2020.	Measured at 25 °C and pH 7.

Property	Value	Reference	Comment (e.g. measured or estimated)
Flash point	No flash point up to the boiling point	ECHA, 2020.	Measured.
Flammability	No data.		
Explosive properties	No data.		
Self-ignition temperature	375 °C.	ECHA, 2020.	Measured. Pressure not reported.
Oxidising properties	Not oxidising.	ECHA, 2020.	Measured using method UN O.2; time for pressure rise from 690 kPa to 2070 kPa was between 9.5 – 17.5 seconds.
Granulometry	Not applicable.		
Stability in organic solvents and identity of relevant degradation products	No data.		
Dissociation constant	Not applicable.		
Viscosity	2.2 mPa/s.	ECHA, 2020.	Measured at 20 °C.

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated as part of this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No data available.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not evaluated as part of this dossier.

10.2 Acute toxicity - dermal route

Not evaluated as part of this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated as part of this dossier.

10.4 Skin corrosion/irritation

Not evaluated as part of this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated as part of this dossier.

10.6 Respiratory sensitisation

Not evaluated as part of this dossier.

10.7 Skin sensitisation

Not evaluated as part of this dossier.

10.8 Germ cell mutagenicity

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

10.8.1.1 *In vitro* mutagenicity/genotoxicity

Table 8: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Similar to OECD 471: Bacterial reverse mutation test. Duplicate plates. GLP compliant. Study did not meet current guideline requirements to include a 5th strain (*S. Typhimurium* TA102 or *E.coli* WP2 uvrA or WP2 uvrA (pKM101).	DMPP (purity > 98 %).	*S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537. 0, 8, 40, 200, 1000 and 5000 µg/plate DMPP. ± metabolic activation with rat liver S9 (Aroclor 1254 induced). Vehicle control: DMSO. Positive controls: Sodium azide, nitrofurantoin and 4-nitro-1,2-phenylene diamine (-S9); 2-aminoanthracene (+ S9). Reliability: reliable.	Result: negative ± metabolic activation. No cytotoxicity observed.	Anonymous, 1993a.
OECD 476: Mammalian cell gene mutation test. GLP compliant.	DMPP (purity > 98 %).	V-79 cell line derived from Chinese hamster lung cells (HPRT locus). 0, 500, 1000, 2000, 3000, 4000 and 5000 µg/ml DMPP for 5 hours ± metabolic activation with rat liver S9 (Aroclor 1254 induced). Expression period: 6 days. Vehicle control: DMSO. Negative control: untreated cells.	Result: negative ± metabolic activation. No cytotoxicity observed.	Anonymous, 1993b.

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		Positive control: ethylmethanesulfonate (- S9) and dimethylbenzanthracene (+ S9). Reliability: reliable.		

10.8.1.2 *In vivo* mutagenicity/genotoxicity

Table 9: Summary table of mutagenicity tests in mammalian somatic and germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
OECD 478: Rodent dominant lethal test. GLP compliant. Highest dose exceeded the maximum tolerated dose. Animals mated 1 male: 2 females. No statistical analysis of the data performed. No historical control data reported.	DMPP (purity > 99 %).	Pilot study: 5 male B6C3F1/BOM mice/dose. 0, 250, 500, 1000 and 2000 mg/kg bw/day administered by oral gavage for 14 days. Main study: 20 male B6C3F1/BOM mice/group; 40 female CRL: CD1 mice/group/mating interval. Males: 0, 500, 1000 and 2000 mg/kg bw/day DMPP via oral gavage 5 days/ week for 13 weeks. Females: untreated. Mating intervals: 5, 9 and 13 weeks. Vehicle: Deionised water. Positive control: 2000 mg/kg bw/day dimethyl methylphosphonate. Females sacrificed 16 days post-mating. Living implants, dead implants, total implants and corpora lutea were recorded. Reliability: reliable.	Clinical signs included apathy, ↑ & ↓ motility, staggered gait, sternal recumbency and difficulty breathing at ≥ 500 mg/kg bw/day. Result: positive. 1/20 males at 1000 mg/kg bw/day died prior to the second mating interval at week 9. 12/20 males at 2000 mg/kg bw/day died prior to study termination. ↓ fertilisation rate in females mated with males at 2000 mg/kg bw/day. ↑ pre-implantation loss per fertilized female at ≥ 1000 mg/kg bw/day. ↑ post-implantation loss per fertilized female at ≥ 500 mg/kg bw/day.	Anonymous, 1995a.
Supporting study on structural analogue. Similar to OECD 478: Rodent dominant test. Not GLP compliant. Animals mated 1 male: 2 females. No concurrent positive control group. No historical control data reported. Corpora lutea not counted.	Dimethyl methylphospho nate. (purity > 99 %).	20 male B6C3F1 mice/group; 40 female CD-1 mice/group. A further 20 male mice/group were assigned to 0, 1000 and 2000 mg/kg bw/day recovery groups. Males: 0, 250, 500, 1000 and 2000 mg/kg bw/day dimethyl methylphosphonate via oral gavage 5 days/ week for 13 weeks. Females: untreated. Mating intervals: 4, 8 and 12 weeks. Males in the recovery groups were treated to week 13 and then mated at the end of the 15 week recovery period. Vehicle: water.	Result: positive. ↑ number of dead implants (early resorptions) per female at all mating intervals at 2000 mg/kg bw/day and at 4 and 12 week mating intervals at 1000 mg/kg bw/day. ↓ number of live foetuses per female at all mating intervals at 2000 mg/kg bw/day and at 4 and 12 week mating intervals at 1000 mg/kg bw/day. No increase in number of dead implants per	Dunnick, *et.al.*, 1984a

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Positive control: none. Females sacrificed 16 days from the middle of the mating interval and the uterine contents examined. Number of live and dead implants, and percentage resorptions recorded. Males sacrificed after 13 weeks or at the end of the recovery period. Analysis of epididymal sperm concentrations, and luteinising hormone (LH) and follicle stimulating hormone (FSH) levels. Histopathological examination of a number of organs including of kidney, prostate, coagulating gland, preputial gland, ductus deferens, seminal vesicle, penis, testes and epididymis. Reliability: reliable.	female and live foetuses per female at 1000 or 2000 mg/kg bw/day following 15 week recovery period. Other examinations: No treatment related effects on sperm concentrations, LH or FSH levels. No treatment related microscopic findings in any organ.	
Supporting study on structural analogue. Similar to OECD 478: Rodent dominant test. Not GLP compliant. Animals mated 1 male: 2 females. No concurrent positive control group. No historical control data reported. Corpora lutea not counted.	Dimethyl methylphosphonate (purity > 99 %).	20 male Fischer 344 rats/group; 40 female Fischer 344 rats/group. Males: 0, 250, 500, 1000 and 2000 mg/kg bw/day dimethyl methylphosphonate via oral gavage 5 days/ week for 90 days. Females: untreated. Mating interval: days 84 – 88. Vehicle: water. Positive control: none. Females sacrificed 14 days from the middle of the mating interval and uterine contents examined. Number of live pups, dead pups and percentage of resorptions recorded. Males sacrificed after 90 days. Analysis of epididymal sperm, and LH and FSH levels. Histopathological examination of a number of organs including of kidney, prostate, testes and epididymis. Reliability: reliable.	Result: positive. ↓ male fertility index at 2000 mg/kg bw/day. 0/20 females were pregnant at 2000 mg/kg bw/day. ↓ total number of pregnant females at ≥ 1000 mg/kg bw/day. ↓ number of live foetuses per litter at ≥ 500 mg/kg bw/day. ↑ number of resorptions at ≥ 250 mg/kg bw/day. Other examinations: ↓ body weight gain in males at 2000 mg/kg bw/day. ↓ relative epididymis weight at 2000 mg/kg bw/day and kidney weight at ≥ 1000 mg/kg bw/day. ↑ incidence of regeneration, hyaline droplet generation, cytoplasmic hyaline bodies and cellular infiltrate into the interstitum of kidney in	Dunnick, *et.al.*, 1984b

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			males at ≥ 250 mg/kg bw/day. Testicular lesions observed in 18/20 males at 2000 mg/kg bw/day (0/20 in control). Prostate lesions observed in 1/20 and 4/20 males at 1000 and 2000 mg/kg bw/day (0/20 in control). ↓ % motile sperm at ≥ 1000 mg/kg bw/day; ↓ sperm count and ↑ incidence of sperm head abnormalities at 2000 mg/kg bw/day. No treatment related effects LH or FSH levels.	
Similar to OECD 483: mammalian spermatogonial chromosome aberration test with deviations. Minimum number of animals in the high dose group was not in line with current guideline requirements. Study did not meet current guideline requirements requiring scoring of at least 200 metaphases per animal. Positive control did not produce an increase in chromosomal aberrations. No results tables reported. GLP compliant.	DMPP (purity > 99 %).	Following 13 weeks of treatment in a rodent dominant lethal study (Anonymous, 1995a), 3 - 5 male B6C3F1/BOM mice from each group were selected for this study. An additional single dose of DMPP administered via oral gavage to 5 mice/group at 0, 500, 1000 mg/kg/day DMPP and 3 mice/group at 2000 mg/kg bw/day. Vehicle: Deionised water. Positive control: 2000 mg/kg bw/day dimethyl methylphosphonate. Colcemid administered 20 hours after treatment and animals sacrificed 4 hours later. Spermatogonial cells from the testicular tubules were isolated and 100 metaphases examined microscopically for structural chromosomal aberrations. Reliability: unreliable.	Result: negative. 1/3 males at 2000 mg/kg bw/day DMPP died prior to sacrifice. No ↑ chromosomal aberrations in spermatogonial cells in DMPP groups. No ↑ chromosomal aberrations in spermatogonial cells in positive control.	Anonymous, 1998a.
Similar to OECD 474: mammalian erythrocyte micronucleus test with deviations. Bone marrow samples were taken at only one time point. Study did not meet	DMPP (purity > 99 %).	Following 13 weeks of treatment in a rodent dominant lethal study (Anonymous, 1995a), 5 male B6C3F1/BOM mice from each group were selected for this study. An additional single dose of 0, 500, 1000 and 2000 mg/kg/day DMPP via oral gavage.	Result: negative. No ↑ in micronucleated PCEs in DMPP treated groups. No ↑ in micronucleated PCEs in positive control. ↑ in micronucleated	Anonymous, 1995b.

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
current guideline requirements to score at least 4000 polychromatic erythrocytes (PCEs) per animal. Positive control did not produce the expected response. GLP compliant.		Vehicle: Deionised water. Positive control: 2000 mg/kg bw/day dimethyl methylphosphonate. Animals sacrificed 24 hours after final treatment. Bone marrow collected from femur, stained and fixed. 2000 PCEs and normochromatic erythrocytes (NCE) scored for micronuclei & number of NCE per 1000 PCE reported. Reliability: unreliable.	NCEs at 1000 mg/kg bw/day DMPP and in the positive control group.	
Similar to OECD 475: mammalian bone marrow chromosome aberration test with deviations. Minimum number of animals and dose groups not in line with current guideline requirements. Study did not meet current guideline requirements requiring analysis of at least 200 metaphases per animal. Mitotic index was not reported. Positive control did not produce the expected response. GLP compliant.	DMPP (purity > 99 %).	Following 13 weeks of treatment in a rodent dominant lethal study (Anonymous, 1995a), 4 - 5 male B6C3F1/BOM mice from each group were selected for this study. 2 additional doses of DMPP administered via oral gavage to 5 mice/group at 0 and 500 mg/kg/day DMPP and 4 mice/group at 1000 mg/kg bw/day. Vehicle: Deionised water. Positive control: 2000 mg/kg bw/day dimethyl methylphosphonate. Following sacrifice of the animals, bone marrow was extracted from the femur. 100 metaphases per animal were examined for structural chromosomal aberrations. Reliability: unreliable.	Result: negative No ↑ in structural chromosomal aberrations in DMPP treated groups. No ↑ in structural chromosomal aberrations in the positive control group.	Anonymous, 1996.
Non-guideline: alkaline elution assay in mouse testes. DNA was eluted under alkaline conditions from a suspension of testicular cells and the DNA concentration in the eluted and filtered fractions was determined. Positive control did not increase DNA strand breakage.	DMPP (purity > 99 %).	Following 13 weeks of treatment in a rodent dominant lethal study (Anonymous, 1995a), 5 male B6C3F1/BOM mice from each group were selected for this study. An additional single dose of 0, 500 and 1000 mg/kg/day DMPP administered by oral gavage. Vehicle: Deionised water. Positive control: 2000 mg/kg bw/day dimethyl methylphosphonate. Animals sacrificed 24 hours after last treatment. DNA prepared from	Result: negative No ↑ in DNA strand breaks in DMPP treated groups. No ↑ in DNA strand breaks in positive control group. Cell viability for DMPP & positive control groups comparable with the negative control.	Anonymous, 1998b.

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
GLP compliant.		testicular cells. Assessment criteria: Negative if none of the doses tested induced a biologically relevant and significant increase in DNA single strand breaks. Positive if a dose-dependent, significant and in parallel treated animals reproducible increase in DNA single strand break induction was observed. Reliability: unreliable.		

Table 10: Summary table of other tests relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Histopathological analysis of the testes and epididymides of male mice. Limited reporting of method and results.	DMPP (purity > 99 %).	Following 13 weeks of treatment in a rodent dominant lethal study (Anonymous 1995a), 5 male B6C3F1/BOM mice from each group were selected for the mammalian erythrocyte micronucleus test and received an additional single dose of 0, 500, 1000 and 2000 mg/kg/day DMPP (Anonymous, 1995b). At the end of the micronucleus test, the testes & epididymides from males in the vehicle control, dimethyl propylphosphonate and positive control groups were fixed and stained for histopathological analysis. Reliability: unreliable.	↑ incidence of testicular atypic cells & giant cells at 2000 mg/kg bw/day DMPP & in positive control.	Anonymous, 1995c.

10.8.2 Short summary and overall relevance of the provided information on germ cell mutagenicity

***In vitro* studies**

In a bacterial reverse mutation test, dimethyl propylphosphonate was not mutagenic in four strains of *S. typhimurium* (TA 98, TA 100, TA 1535 and TA 1537) when tested up to 5000 µg/plate with and without metabolic activation. The dossier submitter notes that in accordance with the most recent version of OECD 471, the study did not include a fifth strain (*S. Typhimurium TA102* or *E.coli* WP2 uvrA or WP2 uvrA (pKM101) to detect DNA cross-linking. In an *in vitro* mammalian cell gene mutation test, dimethyl propylphosphonate was not mutagenic in Chinese hamster lung cells at the *hprt* locus at doses up to 5000 µg/ml with and without metabolic activation.

***In vivo* studies**

In a rodent dominant lethal test conducted in accordance with OECD 478 and to GLP, dimethyl propylphosphonate was administered to groups of 20 male B6C3F1/BOM mice at 0, 500, 1000 and 2000 mg/kg bw/day for 13 weeks (Anonymous, 1995a). Males were mated with untreated females (40 females/group) at mating intervals of 5, 9 and 13 weeks. 1/20 males at 1000 mg/kg bw/day died prior to the second mating interval at 9 weeks. 12/20 males at 2000 mg/kg bw/day died prior to study termination: 5/20 males prior to the first mating interval at 5 weeks, 3/20 males prior to the second mating interval at 9 weeks and 4/20 males prior to the third mating interval at 13 weeks. Clinical signs observed following dosing of males at 1000 mg/kg bw/day and above included apathy, semi-anaesthetised state, reduced reflexes, recumbency and difficulty breathing.

The fertilisation rates, averaged over the three mating intervals, were reported as 88.3 %, 90 %, 81 % and 34.7 % for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. The study report notes that the reduced motility and the decrease in body temperature observed in males at 2000 mg/kg bw/day dimethyl propylphosphonate group would have resulted in a lower DNA synthesis rate and sperm production, which may have impacted on the fertility rate observed at this dose. The dossier submitter notes high mortality and clinical signs of toxicity were observed in males at 2000 mg/kg bw/day dimethyl propylphosphonate and therefore considers that it cannot be excluded that the lower fertilisation rates in this group may be attributed to the systemic toxicity of dimethyl propylphosphonate to males rather than a specific genotoxic effect.

Due to the high mortality rate in males at 2000 mg/kg bw/day, the results for all groups are presented per fertilised female. A decrease in the number of corpora lutea per fertilised female was observed at 2000 mg/kg bw/day: the averages over the three mating intervals were 14.4, 13.2, 12.4 and 8.3 for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. The number of implantations per fertilised female were also reduced at 2000 mg/kg bw/day: the averages over the three mating intervals were 13.4, 12.0, 11.0, 5.9 for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. There was an overall increase in the pre-implantation loss per fertilised female at 1000 mg/kg bw/day and above: the average over the three mating intervals was 0.9, 1.1, 1.5 and 2.4 for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. No significant effect on pre-implantation loss was observed in the positive control group.

A dose dependent decrease in the number of living implants per fertilised female was observed. The averages over the three mating intervals were 12.7, 9.1, 4.9 and 1.0 for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. A conversely dose dependent increase in the number of dead implants per fertilised female was observed. The average number over the three mating intervals were 0.8, 3.0, 6.0 and 4.9 for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. There was an overall dose dependent increase in the rate of post implantation loss per fertilised females: the average over the three mating intervals was 5.6 %, 24.5%, 55.0 % and 82.6 % for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. There was also an increase in the rate of post-implantation loss in the positive control (24.8 %).

The study report states that due to the “clear-cut” results, no statistical analysis of the data was performed and the study authors concluded that there was a clear indication of a mutagenic effect of dimethyl propylphosphonate under the conditions of the study. The dossier submitter acknowledges that the lack of statistical analysis performed could be considered a limitation of the study. However, a clear biologically significant response was observed in the dimethyl propylphosphonate treated groups which is indicative of a treatment related effect.

The dossier submitter considers that the study was well-conducted and reliable, and that the clear increase in pre- and post-implantation loss in untreated females mated with dimethyl propylphosphonate treated males are indicative of a treatment related effect. The dossier submitter concludes that under the conditions of this study,

dimethyl propylphosphonate induced dominant lethal mutations in mice. A summary of the results are presented in Table 11 below.

Table 11: Summary of the effects observed in the rodent dominant lethal test with dimethyl propylphosphonate (Anonymous, 1995a)

Dose (mg/kg bw/day)	Mating interval	Number of corpora lutea per fertilised female	Implantations per fertilised female	Pre-implantation loss per fertilised female	Living implants per fertilised female	Dead implants per fertilised female
0	1	14.8	13.6	1.14	12.7	0.95
	2	13.8	13.1	0.69	12.3	0.72
	3	14.5	13.6	0.91	13.0	0.58
	Mean	**14.4**	**13.4**	**0.9**	**12.7**	**0.8**
500	1	13.3	12.4	0.89	9.6	2.75
	2	12.8	11.4	1.41	8.6	2.85
	3	13.4	12.3	1.11	9.0	3.24
	Mean	**13.2**	**12.0**	**1.1**	**9.1**	**3.0**
1000	1	12.5	11.6	0.9	5.4	6.2
	2	12.3	11.0	1.28	4.7	6.31
	3	12.5	10.3	2.22	4.7	5.59
	Mean	**12.4**	**11.0**	**1.5**	**4.9**	**6.0**
2000	1	6.6	4.79	1.79	0.8	4.0
	2	7.6	6.0	1.57	1.1	4.86
	3	10.8	7.0	3.8	1.2	5.8
	Mean	**8.3**	**5.9**	**2.4**	**1.0**	**4.9**
Positive control	1	13.9	12.5	1.36	9.1	3.44
	2	13.6	13.0	0.55	9.7	3
	3	13.8	12.1	1.65	9.3	2.82
	Mean	**13.8**	**12.5**	**1.2**	**9.4**	**3.1**

Two studies with the structurally similar substance, dimethyl methylphosphonate, investigating dominant lethal effects in mice and rats are provided as supporting evidence. Dimethyl methylphosphonate was selected as a "class specific positive control" in the dominant lethal study with dimethyl propylphosphonate (Anonymous, 1995a, described above). In the first study, dimethyl methylphosphonate was administered to groups of male mice via oral gavage at 0, 250, 500, 1000 and 2000 mg/kg bw/day for 13 weeks (Dunnick *et al*., 1984a). Males were mated with untreated females at mating intervals of 4, 8 and 12 weeks. A further 20 males at 0, 1000 and 2000 mg/kg bw/day were subject to a 15 week recovery period and then mated with untreated females. No effect on fertilisation rates was observed at any dose. A statistically significant decrease in the number of live implants per female was observed at 1000 mg/kg bw/day (mating interval 1 and 3) and

2000 mg/kg bw/day (all three mating intervals). The average number of living implants per female over the three mating intervals was 11.1, 11.5, 11.2, 10.2 and 6.7 for the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. There was a converse statistically significant increase in the number of dead implants (classified as early resorptions) per female at 1000 mg/kg bw/day (mating interval 1 and 3) and 2000 mg/kg bw/day (all three mating intervals). The average number of dead implants per female over the three mating intervals was 0.8, 0.8, 0.9, 1.5 and 3.9 for the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. The percentage of resorptions was statistically significantly increased at 1000 mg/kg bw/day (mating interval 1 and 3) and 2000 mg/kg bw/day (all three mating intervals). The average percentage resorptions over the three mating intervals was 7.8, 6.9, 7.4, 12.9 and 36.6 for the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. A statistically significant increase in the percentage of dominant lethal mutations[1] was reported at 2000 mg/kg bw/day at all three mating intervals and at 1000 mg/kg at the 4 and 12 week mating intervals. The average percentage of dominant lethal mutations over the three mating intervals was -3.3, -1, 7.7 and 44 for the 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. At the mating interval following the 15 week recovery period, no increase in the number of dead implants per female or decrease in live foetuses per female were observed at 1000 or 2000 mg/kg bw/day, which indicates that there was some recovery in males following cessation of treatment.

In the second study, dimethyl methylphosphonate was administered via oral gavage to male rats at 0, 250, 500, 1000 and 2000 mg/kg bw/day for 90 days and males were then mated with untreated females (mating interval 84 days) (Dunnick *et al*., 1984b). A lack of spermatogenesis, and degeneration, vacuolisation and necrosis of spermatogonial cells was observed in the testes of 18/20 males at 2000 mg/kg bw/day (compared with 0/20 in the control group). The percentage of motile sperm was statistically significantly reduced from 1000 mg/kg bw/day: 80.2 %, 80.5 %, 79.7 %, 71.5 % and 35.8 % in the 0, 200, 500, 1000 and 2000 mg/kg bw/day groups, respectively. The epididymal sperm count was statistically significantly decreased at 2000 mg/kg bw/day (219 x 10^6 per g caudal epididymal tissue compared with 541 x 10^6 per g caudal epididymal tissue in the control). There was also a statistically significant increase in the incidence of sperm head abnormalities at 2000 mg/kg bw/day (42 compared with 5 in the control).

The male fertility index was statistically significantly reduced at 2000 mg/kg bw/day due to no females at this dose becoming pregnant (0/40 compared with 20/40 in the control group). The male fertility indices were 70 %, 75 %, 60 %, 40 % and 0 % in the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. The publication reports that there was evidence of mating in the 2000 mg/kg bw/day group as 11/20 males had sperm positive females (the number of sperm positive females per group was not reported).

There was a statistically significant decrease in the percentage of pregnant females at 1000 mg/kg bw/day and above. The percentage of pregnant females were 50 %, 47.5 %, 42.5 %, 27.5 % and 0 % in the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. There was a statistically significant decrease in the number of live foetuses per litter from 500 mg/kg bw/day. The incidences were 7.6, 7.8, 5.7, 0.82 and 0 in the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. The percentage of resorptions was statistically significantly increased from 250 mg/kg bw/day. The incidences were 6.1 %, 14.9 %, 39.4 %, and 79.1 % in the 0, 250, 500 and 1000 mg/kg bw/day groups, respectively (no data is reported for the 2000 mg/kg bw/day since no females were impregnated).

Although the studies with dimethyl methylphosphonate had some limitations including the lack of concurrent positive control and limited reporting, they provide evidence of a treatment related effect on post implantation loss in both mice and rats and add to the concern for germ cell mutagenicity for dimethyl propylphosphonate.

[1] Dunnick *et al.* 1984a reports that the percentage of dominant lethal mutations was calculated as 1 minus (average number of implants in the test group ÷ average number of implants in the control group) x 100.

At the end of the 13 week treatment period in the dominant lethal test (Anonymous, 1995a, described above), 4-5 males from each treatment group were selected for a number of follow up *in vivo* genotoxicity studies. Due to the high mortality rate of males in the 2000 mg/kg bw/day group in the dominant lethal test, some of the follow up *in vivo* genotoxicity studies had either a lower number of animals assigned to the 2000 mg/kg bw/day group or the studies were performed with only two doses (500 and 1000 mg/kg bw/day). These follow up *in vivo* genotoxicity studies are described below.

In a mammalian spermatogonial chromosome aberration test, similar to OECD 483 but with deviations, a single additional dose of dimethyl propylphosphonate was administered to 5 males at 0, 500 and 1000 mg/kg bw/day and 3 males at 2000 mg/kg bw/day. 5 males in the positive control group were similarly treated. No increase in chromosome aberrations were observed in spermatogonial cells in the dimethyl propylphosphonate treated groups or in the positive control group.

In a mammalian erythrocyte micronucleus test, similar to OECD 474 but with deviations, a single additional dose of dimethyl propylphosphonate was administered to 5 males at 0, 500, 1000 and 2000 mg/kg bw/day. 5 males in the positive control group were similarly treated. No increase in the incidence of micronucleated polychromatic erythrocytes was observed in the dimethyl propylphosphonate treated groups or in the positive control. Histopathological examination of the testes and epididymides from animals in this study was performed. A treatment related increase in the incidence of atypic cells (2/5) and giant cells (3/5), graded minimal to slight, in the germinal epithelium or the tubular lumen of the testes of males treated with 2000 mg/kg bw/day dimethyl propylphosphonate was observed when compared with the negative control (0/5). The incidence of atypic cells and giant cells of the testes in the positive control group was 3/5 and 1/5, respectively. No abnormalities were reported in the epididymides at any dose. The dossier submitter considers that these findings may indicate that dimethyl propylphosphonate reaches the testes. However, the dossier submitter notes that only a limited histopathological examination was performed on a small number of animals and therefore considers that no firm conclusions can be drawn from this data.

In a mammalian bone marrow chromosome aberration test, similar to OECD 478 but with deviations, two additional doses of dimethyl propylphosphonate were administered to 5 males at 0, 500 mg/kg bw/day and 4 males at 1000 mg/kg bw/day. 5 males in the positive control group were similarly treated. No increase in the frequency of cells with structural chromosome aberrations was observed in the dimethyl propylphosphonate groups or in the positive control group.

In a non-guideline alkaline elution assay, a single additional dose of dimethyl propylphosphonate was administered to 5 males at 0, 500 and 1000 mg/kg bw/day. 5 males in the positive control group were similarly treated. No increase in DNA strand breaks was observed in the dimethyl propylphosphonate groups or in the positive control group.

The dossier submitter notes that the follow up *in vivo* genotoxicity studies had a number of limitations. Of note was that the positive control, dimethyl methylphosphonate, did not elicit a positive response in any of the studies. Therefore, the acceptability criteria for the studies are not met. In addition, in some of the studies there was an insufficient number of dose groups or number of animals per dose group when compared with the relevant test guideline. Also, the number of cells counted in some of the studies was lower than that recommended in the relevant test guideline. The dossier submitter concludes that the follow up *in vivo* genotoxicity studies are not reliable and therefore do not negate the positive result observed in the dominant lethal test with dimethyl propylphosphonate.

Further details on the above studies are provided in Annex I to this report.

10.8.3 Comparison with the CLP criteria

According to Annex I to the CLP Regulation, substances may be classified as category 1A germ cell mutagens *"if they induce heritable mutations in the germ cells of humans"* and that classification is based on positive evidence from human epidemiological studies. No epidemiological data are available to demonstrate heritable gene mutations in humans. Therefore, classification in category 1A is not warranted.

According to Annex I to the CLP Regulation, substances may be classified as category 1B germ cell mutagens if there are *"positive results from in vivo heritable germ cell mutagenicity tests in mammals or positive results from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells…or positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny…".*

In the available dominant lethal test with dimethyl propylphosphonate, a clear increase in pre- and post-implantation loss were observed in untreated females mated with treated males, indicating that dimethyl propylphosphonate produced dominant lethal mutations under the conditions of the study. Therefore, classification in category 1B is warranted.

According to Annex I of the CLP Regulation, substances may be classified as category 2 germ cell mutagens if positive results are obtained *in vivo* somatic cell mutagenicity tests or somatic cell genotoxicity tests supported by positive results from *in vitro* mutagenicity assays. The available positive results from a rodent dominant lethal mutation test with dimethyl propylphosphonate provide evidence of *in vivo* heritable germ cell mutation in the mouse. Therefore, classification in category 2 is not appropriate.

10.8.4 Conclusion on classification and labelling for germ cell mutagenicity

Based on the available data, classification of dimethyl propylphosphonate as a category 1B germ cell mutagen is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Dimethyl propylphosphonate (DMPP) did not induce mutagenic effects in bacteria and in mammalian cells *in vitro*.

In vivo, dominant lethal mutations (increased pre- and post-implantation losses) were induced by DMPP in the mouse, indicative of germ cell mutagenicity. Negative results were obtained in follow-up *in vivo* genotoxicity studies of the dominant lethal assay, in the same surviving mice at the end of the 13-week period (cytogenics test in bone marrow and spermatogonia, alkaline elution test in testes, micronucleus test in bone marrow and histopathology of male gonads). Nevertheless, the negative results were considered unreliable by the dossier submitter (DS), as the positive control dimethyl methylphosphonate (DMMP) did not provide the expected positive response.

The DS also reported two positive *in vivo* dominant lethal assays in mice and rats with the structurally similar substance DMMP, used as the positive control in the DMPP studies. Although these two dominant lethal studies had limitations (lack of positive control), they added to the concern of germ cell mutagenicity of DMPP.

Based on the positive *in vivo* germ cell study (dominant lethal test) with DMPP, the DS proposed to classify DMPP in Muta. 1B, H340.

Comments received during consultation

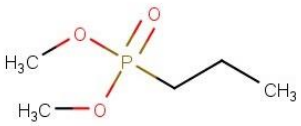
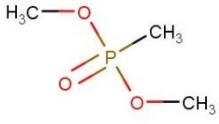
Two member states (MS) agreed with the DS's proposal based on the positive germ cell mutagenicity test. One MS highlighted that no reliable micronucleus or chromosomal aberration tests were available *in vitro* or *in vivo* (somatic cells) to confirm the mechanism of mutagenicity. In addition, as decreased fertilisation rates and increased pre-implantation losses were noted in the rodent dominant lethal assay at 2000 mg/kg, it was not possible to conclude if the observed pre-implantation losses were due to dominant lethal effects or not. The DS was also requested to provide further justification of the read-across between DMMP and DMPP.

The DS agreed that no conclusion on the mechanism of mutagenicity could be drawn. The DS agreed that the dominant lethal test is designed to detect dominant lethal mutations fixed post fertilisation in the early embryo, and that the test design does not allow a definitive conclusion regarding whether the increase in pre-implantation loss observed with DMPP is only due to a dominant lethal effect. The DS also considered that the effect on pre-implantation losses were presented "per fertilised female" and was thus independent of the reduced fertility rate. The DS highlighted that no read-across was proposed with DMMP. The DS considered that the positive results on the dominant lethal assay using DMPP is sufficient to support classification. Nevertheless, in response to the MS's comment a brief profile of both substances was provided based on their respective registration dossiers (See additional key elements below).

Additional key elements

Information for DMPP and DMMP provided by the DS during the consultation or provided in the ECHA dissemination website (as available on June 2021) are summarised in the table below. The DS highlighted that the 2 substances are both organophosphorus compounds. DMMP has no harmonised classification but is self-classified Muta. 1B, H340. Overall, based on the available data, the DS concluded that DMPP and DMMP were structurally and toxicologically similar and data provided on DMMP can be used as supportive.

***Table**: Data matrix*

Chemical name	Dimethyl propylphosphonate (DMPP)	Dimethyl methylphosphonate (DMMP)
CAS no.	187554-13-6	756-79-6
Structural formula		
Molecular weight	152 g/mol	124 g/mol
Vapour pressure	0.00022 Pa at 20°C	128 Pa at 25°C
Partition coefficient	0.5	-0.61
Water solubility	> 900 g/l at 15°C	> 100 g/l at 21 °C
Acute oral	LD_{50} (rat) > 2000 mg/kg (OECD TG 401)	LD_{50} (rat) > 2000 mg/kg (Non-guideline study)
Skin irritation/corrosion	Not irritating (OECD TG 404)	Not irritating (non-guideline study)

Eye irritation	Eye irritant (OECD TG 405)	Eye irritant (non-guideline study)
Skin sensitisation	Not sensitising (OECD TG 429)	Not sensitising (similar to OECD TG 406)
Repeated dose	Oral (rat): LOAEL = 5 mg/kg bw/day (nephropathy at 5 mg/kg bw/day in males, hepatocellular hypertrophy at 40 mg/kg bw/day, ↓ grip strength at 1000 mg/kg bw/day in females) OECD TG 407 (28-day study)	Oral (rat): LOAEL = 535 mg/kg bw/day (nephropathy, liver weight increase at 1790 mg/kg bw/day) Similar to OECD TG 407 (28-day study) LOAEL = 65 mg/kg bw/day (nephropathy, hepatocellular hypertrophy at ≥ 195 mg/kg bw/day, testicular atrophy at ≥ 250 mg/kg bw/day) Similar to OECD TG 408 (90-day study)
Genetic toxicity *in vitro*	Ames: negative (similar to OECD TG 471) Chromosomal aberration: No data Gene Mutation in mammalian cells: negative (OECD TG 476)	Ames: negative (similar to OECD TG 471) Gene Mutation in mammalian cells: positive (OECD TG 476) Chromosomal aberration: negative (OECD TG 472) Sister chromatid exchanges: positive (OECD TG 479)
Genetic toxicity *in vivo*	Dominant lethal assay: positive in mice Additional investigations: negative (micronucleus, cytogenicity in bone marrow and spermatogonia, alkaline elution with testes, histopathology of tested and epididymides)	Dominant lethal assay: positive in rats and mice
Carcinogenicity	No data	Female rats and mice: no effects LOAEL male rats = 500 mg/kg bw/day (kidney tubular cell adenocarcinoma and papillomas, mononuclear cell leukemia) Male mice: inadequate (low survival) Similar to OECD TG 451
Prenatal developmental toxicity	LOAEL = 500 mg/kg bw/day (Decreased live birth index, mean litter size, percentage of male pups, viability index, fertility index, number of implantation sites, number of pups at birth) pilot reproductive toxicity study	LOAEL = 1000 mg/kg bw/day (delayed development in absence of marked maternal toxicity) Similar to OECD TG 414
Fertility and sexual function		No data

RAC notes that the substances target the same organs but some differences in potency are noted between the compounds.

Assessment and comparison with the classification criteria

The potential mutagenicity of DMPP has been studied both *in vitro* and *in vivo*.

In vitro studies

One negative mutagenicity assay with standard strains of *S. typhimurium* was reported in the CLH report. The assay gave negative results. The assay was similar to OECD TG 471 but a fifth strain to detect DNA cross-linking (*S. Typhimurium* TA102 or *E.coli* WP2) was not included.

A negative gene mutation test (HPRT locus) in hamster V79 cells, performed according to OECD TG 476, tested DMPP at 400 to 5000 µg/ml with and without metabolic activation.

In conclusion, DMPP showed no mutagenic potential when tested *in vitro*. Nevertheless, the available amount/type of *in vitro* data is very limited and no cytogenicity assay in mammalian cells is available.

In vivo studies with DMPP

The *in vivo* mutagenic potential of DMPP was assessed in a rodent dominant lethal test in mice following 13-week treatment. Due to the positive results obtained in this assay, the surviving mice at the end of the 13-week period (4-5 animals per group) were used for a follow up. The following tests were performed:
- a mammalian spermatogonial chromosome aberration test,
- a mammalian bone marrow micronucleus test,
- a mammalian bone marrow chromosome aberration test,
- an alkaline elution assay in testes,
- histopathology of gonads (testes and epididymides).

Rodent dominant lethal assay

In the rodent dominant lethal test (GLP-compliant), twenty male C6B3F1-mice per group received DMPP at 0, 500, 1000 and 2000 mg/kg bw/day by gavage, 5 days per weeks for 13 weeks. Treated males were mated with 40 untreated females per group and per mating interval. The mating intervals were 5, 9 and 13 weeks. The dominant lethal assay was performed according to OECD TG 478 except that statistical analysis was not performed and no historical controls were provided. In addition, the positive control used in the study was DMMP. This positive control was considered class specific in the study report. Although this positive control is not proposed in the OECD test guideline, the substance provided positive results in rodent dominant lethal assays (Dunnick et al., 1984 a and b), supporting the suitability of the positive control to demonstrate the sensitivity of the assay.

The top dose exceeded the maximum tolerable dose as 12/20 males died at 2000 mg/kg bw/day before the study termination. It is also reported that males, at this dose, had reduced motility and decreased body temperature. At 1000 mg/kg bw/day, one male died before the study termination. Clinical signs in males at 1000 mg/kg included apathy, semi-anaesthetised state, reduced reflexes, recumbence and difficulty in breathing. At 500 mg/kg bw/day, no mortality or clinical signs were observed. No effects on male body weight were reported at any dose.

Fertilisation rates were decreased in females at medium and top dose, being 88%, 90%, 81% and 35% at 0, 500, 1000 and 2000 mg/kg bw/day. The decrease fertilisation rate observed at the top

dose may be accounted for to the general toxicity observed at 2000 mg/kg bw/day and not to specific effect on the germ cells.

An increase in dead implants, pre-implantation loss and post-implantation losses and a dose-related decrease in viable implants and total implants was noted following DMPP exposure for the three mating trials. Due to the high general toxicity in males at 2000 mg/kg bw/day, cytotoxicity cannot be excluded. RAC agrees with the DS that although no statistical analysis was performed, a clear trend for the parameters related to a mutagenic effect was observed after exposure to DMPP at ≥ 500 mg/kg bw/day onward. In addition, RAC agrees with the DS that as the results were expressed "per fertilised females", the pre-implantation losses could be due to dominant lethal effects independently of the low fertilisation rate observed in the study.

***Table**: Pre- and post-implantation losses in fertilised female mice in the rodent lethal test with DMPP (Anonymous, 1995a)*

Dose group (mg/kg bw/day)	**0**	**500**	**1000**	**2000**	**Positive control**
Fertilisation rates (%)	88	90	81	35	87
Number of corpora lutea[1]	14.4	13.2	12.4	8.3	13.8
Total implants[1]	13.4	12.0	10.9	5.54	12.6
Pre-implantation losses[1]	0.92	1.1	1.5	2.1	1.2
Living implants[1]	12.7	9.1	4.9	1.0	9.4
Dead implants[1]	0.75	3.0	6.0	4.9	3.1
Post-implantation losses (%)	5.6	25	55	83	25

[1]Mean per fertilised female mice over 3 matings

RAC agrees with the DS that under the condition of the study, DMPP induced dominant lethal mutation in male mice.

In vivo follow-up studies

All the *in vivo* follow-up studies had limitations compared to recommended test guidelines: absence of historical control data, only two dose levels or low number of animals at the top dose, etc. In addition, RAC agrees with the DS that the follow-up studies are unreliable as the positive control DMMP failed to produce positive responses. RAC notes that it is unclear whether the use of DMMP as a positive control was appropriate in these studies as there are no data in the DMMP database to confirm that the substance would be positive in these assays.

In the mammalian spermatogonial chromosome aberration test (similar to OECD TG 483 with limitations), single additional dose of DMPP was administered to 5 males at 0, 500 and 1000 mg/kg bw and 3 males at 2000 mg/kg bw. 5 males in the positive control group were similarly treated. No increase in chromosome aberrations were observed in spermatogonial cells in DMPP groups or in the positive control group (DMMP).

In the mammalian erythrocyte micronucleus test, similar to OECD TG 474 but with limitations, a single additional dose of DMPP was administered to 5 males at 0, 500, 1000 and 2000 mg/kg bw and in the positive control group (DMMP). Males were euthanised 24 hours following treatment. As noted by the DS, only one time point was analysed. No increase in the incidence of micronucleated polychromatic erythrocytes was observed in the DMPP treated groups or in the positive control.

In the mammalian bone marrow chromosome aberration test, similar to OECD TG 475 but with limitations, two additional doses of DMPP were administered to 5 males at 0, 500 mg/kg bw/day

and in the positive control group and 4 males at 1000 mg/kg bw/day. No increase in the frequency of cells with structural chromosome aberrations was observed in the DMPP groups or in the positive control group.

In the non-guideline alkaline elution assay in testes, a single additional dose of DMPP was administered to 5 males at 0, 500 and 1000 mg/kg bw or DMMP (positive control). Animals were sacrificed 24 hours after the final treatment. No increase in DNA strand breaks was observed in the DMMP groups or in the positive control group. RAC notes that harvesting 24 hours after the last treatment is not appropriate as DNA strand breaks may have already been removed, repaired or lead to cell death (as stated in the OECD TG 489).

Histopathological examination of the testes and epididymides was performed at the end of the micronucleus assay. The analysis revealed a treatment-related increase in the incidence of atypic cells (2/5) and giant cells (3/5), graded minimal to slight, in the germinal epithelium or the tubular lumen of the testes of males treated with 2000 mg/kg bw compared with control (0/5). According to the DS, at 2000 mg/kg bw, spermatogenesis was apparently not affected in most of tubules and epididymides contained plenty of sperm. The incidence of atypic cells and giant cells of the testes in the positive control group (DMMP) was 3/5 and 1/5, respectively. No effects were reported in the epididymides at any dose. RAC agrees with the DS as a low number of animals were investigated and as the reporting was limited, no firm conclusion can be drawn based on these data.

RAC considers that as negative results were obtained in the follow-up studies, no conclusion on the mechanism of mutagenicity can be drawn. Moreover, the negative results obtained in the follow up studies had limitations and do not overrule the positive results obtained in the dominant lethal assay.

In vivo studies with DMMP

Two dominant lethal assays are available in mice and rats with the structural analogue DMMP as supporting information. Several limitations were noted in these studies: no positive controls were used, the number of corpora lutea was not counted and no historical control data were reported. The DS also highlighted the limited reporting of the method and results.

Male rats and mice were treated for 90 days, 5 days per week at 0, 250, 500, 1000 and 2000 mg/kg bw/day of DMMP. 20 males and 40 females were used per groups. The mating intervals in mice were 4, 8 and 12 weeks. There was only one mating between days 85 to 88 in rats. A recovery group was included in the mice study. This recovery group was kept for additional 15 week without treatment and were mated to untreated mice at week 29.

In male rats, up to 2000 mg/kg bw/day, no effect on survival was noted. Decreased body weight, histopathological findings in kidney, testes and prostate gland as well as changes in sperm analysis were noted at 2000 mg/kg bw/day. A dose-related decrease in fertility index was noted and at the top dose, male rats failed to impregnate females. A statistically significant decrease in the number of live implants was noted at ≥ 500 mg/kg bw/day and resorptions were reported at ≥250 mg/kg bw/day.

In male mice, no general toxicity and no histopathological findings in male reproductive organs were noted up to 2000 mg/kg bw/day. No effects on fertilisation rates were observed. A dose-related statistically significant decrease in dead implants (mainly early resorptions) and a decreased number of live foetuses were noted in the female mice at ≥1000 mg/kg bw/day. These effects were

not observed in the recovery group. Based on these two published studies, the male rats were more sensitive than the male mice to the effects of DMMP.

RAC agrees with the DS that the positive results of the dominant lethal assays on DMMP support the mutagenic potential of DMPP.

Comparison with criteria

In vitro data: The available *in vitro* gene mutation assays were negative in bacteria and mammalian cells. No *in vitro* chromosomal aberration or micronucleus assays were available.

In vivo data in somatic cells: There is no evidence that DMPP was mutagenic in somatic cells. Nevertheless, RAC agrees with the DS that the negative results obtained in the *in vivo* follow-up studies of the dominant lethal assay should be interpreted with care as the positive control used in the study failed to induce the expected response and as several limitations were noted in the somatic cell studies.

In vivo data in germ cells: there is evidence that DMPP induced heritable mutations *in vivo* based on the positive rodent dominant lethal assay in mice. Although some limits were noted in the assay (e.g. no statistical analysis), the study is considered acceptable for classification purposes. The dominant lethal assay is designed to detect dominant lethal mutations resulting from chromosomal aberration (not excluding gene mutation). RAC notes that there are no data available to confirm the potential mechanism of action of the substance. Nevertheless, the positive results observed in the dominant lethal studies in both rat and mice with the structurally similar substance DMMP provide supportive evidence that a classification is warranted. In addition, a decrease in the total number of implants and pup viability and an increase in post-implantation losses was observed in a pilot reproductive toxicity assay performed with DMPP in rats (see reproductive toxicity section). These findings would be expected in case of a germ cell mutagen and the results of this pilot study also provide supportive evidence that DMPP is a germ cell mutagen.

Therefore, based on the overall available information, RAC agrees with the DS's proposal that DMPP warrants classification as Muta. 1B, H340.

10.9 Carcinogenicity

Not evaluated as part of this dossier. No carcinogenicity data is available for dimethyl propylphosphonate.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 12: 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
Non-guideline: pilot study for an OECD 408/422.	DMPP (purity 97%). Oral gavage.	Parental animals general: ↓ body weight gain in females at	Anonymous, 2012

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Not GLP compliant. 5 Wistar HsdRCCHan:Wist rats/sex/dose. Animals mated 1 male:1 female. Pregnant females were allowed to litter and nurse pups until at least PND 4. Number of live and dead pups and sex ratio of pups determined on PND 0 and 4. Parental animals and pups necropsied at end of study. Reliability: reliable.	0, 20, 100 and 500 mg/kg bw/day. Vehicle: corn oil. Treatment for 2 weeks prior to the 2 week mating period, and to Day 44 in males and to PND 4 in females.	500 mg/kg bw/day on GD 14 – 20. ↑ food consumption in males at 500 mg/kg bw/day in weeks 5 and 6 and in females at 500 mg/kg bw/day in week 2 (pre-mating) and during gestation. ↑ incidence of renal pelvic dilation in females at 500 mg/kg bw/day. ↑ incidence of renal cortical basophilic tubules and tubular dilation in males at ≥ 20 mg/kg bw/day. ↑ incidence hyaline droplets in kidneys of males at ≥ 100 mg/kg bw/day. Reproductive parameters: At 500 mg/kg bw/day: ↓ fertility index, ↓ no. of implantation sites, ↓ no. of pups at birth, ↑ in pre-natal loss. F1 pups: At 500 mg/kg bw/day: ↓ live birth index, ↓ mean litter size, ↓ in % of male pups. No pups survived to PND 1.	
OECD 478: Rodent dominant lethal test. GLP compliant. 20 male B6C3F1/BOM mice/group; 40 female CRL:CD1 mice/group/mating interval. Animals mated 1 male: 2 females. Mating intervals were 5, 9 and 13 weeks. Females were sacrificed 16 days post mating. Living implants, dead implants, total implants and corpora lutea were recorded. Highest dose exceeded the maximum tolerated dose. No statistical analysis of the data performed. No historical control data.	DMPP (purity > 99 %). Oral gavage. Males: 0,500, 1000 and 2000 mg/kg bw day DMPP via oral gavage 5 days/ week for 13 weeks. Females: untreated. Vehicle: deionised water. Positive control: 2000 mg/kg bw/day dimethyl methylphosphonate.	Result: positive. 1/20 males at 1000 mg/kg bw/day died prior to the second mating interval at week 9. 12/20 males at 2000 mg/kg bw/day died prior to study termination. ↓ fertilisation rate in females mated with males at 2000 mg/kg bw/day. ↑ pre-implantation loss per fertilized female at ≥ 1000 mg/kg bw/day. ↑ post-implantation loss per fertilized female at ≥ 500 mg/kg bw/day.	Anonymous, 1995a

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reliability: reliable.			

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

In a non-guideline pilot reproductive toxicity study, 5 Wistar rats/sex/group were administered 0, 20, 100 and 500 mg/kg bw/day dimethyl propylphosphonate via oral gavage. Treatment began two weeks prior to mating and up to 44 days in males and to post-natal day (PND) 4 (6-7 weeks) in females.

At 500 mg/kg bw/day, a statistically significant decrease in maternal body weight was observed on gestational days (GD) 18 to 20, with a corresponding statistically significant decrease in body weight gain in the same group during GD 14 to 20. The mean maternal body weight during gestation is reported in Table 13 below.

Table 13: Mean maternal body weight during gestation from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg bw/day)	Mean maternal body weight (g)					
	GD 0	GD 7	GD 14	GD 18	GD 19	GD 20
0	246	272.3	302.5	348.3	362.3	380.3
20	241.8	269.0	299.8	347.3	362.5	380.5
100	243.8	271.0	296.0	337.6	348.8	361.8
500	235.7	270.0	293.3	321.7*	325.7**	332.7**

* = $p < 0.05$; ** = $p < 0.01$

In order to further assess whether the effect on material body weight observed in the high dose group was due to maternal toxicity or an intrauterine effect, the corrected mean maternal body weight changes were calculated in accordance with Annex I, 3.7.2.4.4 of CLP. These are reported in Table 14 below. No significant effect on the calculated mean corrected maternal body weight change was observed at any dose.

Table 14: Calculated corrected mean maternal body weight change using maternal body weight on GD 21/22 from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg bw/day)	Mean initial maternal body weight on study day 1 (g)	Mean terminal maternal body weight on GD 21/22 (g)	Mean maternal body weight change (g)	Mean pup weight (g)	Mean corrected maternal body weight change (g)*
0	222.6	369.8	147.2	69.28	77.92
20	219.2	374.4	155.2	66.62	88.58

100	217.4	375.4	158.0	70.92	87.08
500	216.6	306.0	89.4	8.5	80.9

*Calculated as the difference between the maternal body weight on study day 1 and terminal maternal body weight (GD 21/22), minus pup weights.

It is noted that at 500 mg/kg bw/day, there is a large variation in the individual corrected maternal body weight changes (44 g – 116 g, mean value 80.9 g). This variation is due to 2/5 females with no implantation sites and 2/5 females with pups that died. Of the two females with pups that died, one had one pup (pup weight not recorded) and the other had three pups, where the weight was recorded for only one pup (5.3 g). The absence of the recorded pup weights for these females may result in a slight error in the mean corrected maternal body weight for this group. In addition, it is noted that the weight of the placentas was not recorded and thus was not considered in the corrected maternal body weight change calculation at any dose. In order to correct for these aspects, the mean corrected maternal body weight was also calculated using the maternal body weight on lactation day 0 (LD 0), which are reported in Table 15 below. No significant effect on the mean corrected maternal body weight change was observed when the maternal body weight on LD 0 was used for the calculation. Further detail, including individual maternal body weight data, is included in Annex I to this report.

Table 15: Calculated corrected mean maternal body weight change using maternal body weight on LD 0 from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg bw/day)	Mean initial maternal body weight on study day 1 (g)	Mean maternal body weight on LD 0 (g)	Mean corrected maternal body weight change (g)*
0	222.6	290.5	67.9
20	219.2	286.8	67.6
100	217.4	282.4	65.0
500	216.6	282.7	66.1

* Calculated as the difference between maternal body weight on study day 1 and LD 0

Mean food consumption was statistically significantly increased in females at 500 mg/kg bw/day during week 2 of the pre-mating period (81.3 g/kg bw/day compared with 63.3 g/kg bw/day in the control group) and during gestation days 0 to 7 (93.4 g/kg bw/day compared with 72.7 g/kg bw/day in the control group) and 7 to 14 (91.2 g/kg bw/day compared with 76.6 g/kg bw/day in the control group). Mean food consumption was also increased in males at 500 mg/kg bw/day during week 5 (57.9 g/kg bw/day compared with 49.8 g/kg bw/day in the control group) and week 6 (56.6 g/kg bw/day compared with 47.6 g/kg bw/day in the control group) of the pre-mating period.

Table 16: Mean food consumption in females from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg	Mean food consumption (g/kg bw/day)

bw/day)	Premating week 1	Premating week 2	GD 0-7	GD 7-14	GD 14-20	LD 0-4
0	67.2	63.3	72.7	76.6	76.3	99.6
20	74.2	71.3	77.3	79.1	79.1	106.0
100	72.3	71.0	74.7	74.4	78.4	101.2
500	84.7	81.3**	93.4*	91.2*	91.0	-[a]

* = $p < 0.05$; **=$p < 0.01$

a= No data reported (females in this group had no live litters and were necropsied at the end of the gestation period).

At 500 mg/kg bw/day, pelvic dilation of the kidneys was observed in 4/5 females compared with 0/5 in the control group. Renal tubular dilation, degeneration, papillary necrosis, pelvic degeneration and transitional cell hyperplasia was also observed in 1/5 females at 500 mg/kg bw/day. In males, an increased incidence of renal tubular dilation was observed from 20 mg/kg bw/day. The incidences were 1/5, 5/5, 5/5 and 3/5 in the 0, 20, 100 and 500 mg/kg bw/day groups, respectively.

A biologically significant decrease in the fertility index was observed in females at 500 mg/kg bw/day (60 % compared with 80 % in the control group) due to 2/5 females in this group not conceiving. No effect on mating or gestation indices or mating performance was observed in any of the dimethyl propylphosphonate groups.

Table 17: Summary of insemination, fertility and gestation indices from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg bw/day)	Mating index	Fertility index	Gestation index	No. of litters with live born pups
0	100 %	80 %	100 %	4
20	100 %	80 %	100 %	4
100	100 %	100 %	100 %	5
500	100 %	60 %	100 %	2

At 500 mg/kg bw/day, there was a biologically significant decrease in the total number of implantation sites (33 compared with 56 in the control group). At this dose, there was also a statistically significant decrease in the total number of pups delivered (12 compared with 53 in the control group) and litter size (5.0 compared with 13.25 in the control group), which resulted in a significant increase in the post implantation loss (21 compared with 3 in the control group).

Table 18: Summary of data relating to post implantation loss from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg bw/day)	No. of implantation sites (total)	No. of implantation sites (per litter)	No. of pups at birth (total)	Post implantation loss (total)	Post implantation loss (per litter)
0	56	14.0	53	3	0.75
20	58	14.5	54	4	1.00

100	65	13.0	60	5	1.00
500	33	11.0	12*	21	7.00*

* = $p < 0.01$

In a dominant lethal test where untreated females were mated with males treated with dimethyl propylphosphonate (Anonymous, 1995a see also section 10.8), a significant effect on pre- and post-implantation loss per fertilised female was observed at 1000 mg/kg bw/day and above.

On the basis of the pilot reproductive toxicity study, the REACH registration dossier for dimethyl propylphosphonate applies a self-classification of category 1B reproductive toxicant and proposes no further testing for the endpoint of toxicity to reproduction.

The dossier submitter notes that the available pilot reproductive toxicity study with dimethyl propylphosphonate has a number of limitations, in particular the group size was lower than that recommended in OECD 421 thus decreasing the sensitivity of the study to detect effects on sexual function and fertility. However, despite this, a significant effect on the fertility index and post implantation loss was observed at 500 mg/kg bw/day. The dossier submitter considers these effects to be treatment related.

10.10.3 Comparison with the CLP criteria

According to Annex I to the CLP Regulation, substances may be classified as category 1A reproductive toxicants if they are known "*human reproductive toxicants*".

No epidemiological data are available to demonstrate reproductive toxicity in humans. Therefore, classification in category 1A is not warranted.

According to Annex I to the CLP Regulation, substances may be classified as category 1B if presumed to be a human reproductive toxicant. The classification of a substance as category IB reproductive toxicant "…*is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function or 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".*

In the available pilot reproductive toxicity study with dimethyl propylphosphonate, a biologically significant decrease in the fertility index was observed in females at 500 mg/kg bw/day when compared with the concurrent control group. At the same dose, there was a significant decrease in the number of implantation sites and the total number of pups born, leading to an increase in post implantation loss. These effects are indicative of an effect on sexual function and fertility. In addition, an increase in the incidence of pre- and post-implantation loss was observed in untreated females mated with dimethyl propylphosphonate treated males in a dominant lethal test, again indicative of an effect on sexual function and fertility.

The dossier submitter notes that the pilot reproductive toxicity study with dimethyl propylphosphonate has a number of limitations, in particular the group size was lower than that recommended in OECD 421 thus decreasing the sensitivity of the study to detect effects. However, despite these limitations, the study provides clear evidence of an effect on sexual function and fertility in the high dose group (500 mg/kg bw/day). These effects were not considered to be secondary non-specific consequence of other toxic effects. In addition, the effect on pre- and post-implantation loss in the dominant lethal test provides supporting evidence for an effect

on sexual function and fertility. Therefore, based on the available information, the dossier submitter considers that classification in category 1B is warranted for effects on sexual function and fertility.

According to Annex I to the CLP Regulation, a substance may be classified as category 2 if it is a suspected human reproductive toxicant. The classification of a substance as category 2 reproductive toxicant is warranted "*…where there is some evidence from humans or experimental animals…of an adverse effect on sexual function and fertility, or on development…if deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification".*

The available pilot reproductive toxicity study with dimethyl propylphosphonate provides clear evidence of an effect on sexual function and fertility which is not considered to be a secondary non-specific consequence of other toxic effects. Therefore, classification in category 2 is not considered appropriate.

10.10.4 Adverse effects on development

Table 19: 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
Non-guideline: pilot study for an OECD 408/422. Not GLP compliant. 5 Wistar HsdRCCHan:Wist rats/sex/dose. Animals mated 1 male:1 female. Pregnant females were allowed to litter and nurse pups until at least PND 4. Number of live and dead pups and sex ratio of pups determined on PND 0 and 4. Parental animals and pups necropsied at end of study. Reliability: reliable.	DMPP (purity 97%). Oral gavage. 0, 20, 100 and 500 mg/kg bw/day. Vehicle: corn oil. Treatment for 2 weeks prior to the 2 week mating period, and to Day 44 in males and to PND 4 in females.	Parental animals general: ↓ body weight gain in females at 500 mg/kg bw/day on GD 14 – 20. ↑ food consumption in males at 500 mg/kg bw/day in weeks 5 and 6 and in females at 500 mg/kg bw/day in week 2 (pre-mating) and during gestation. ↑ incidence of renal pelvic dilation in females at 500 mg/kg bw/day. ↑ incidence of renal cortical basophilic tubules and tubular dilation in males at ≥ 20 mg/kg bw/day. ↑ incidence hyaline droplets in kidneys of males at ≥ 100 mg/kg bw/day. Reproductive parameters: At 500 mg/kg bw/day: ↓ fertility index, ↓ no. of implantation sites, ↓ no. of pups at birth, ↑ in pre-natal loss. F1 pups: At 500 mg/kg bw/day: ↓ live birth index, ↓ mean litter size, ↓ in % of male pups. No pups survived to PND 1.	Anonymous, 2012
OECD 478: Rodent dominant lethal test. GLP compliant. 20 male B6C3F1/BOM mice/group; 40 female CRL:CD1 mice/group/mating interval. Animals mated 1 male: 2 females. Mating intervals were 5, 9 and 13 weeks. Females were sacrificed 16 days post mating. Living implants, dead implants, total implants and corpora lutea were recorded.	DMPP (purity > 99 %). Oral gavage. Males: 0,500, 1000 and 2000 mg/kg bw day DMPP via oral gavage 5 days/ week for 13 weeks. Females: untreated. Vehicle: deionised water. Positive control: 2000 mg/kg bw/day dimethyl methylphosphonate.	Result: positive. 1/20 males at 1000 mg/kg bw/day died prior to the second mating interval at week 9. 12/20 males at 2000 mg/kg bw/day died prior to study termination. ↓ fertilisation rate in females mated with males at 2000 mg/kg bw/day. ↑ pre-implantation loss per fertilized female at ≥ 1000 mg/kg bw/day. ↑ post-implantation loss per fertilized female at ≥ 500 mg/kg bw/day.	Anonymous, 1995a

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Highest dose exceeded the maximum tolerated dose. No statistical analysis of the data performed. No historical control data. Reliability: reliable.			

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

In a non-guideline pilot reproductive toxicity study, 5 Wistar rats/sex/group were administered 0, 20, 100 and 500 mg/kg bw/day dimethyl propylphosphonate via oral gavage. Treatment began two weeks prior to mating and up to 44 days in males and to post-natal day (PND) 4 (6-7 weeks) in females.

A statistically significant decrease in maternal body weight was observed at 500 mg/kg bw/day on gestational days (GD) 18 to 20, with a corresponding statistically significant decrease in body weight gain in the same group during GD 14 to 20 (see Table 13 in section 10.10.2).

In order to further assess whether the effect on maternal body weight observed in the high dose group was due to maternal toxicity or an intrauterine effect, the corrected mean maternal body weight changes were calculated in accordance with Annex I, 3.7.2.4.4 of CLP. These are reported in Table 20 below. No significant effect on the calculated mean corrected maternal body weight change was observed at any dose.

Table 20: Calculated corrected mean maternal body weight change using maternal body weight on GD 21/22 from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg bw/day)	Mean initial maternal body weight on study day 1 (g)	Mean terminal maternal body weight on GD 21/22 (g)	Mean maternal body weight change (g)	Mean pup weight (g)	Mean corrected maternal body weight change (g)*
0	222.6	369.8	147.2	69.28	77.92
20	219.2	374.4	155.2	66.62	88.58
100	217.4	375.4	158.0	70.92	87.08
500	216.6	306.0	89.4	8.5	80.9

*Calculated as the difference between the maternal body weight on study day 1 and terminal maternal body weight (GD 21/22), minus pup weights.

As discussed in section 10.10.2, there is a large variation in the individual corrected maternal body weight changes (44 g – 116 g, mean value 80.9 g) at 500 mg/kg bw/day. In order to correct for these aspects, the mean corrected maternal body weight was also calculated using the maternal body weight on lactation day 0 (LD 0), which are reported in Table 21 below. No significant effect on the mean corrected maternal body weight change

was observed when the maternal body weight on LD 0 was used for the calculation. Further detail, including individual maternal body weight data, is included in Annex I to this report.

Table 21: Calculated corrected mean maternal body weight change using maternal body weight on LD 0 from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg bw/day)	Mean initial maternal body weight on study day 1 (g)	Mean maternal body weight on LD 0 (g)	Mean corrected maternal body weight change (g)*
0	222.6	290.5	67.9
20	219.2	286.8	67.6
100	217.4	282.4	65.0
500	216.6	282.7	66.1

* Calculated as the difference between maternal body weight on study day 1 and LD 0

Mean food consumption was statistically significantly increased in females at 500 mg/kg bw/day during week 2 of the pre-mating period (81.3 g/kg bw/day compared with 63.3 g/kg bw/day in the control group) and during gestation days 0 to 7 (93.4 g/kg bw/day compared with 72.7 g/kg bw/day in the control group) and 7 to 14 (91.2 g/kg bw/day compared with 76.6 g/kg bw/day in the control group). Mean food consumption was also increased in males at 500 mg/kg bw/day during week 5 (57.9 g/kg bw/day compared with 49.8 g/kg bw/day in the control group) and week 6 (56.6 g/kg bw/day compared with 47.6 g/kg bw/day in the control group) of the pre-mating period (see Table 16 in section 10.10.2).

At 500 mg/kg bw/day, pelvic dilation of the kidneys was observed in 4/5 females compared with 0/5 in the control group. Renal tubular dilation, degeneration, papillary necrosis, pelvic degeneration and transitional cell hyperplasia was also observed in 1/5 females at 500 mg/kg bw/day. In males, an increased incidence of renal tubular dilation was observed from 20 mg/kg bw/day. The incidences were 1/5, 5/5, 5/5 and 3/5 in the 0, 20, 100 and 500 mg/kg bw/day groups, respectively.

At 500 mg/kg bw/day, the number of live born pups was reduced (10 compared with 53 in the control group), resulting in a biologically significant decrease in the live birth index (62.5 % compared with 100 % in the control group). At this dose, the mean litter size was also statistically significantly reduced (5 compared with 13.25 in the control group). No pups at 500 mg/kg bw/day survived beyond PND 1 and thus the viability index at PND 4 at 500 mg/kg bw/day was 0 % (compared with 100 % in the control group). There was a statistically significant decrease in the percentage of male pups at 500 mg/kg bw/day (14 % compared with 66 % in the control group).

Table 22: Summary of litter parameters from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose mg/kg bw/day	No. of pups at birth	No. of live pups	No. of dead pups (PND 0)	No. of dead pups (PND 4)	Live birth index	Mean litter size (PND 0)	% Male pups	Viability index (PND 4)
0	53	53	0	0	100 %	13.25	66.14	100 %
20	54	54	0	0	100 %	13.50	44.64	100 %
100	60	60	0	1	100 %	12.00	43.08	98.46 %
500	12*	10	2	8*	62.50 %	5.00*	14.29*	0 %

* = $p<0.01$

No clinical signs were reported in F1 pups at 20 or 100 mg/kg bw/day during the five day lactation period. No assessment of pups at 500 mg/kg bw/day was possible due to the low survival rate in this group. At necropsy, no macroscopic alterations were noted in F1 pups at 20 or 100 mg/kg bw/day, with the exception of hydronephrosis of the kidney in one pup at 100 mg/kg bw/day. The study report notes that this finding is frequently observed in this strain of rat. Of the three pups which could be necropsied at 500 mg/kg bw/day, one had no findings and two had no milk in their stomachs.

In a dominant lethal test where untreated females were mated with males treated with dimethyl propylphosphonate (Anonymous, 1995a see also section 10.8), a significant effect on post-implantation loss per fertilised female was observed at 1000 mg/kg bw/day and above.

On the basis of this study, the REACH registration dossier for dimethyl propylphosphonate applies a self-classification of category 1B reproductive toxicant and proposes no further testing for the endpoint of toxicity to reproduction.

The dossier submitter notes that although a statistically significant decrease in maternal body weight on GD 18 to 20 was reported at 500 mg/kg bw/day in the pilot reproductive toxicity study with dimethyl propylphosphonate, there was no effect on the corrected mean maternal body weight change at any dose. Therefore, the dossier submitter considers that the observed effect on maternal body weight was due to an intrauterine effect rather than maternal toxicity. This view is supported by the lack of clinical signs of toxicity and the observed increase, rather than decrease, in food consumption in females at 500 mg/kg during the gestation period. Therefore, the dossier submitter concludes that the observed effects on development cannot be considered to be secondary to maternal toxicity.

The dossier submitter notes that the available pilot reproductive toxicity study with dimethyl propylphosphonate has a number of limitations, in particular the group size was lower than that recommended in OECD 421 thus decreasing the sensitivity of the study to detect effects on development. However, despite this, a significant effect on the number of pups born, the number of dead pups, the mean litter size and the viability of pups on PND 4 was observed at 500 mg/kg bw/day. In addition, a statistically significant decrease in the percentage of male pups was also observed at this dose. The dossier submitter considers these effects to be treatment related.

10.10.6 Comparison with the CLP criteria

According to Annex I to the CLP Regulation, substances may be classified as category 1A reproductive toxicants if they are known "*human reproductive toxicants*".

No epidemiological data are available to demonstrate reproductive toxicity in humans. Therefore, classification in category 1A is not warranted.

According to Annex I to the CLP Regulation, substances may be classified as category 1B if presumed to be a human reproductive toxicant. The classification of a substance as category IB reproductive toxicant "…*is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function or 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".*

In the available pilot reproductive toxicity study with dimethyl propylphosphonate, a significant decrease in the number of live born pups and live birth index was observed at 500 mg/kg bw/day. No pups at 500 mg/kg bw/day survived beyond PND 1 and thus the viability index at PND 4 at 500 mg/kg bw/day was 0 %. At the same dose, there was also a significant effect on the pup sex ratio, where the percentage of male pups was statistically significantly reduced. These effects are indicative of an effect on development. Also as described in section 10.8, an increase in the incidence of post-implantation loss was observed in untreated females mated with dimethyl propylphosphonate treated males in a dominant lethal test.

The dossier submitter notes that the pilot reproductive toxicity study with dimethyl propylphosphonate has a number of limitations, in particular the group size was lower than that recommended in OECD 421 thus decreasing the sensitivity of the study to detect effects. However, despite these limitations, the study provides clear evidence of an effect on development in the high dose group (500 mg/kg bw/day). These effects were not considered to be a secondary non-specific consequence of other toxic effects. In addition, the effect on post-implantation loss in the dominant lethal test provides supporting evidence for an effect on development.

Based on the available information, the dossier submitter considers that classification in category 1B is warranted for effects on development.

According to Annex I to the CLP Regulation, a substance may be classified as category 2 if it is a suspected human reproductive toxicant. The classification of a substance as category 2 reproductive toxicant is warranted "…*where there is some evidence from humans or experimental animals…of an adverse effect on sexual function and fertility, or on development…if deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification".*

The available pilot reproductive toxicity study with dimethyl propylphosphonate provides clear evidence of an effect on development which is not considered to be a secondary non-specific consequence of other toxic effects. Therefore, classification in category 2 is not considered appropriate.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

In a pilot study for an OECD TG 408/422 study in rats (Anonymous, 2012), a decrease in fertility index, a significant decrease in the number of implantation sites and an increase in post-implantation losses leading to a decrease in total number of pups born was observed at the top dose of 500 mg/kg bw/day. The DS considered the effects not to be secondary non-specific consequence of other toxic effects. The DS noted the limitation of the pilot study and its potentially low sensitivity (limited number of animals and parameter investigated). According to the DS, the pre- and post-implantation losses observed in the dominant lethal study also provide supporting evidence of an effect on fertility.

On this basis, the DS proposed to classify DMPP as **Repr. 1B, H360F**.

Developmental toxicity

In the same pilot study, the following developmental findings were noted: a significant decrease in the number of live born pups, live birth index, decreased in the percentage of male pups and viability index at 500 mg/kg bw/d. The DS considered the effects not to be secondary non-specific consequence of other toxic effects. The DS noted the limitation of the pilot study and its potential low sensitivity (limited number of animals and parameters investigated). The DS considered that the post-implantation losses observed in the dominant lethal test provide supporting evidence of an effect on development.

On this basis, the DS proposed to classify DMPP as **Repr. 1B, H360D.**

Comments received during consultation

One MS agreed with the DS proposal.

One MS commented that the observed effects on fertility (fertility index, pre-implantation losses) and development (post-implantation losses, decreased % male pups and decreased viability index) are more appropriately addressed under the classification of germ cell mutagenicity in accordance with Annex I, 3.7.1.1. of the CLP regulation rather than reproductive toxicity: "*Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. […]. For classification purposes, the known induction of genetically based heritable effects in the offspring is addressed in Germ Cell Mutagenicity (section 3.5), since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity*." The MS also noted that the fertility effects observed in the dominant lethal test were only observed in presence of excessive toxicity and would not fulfil the classification in category 1B.

The DS responded that the fertility index was calculated based on the number of pregnant females/number of sperm positive females and that there was no information on pre-implantation losses in the pilot study. The DS highlighted that the fertility effects observed in the pilot study were noted in absence of maternal toxicity. Although the DS acknowledged the overlap between the effects in the dominant lethal test and the pilot reproductive study, the DS considered the effects on the number of dead pups, mean litter size and viability of pups on post-natal day (PND)4 indicative of developmental toxicity, relevant for classification.

Assessment and comparison with the classification criteria

A pilot reproductive toxicity study was available in which male and female Wistar rats (n=5/sex/dose) received DMPP *via* oral gavage during a 2-week pre-mating period. Dose levels were 0, 20, 100 and 500 mg/kg bw/day. Females were also treated during gestation and up to PND4. Males were treated for 44 days. Animals were mated and pregnant females were allowed to litter. Females and offspring were subject to necropsy on PND4. RAC notes that the study has limitations as a low number of animals were used and as a low number of parameters were investigated compared to OECD TG 421 study. Sperm investigation was not performed and male reproductive organs were not examined. Histopathological examination was limited to kidneys, uterus (number of implantations) and ovaries (number of corpora lutea).

No clinical signs were observed in dams or male rats. Food consumption was increased at the top dose in both males and females. Body weight of males was not affected in the study. A significant decrease in maternal body weight was noted on gestational days (GD) 18 to 20 (max 13% vs controls at GD 20) and marked decrease in body weight gain during GD14-20 was noted at the top dose.

***Table**: Mean maternal body weight during gestation in the pilot reproductive toxicity study (Anonymous, 2012)*

Dose (mg/kg bw/day)	**Mean maternal body weight (g)**					
	GD0	**GD7**	**GD14**	**GD18**	**GD19**	**GD20**
0	246	272	303	348	362	380
20	242	269	300	347	362	381
100	244	271	296	338	349	362
500	236	270	393	322*	326**	333**

*p<0.05, **p<0.01

Corrected maternal body weight change was provided using maternal body weight on GD 21/22. Nevertheless, RAC considered the calculation not appropriate as the placenta weight and gravid uterine weight were not available and as pup weight used for the correction was not recorded for all pups. Nevertheless, no significant effect on maternal mean corrected maternal body weight was observed when the maternal body weight on lactation day (LD) 0 was used for the calculation. Therefore, RAC agrees that the observed effect on body weight of dams was due to intrauterine effects rather than maternal toxicity. At 500 mg/kg bw/day, histopathological findings were noted in the kidney of dams (pelvic dilation in 4/5 and renal tubular dilatation, degeneration, papillary necrosis, pelvic dilatation and transitional cell hyperplasia in 1/5 female). Increased renal tubular dilatation, swelling and/or vacuolation was noted at ≥ 20 mg/kg bw/day in all males.

Sexual function and fertility

A significant effect on the fertility index was noted at the top dose of 500 mg/kg bw/day.

***Table**: Summary of fertility effects observed in the pilot study with DMPP (Anonymous, 2012)*

Dose (mg/kg bw/day)	**0**	**20**	**100**	**500**
Fertility index (%)	80% (4/5)	80% (4/5)	100% (5/5)	60% (3/5)
No. of corpora lutea	No effects			
No. of implantation sites (mean)	56	58	65	33
No. of implantation sites per litter	14.0	14.5	13.0	11.0

*p<0.01

Developmental toxicity

A significant effect on post-implantation losses, number of pups and dead pups, mean litter size and viability index and number of male pups was noted at the top dose of 500 mg/kg bw/day in the pilot study.

***Table**: Summary of developmental toxicity effects observed in the pilot study with DMPP (Anonymous, 2012)*

Dose (mg/kg bw/day)	**0**	**20**	**100**	**500**
No. of pups at birth (total)	53	54	60	12*
No. of live born pups	53	54	60	10
Live born index (%)	100%	100%	100%	62.5%*
Post-implantation losses per litter	0.75	1	1	7 *
No. of dead pups (PND0)	0	0	0	2
No. of dead pups (PND4)	0	0	1	8*
Mean litter size (PND0)	13.3	13.5	12	5*
Mean litter size (PND4)	13.3	13.5	11.8	0
Pup viability index (%)	100%	100%	98.6%	0%
Male pups (%)	66%	45%	43%	14%*
Pup weight (PND0) (g)	6.55	6.21	6.05	5.33

*p<0.01

No clinical signs were noted in F1 pups. At necropsy, 2/3 pups at 500 mg/kg bw/day had no milk in their stomach and 1/59 at 100 mg/kg had hydronephrosis of the left kidney.

Comparison with criteria

<u>Sexual function and fertility</u>

DMPP induces a decrease in implantation sites and a decrease in the number of fertile rats, not secondary to maternal toxicity. On this basis, a classification is warranted for sexual function and fertility.

DMPP is a germ cell mutagen and it is possible that effects observed in the pilot study are mediated by a genotoxic mechanism. Nevertheless, fertility effects were observed at lower dose levels than in the dominant lethal assay. In addition, RAC notes that the effects were observed in studies with different species, different dose levels, differences in pre-mating period and both male and female

were exposed in the pilot study compared to the dominant lethal study (male only). Moreover, it is not possible to exclude a potential fertility effect *via* other mechanism(s) than mutagenicity. Thereby, the fertility effect observed in the pilot study may not be covered by a germ cell mutagenicity classification.
Considering the observed effects and the limitations of the study (e.g. low sensitivity), RAC notes that the data on fertility are not sufficiently conclusive to decide on category 1B. Therefore, RAC considers that the evidence warrants to classify DMPP as **Repr. Cat. 2, H361f**.

Developmental toxicity

Based on the decrease in live born pups, live birth index, viability index and percentage of male pups and the increase in post-implantation losses at 500 mg/kg bw/day in the pilot study, classification of DMPP for developmental toxicity is warranted. Although the observed effects could be due to a dominant lethal effect caused by a genotoxic insult, other mechanisms than germ cell mutagenicity cannot be excluded. The classification is not solely based on DL test data, but rather mainly on a pilot study for reproductive toxicity, where already at small number of animals used, clear effects on development where observed. In addition, RAC notes that there were differences in study design (both male and female exposed in the pilot reproductive toxicity study, differences in pre-mating period), differences in species and dose levels that lead to remaining uncertainties whether the serious effects observed in the pilot study are covered by the germ cell mutagenicity classification. Although RAC notes the limits of the pilot study and its low sensitivity due to the low number of animals, serious developmental effects were observed in the pilot study, not secondary to maternal toxicity. Therefore, RAC considers that overall data on DMPP fulfills the criteria and warrants for classification as **Repr. 1B, H360D**.

10.11 Specific target organ toxicity-single exposure

Not evaluated as part of this dossier.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated as part of this dossier.

10.13 Aspiration hazard

Not evaluated as part of this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated as part of this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

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15 ANNEX 1

Detailed study summaries for the germ cell mutagenicity and reproductive toxicity endpoints.