Annex I to the 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: -

Contact details for dossier submitter: Health & Safety Authority
The Metropolitan building
James Joyce Street
Dublin 1, Ireland.

Version number: 3.0 **Date: August 2020**

CONTENTS

1 PHYSICAL HAZARDS

Not evaluated as part of this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not available.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

Not evaluated as part of this dossier.

3.2 Acute toxicity - dermal route

Not evaluated as part of this dossier.

3.3 Acute toxicity - inhalation route

Not evaluated as part of this dossier.

3.4 Skin corrosion/irritation

Not evaluated as part of this dossier.

3.5 Serious eye damage/eye irritation

Not evaluated as part of this dossier.

3.6 Respiratory sensitisation

Not evaluated as part of this dossier.

3.7 Skin sensitisation

Not evaluated as part of this dossier.

3.8 Germ cell mutagenicity

3.8.1 *In vitro* data

3.8.1.1 Bacterial reverse mutation test

Study reference

Anonymous, 1993a. Dimethylpropanphosphonat *Salmonella* / microsome test (Unpublished report).

Detailed study summary and results

Test type

Similar to OECD 471: Bacterial reverse mutation test. The study did not meet current test guideline requirements to include a fifth strain to detect DNA cross-linking (*S. Typhimurium* TA102 or *E.coli* WP2 uvrA or WP2 uvrA (pKM101). GLP-compliant study.

- *Number of replicates:* Two.
- *Positive controls*
    - + S9 mix: All strains: 2-aminoanthracene (2-AA).
    - – S9 mix: TA 1535: sodium azide (NaN_3)

      TA 100: Nitrofurantoin (NF)

      TA 1537 and TA 98: 4-nitro-1,3-phenylene diamine (4-NPDA).
- *Vehicle control:* Dimethyl sulfoxide.
- *Criteria for evaluating results:* The test was considered positive if a reproducible, dose-related increase in mutant colony numbers was two fold greater than the negative control for TA1535, 100 & 98 or three fold greater for TA 1537.

Test substance

- *Name*: Dimethyl propylphosphonate. Identical to substance identified in CLH dossier.
- *Degree of purity*: > 98 %.
- *Impurities:* Not reported.
- *Batch number:* FHS 599.

Administration/exposure

- *Strain or cell type or cell line: S. typhimurium* strains: TA 98, TA 100, TA 1535 and TA 1537.
- *Type and composition of metabolic activation system*: S9 mix prepared from livers of male Sprague Dawley rats treated with a single i.p. injection of Aroclor 1254.
- *Test concentrations*: 0, 8, 40, 200, 1000 and 5000 µg/plate dimethyl propylphosphonate.
- *Vehicle:* Dimethyl sulfoxide.

Results and discussion

No cytotoxicity was observed up to 5000 µg/plate. The number of revertant colonies per strain is presented in Table A1. No increase in mutation frequency was observed with or without metabolic activation.

Table A1: Number of revertant colonies per bacterial strain following treatment with dimethyl propylphosphonate (Anonymous, 1993a)

	+ S9				- S9			
	TA 1535	**TA 100**	**TA 1537**	**TA 98**	**TA 1535**	**TA 100**	**TA 1537**	**TA 98**
µg/plate	**Mean revertants per plate ± standard deviation**							
1st test								
0	18 ± 4	76 ± 2	13 ± 1	53 ± 7	20 ± 3	75 ± 6	10 ± 1	37 ± 6
8	24 ± 5	91 ± 8	14 ± 2	55 ± 4	14 ± 4	72 ± 4	11 ± 2	43 ± 6
40	26 ± 4	87 ± 9	11 ± 2	55 ± 8	20 ± 6	63 ± 12	12 ± 2	44 ± 6
200	21 ± 2	80 ± 14	11 ± 2	51 ± 8	19 ± 3	73 ± 4	11 ± 3	38 ± 5
1000	26 ± 4	89 ± 4	13 ± 2	53 ± 5	22 ± 2	71 ± 11	10 ± 1	44 ± 6
5000	27 ± 3	82 ± 8	10 ± 2	49 ± 2	20 ± 4	77 ± 8	11 ± 2	41 ± 2
2-AA	280 ± 21 *	1479 ± 105*	481 ± 49*	1578 ± 60*	-	-	-	-
NaN_3	-	-	-	-	825 ± 34*	-	-	-
NF	-	-	-	-	-	246 ± 43*	-	-
4-NPDA		-	-	-	-	-	69 ± 17*	97 ± 11*
2nd test								
0	16 ± 2	50 ± 6	10 ± 1	30 ± 5	15 ± 3	51 ± 7	10 ± 2	24 ± 8
8	14 ± 2	47 ± 6	9 ± 3	29 ± 6	12 ± 3	41 ± 2	11 ± 2	24 ± 7
40	15 ± 3	51 ± 7	10 ± 3	31 ± 3	13 ± 1	42 ± 7	9 ± 3	22 ± 4
200	12 ± 2	49 ± 14	11 ± 2	30 ± 4	12 ± 4	43 ± 3	10 ± 2	24 ± 7
1000	17 ± 5	49 ± 6	12 ± 1	31 ± 10	15 ± 2	49 ± 8	8 ± 3	22 ± 7
5000	17 ± 3	53 ± 5	11 ± 1	33 ± 9	11 ± 3	48 ± 3	7 ± 1	21 ± 4
2-AA	166 ± 23*	677 ± 37*	381 ± 21 *	1581 ± 67	-	-	-	-
NaN_3	-	-	-	-	698 ± 64*	-	-	-
NF	-	-	-	-	-	195 ± 59*	-	-
4-NPDA	-	-	-	-	-	-	46 ± 11*	79 ± 18*

* Mutagenic response

3.8.1.2 Mammalian cell gene mutation test

Study reference

Anonymous (1993b). Mutagenicity study for the detection of induced forward mutations in the V79-*HGPRT* assay *in vitro* (Unpublished report).

Detailed study summary and results

Test type

According to OECD 476: Mammalian cell gene mutation test. GLP-compliant.

- *Number of replicate:* Two.
- *Positive and negative control groups:*
    - *Positive controls:* Ethylmethanesulfonate (-S9); dimethylbenzanthracene (+ S9).
    - *Negative controls:* Untreated cells.
    - *Vehicle control:* Dimethyl sulfoxide.
- *Criteria for evaluating results:* The test was considered positive if a dose-dependent, significant, reproducible increase in mutant frequency was observed.

Test substance

- *Name:* Dimethyl propylphosphonate. Identical to substance identified in CLH dossier.
- *Degree of purity:* > 98 %.
- *Impurities:* Not reported.
- *Batch number:* FHS 599.

Administration/exposure

- *Strain or cell type or cell line, target gene:* V-79 cell line from Chinese hamster lung cells; *hgprt* locus.
- *Type and composition of metabolic activation system*: S9 mix prepared from livers of male Wistar rats treated with Aroclor 1254.
- *Test concentrations*: 0, 500, 1000, 2000, 3000, 4000 and 5000 µg/ml dimethyl propylphosphonate.
- *Vehicle:* Dimethyl sulfoxide.

Results and discussion

No cytotoxicity was observed up to 5000 µg/ml. The mutant frequencies are presented in Table A2. No increase in mutation frequency was observed with or without metabolic activation.

Table A2: Mutant frequencies at *HGPRT* locus in Chinese hamster lung cells following treatment with dimethyl propylphosphonate (Anonymous, 1993b)

µg/ml	Mutant frequency (x 10^{-6} clonable cells) at HGPRT locus			
	+ S9		- S9	
	1st test	2nd test	1st test	2nd test
0	2.3	1.6	4.7	4.7
500	1.8	1.2	7.0	11.7
1000	2.5	1.2	5.8	6.2
1500	2.6	4.7	-	-
2000	5.1	3.8	4.6	9.1
3000	2.6	2.4	5.0	4.3
4000	4.5	3.5	9.9	11.0
5000	3.9	2.2	4.9	7.0
Positive control	110.8	71.7	1160.7	1119.1
Vehicle control	1.2	3.5	4.6	7.4

3.8.2 Animal data

3.8.2.1 Rodent dominant lethal test

Study reference

Anonymous (1995a): Dominant lethal test on the male mouse using subchronic treatment (Unpublished report).

Detailed study summary and results

Test type

OECD 478: Rodent dominant lethal test. GLP compliant study. The study deviated from the test guideline in that the highest dose tested exceeded the maximum tolerated dose, historical control data was not reported, and no statistical analysis of the data was performed.

Test substance

- *Name:* Dimethyl propylphosphonate. Identical to substance identified in CLH dossier.
- *Degree of purity:* > 99 %.
- *Impurities:* Not reported.
- *Batch number:* FHS 599/4.

Test animals

- *Species/strain/sex:* B6C3F1/BOM male mice and CRL:CD1 female mice.
- *No. of animals per sex per dose:* 20 males & 40 females per group.
- *Age and weight at the study initiation:*
    - Males: 7 – 8 weeks old and 22 – 31g.

- Females: 9 weeks old and 25 – 30 g.

Administration/exposure

- *Doses/concentration levels*: 0, 500, 1000, and 2000 mg/kg bw/day dimethyl propylphosphonate. Doses were selected based on the results of a range finding study where 5 male B6C3F1/BOM mice/dose were administered dimethyl propylphosphonate at 0, 250, 500, 1000 and 2000 mg/kg bw/day by oral gavage for 14 days. Apathy, reduced and increased motility, staggered gait, sternal recumbency and difficulty breathing were observed at 500 mg/kg bw/day and above.
- *Vehicle:* Deionised water.
- *Details on test system and conditions, and details on route of administration, exposure:* Males were administered dimethyl propylphosphonate or the positive control via oral gavage. Females were untreated.
- *Duration of treatment:* 5 days/ week for 13 weeks.
- *Positive control groups and treatment:* 2000 mg/kg bw/day dimethyl methylphosphonate.
- *Historical control data:* None reported.
- *Mating and mating intervals:* Animals were mated 1 male to 2 females. The mating intervals were 5, 9 and 13 weeks.
- *Assessment:* Females were sacrificed 16 days after each mating interval and the uterine contents were assessed to determine the total number of implantations, the number of living and dead implants and the number of corpora lutea.
- *Statistical methods:* No statistical analysis performed.

Results and discussion

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 88.3 %, 90 %, 81 % and 34.7 % for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. The average fertilisation rate in the positive control group was 86.7 %. 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 in the 2000 mg/kg bw/day group and therefore considers that it cannot be excluded that the lower fertilisation rates observed this group may be attributed to the systemic toxicity of dimethyl propylphosphonate to males rather than a specific genotoxic effect. The fertilisation rates in females are reported in the Table A3.

Table A3: Reported fertilisation rates in female mice in the rodent dominant lethal test with dimethyl propylphosphonate (Anonymous, 1995a)

	Number of females fertilised					Fertilisation rate				
Dose group (mg/kg bw/day)	**0**	**500**	**1000**	**2000**	**+ve control**	**0**	**500**	**1000**	**2000**	**+ve control**
Mating interval 1	37/40	36/40	30/40	14/31	39/40	92.5 %	90.0 %	75.0 %	45.2 %	97.5 %
Mating interval 2	36/40	34/40	32/38	7/26	31/40	90.0 %	85.0 %	84.2 %	26.9 %	77.5 %
Mating interval 3	33/40	38/40	32/38	5/18	34/40	82.5 %	95.0 %	84.2 %	27.8 %	85.0 %
Average	106/120	108/120	94/116	26/75	104/120	88.3 %	90.0 %	81.0 %	34.7 %	86.7 %

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 14.3, 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. Data relating to pre-implantation loss are reported in Table A4.

Table A4: Pre-implantation loss in fertilised female mice in the rodent dominant lethal test with dimethyl propylphosphonate (Anonymous, 1995a)

Dose group (mg/kg bw/day)	Mating interval	Number of corpora lutea per fertilised female	Implantations per fertilised female*	Pre-implantation loss per fertilised female
0	1	14.8	13.6	1.14
	2	13.8	13.1	0.69
	3	14.5	13.6	0.91
	Mean	**14.4**	**13.4**	**0.9**
500	1	13.3	12.4	0.89
	2	12.8	11.4	1.41
	3	13.4	12.3	1.11
	Mean	**13.2**	**12.0**	**1.1**
1000	1	12.5	11.6	0.9
	2	12.3	11.0	1.28
	3	12.5	10.3	2.22
	Mean	**12.4**	**11.0**	**1.5**
2000	1	6.6	4.79	1.79
	2	7.6	6.0	1.57
	3	10.8	7.0	3.8
	Mean	**8.3**	**5.9**	**2.4**
Positive control	1	13.9	12.5	1.36
	2	13.6	13.0	0.55
	3	13.8	12.1	1.65
	Mean	**13.8**	**12.5**	**1.2**

*The study report notes that the number of implantation sites reported in this table may be slightly different to the "total of living and dead implants" (as presented in Table A5 below) as a placenta with two embryos may be found at one implantation site.

A dose dependent decrease in the number of living implants per fertilised female was observed, with the average over the three mating intervals reported as 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, with the average number over the three mating intervals reported as 0.8, 3.0, 6.0 and 4.9 for the 0, 500, 1000 and 2000 mg/kg bw/day groups, respectively. There was a dose dependent increase in the percentage of dead implants/total number of implants per fertilised females, corresponding to the rate of post-implantation loss: 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. A number of dead implants per fertilised female (3.1) and the percentage of dead implants /total number of implants per fertilised female (24.8 %) was also increased in the positive control group when compared with the concurrent negative control group. Data relating to post-implantation loss are reported in Table A5.

Table A5: Post-implantation loss in fertilised female mice in the rodent dominant lethal test with dimethyl propylphosphonate (Anonymous, 1995a)

Dose group (mg/kg bw/day)	Mating interval	Total living and dead implants per fertilized female	Living implants per fertilised female	Dead implants per fertilised female	% dead implants per fertilized female*
0	1	13.65	12.7	0.95	6.96 %
	2	13.02	12.3	0.72	5.53 %
	3	13.58	13.0	0.58	4.27 %
	Mean	**13.4**	**12.7**	**0.8**	**5.6 %**
500	1	12.35	9.6	2.75	22.27 %
	2	11.45	8.6	2.85	24.89 %
	3	12.24	9.0	3.24	26.47 %
	Mean	**12.0**	**9.1**	**3.0**	**24.5 %**
1000	1	11.6	5.4	6.2	53.45 %
	2	11.01	4.7	6.31	57.31 %
	3	10.29	4.7	5.59	54.32 %
	mean	**11.0**	**4.9**	**6.0**	**55.0 %**
2000	1	4.8	0.8	4.0	83.33 %
	2	5.96	1.1	4.86	81.54 %
	3	7.0	1.2	5.8	82.86 %
	Mean	**5.9**	**1.0**	**4.9**	**82.6 %**
Positive control	1	12.54	9.1	3.44	27.43 %
	2	12.7	9.7	3.0	23.62 %
	3	12.12	9.3	2.82	23.27 %
	Mean	**12.5**	**9.4**	**3.1**	**24.8 %**

* calculated as % of dead implants/total living and dead implants

No historical control data was reported in the study report. However, data from the vehicle control (water) group from a dominant lethal test conducted with the same strains of mice, namely B6C3F1 males and CD1 females, as used in this study are reported in Table A6 (Dunnick *et.al.*, 1984a). These results are comparable to those of the vehicle control group in the dominant lethal test with dimethyl propylphosphonate, supporting the validity of the study.

Table A6: Data from water vehicle control group in a dominant lethal test conducted with B6C3F1 male and CD1 female mice (Dunnick *et.al,* 1984a)

Mating interval	Fertilisation rate	Live implants per female	Dead implants per female	% dead implants per female
4 weeks	85 % (34/40)	11.9 ± 0.3	0.6 ± 0.2	5.3 ± 1.3
8 weeks	88 % (34/40)	10.3 ± 0.5	1.1 ± 0.2	9.8 ± 2.1
12 weeks	94 % (74/80)	11.2 ± 0.4	0.8 ± 0.1	8.3 ±1.5

The study report states that the concurrent positive control, dimethyl methylphosphonate, was selected as a "class-specific positive control" based on the results of a dominant lethal test in which the positive control substance induced dominant lethal mutations in germ cells of the same strain of mouse (Dunnick *et. al.* 1984a, described in section 3.8.2.2). The dossier submitter notes the positive control substance is not included in Annex I of CLP but is self-classified by the REACH registrant as Muta. 1B H340. In the dominant lethal test with dimethyl propylphosphonate described above, the positive control elicited the expected increase in post-implantation loss confirming the sensitivity of the test system.

The study report also 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 groups, which is indicative of a treatment related effect.

Under the conditions of the study, dimethyl propyl phosphonate induced dominant lethal mutations in mice.

3.8.2.2 Rodent dominant lethal test with structural analogue

Study reference

Dunnick JK, Solleveld HA, Harris, MW, Chapin R and Lamb JC (1984a). Dimethyl methyl phosphonate induction of dominant lethal mutations in male mice. Mutat. Res.138: 213-218.

Detailed study summary and results

Test type

Published study similar to OECD 478: Rodent dominant lethal test. Non-GLP compliant study. The study deviated from the test guideline in that there was no concurrent positive control group and the number of corpora lutea were not counted. There was limited reporting of results, in particular histopathological evaluations.

Test substance

- *Name:* Dimethyl methylphosphonate. Substance is a structural analogue of the substance identified in CLH dossier.
- *Degree of purity:* > 99 %.
- *Impurities:* Not reported.
- *Batch number:* Not reported.

Test animals

- *Species/strain/sex:* B6C3F1 male mice and CD1 female mice.
- *No. of animals per sex per dose:* 20 males & 40 females per group. A further 20 male mice/group assigned to 0, 1000 and 2000 mg/kg bw/day recovery groups.
- *Age and weight at the study initiation:*
    - Males: 7 – 8 weeks old on first day of dosing. Weight not reported.
    - Females: Age and weight at study initiation not reported. Females were reported to be 9 weeks old at mating.

Administration/exposure

- *Doses/concentration levels*: 0, 250, 500, 1000 and 2000 mg/kg bw/day dimethyl methylphosphonate.
- *Vehicle:* Water.
- *Details on test system and conditions, and details on route of administration, exposure:* Males were administered dimethyl methylphosphonate via oral gavage. Females were untreated.
- *Duration of treatment:* 5 days/ week for 13 weeks.
- *Positive control groups and treatment:* None.
- *Historical control data:* None reported.
- *Mating and mating intervals:* Animals were mated 1 male to 2 females. The mating intervals were 4, 8 and 12 weeks. Males in the recovery groups (0, 1000 and 2000 mg/kg bw/day) were treated to week 13 and then mated with untreated females at the end of the 15 week recovery period.
- *Assessment:*
    - Females were sacrificed 16 days after the middle of each mating interval and the uterine contents were assessed to determine the number of live and dead implants and the percentage resorptions.
    - Males were sacrificed after 13 weeks or at the end of the 15 week recovery period. Epididymal sperm concentrations, and luteinising hormone (LH) and follicle stimulating hormone (FSH) levels were assessed. Kidney, prostrate, testes and epididymis weights were recorded. Histopathological examination was performed on kidney, prostate, coagulating gland, preputial gland, urinary bladder, ductus deferens, seminal vesicle, penis, testes, epididymis, zymbal gland, salivary gland, pituitary and thymus.
- *Statistical methods:* Kruskal-Willis one way analysis of variance and Wilcoxon rank sum.

Results and discussion

In males, no mortality or clinical signs of toxicity were reported. No effect on body weight or in the relative weights of testes, epididymis, prostate or testes were observed (absolute organ weights were not reported). No histopathological changes in genitourinary system were reported. A slight, non-statistically significant decrease in mean sperm concentrations were observed at 2000 mg/kg bw/day. The mean sperm concentrations were 457.8 x 10^6 , 459.2 x 10^6, 405.9 x 10^6, 469.6 x 10^6 and 405.7 x 10^6 per g caudal epididymal tissue in the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. No dose-related effect on FSH or LH plasma levels were reported.

No effect on fertilisation rates were observed at any dose group: the fertilisation rates, averaged over the three mating intervals, were 89.0 %, 85.7 %, 88.7 %, 89.3 % and 88.7 % for the 0, 250, 500, 1000 and 2000

mg/kg bw/day groups, respectively. No effect on the fertilisation rate was noted for the mating interval after 15 week recovery period. The fertilisation rates are reported in the Table A7.

Table A7: Fertilisation rates in female mice in the rodent dominant lethal test with dimethyl methylphosphonate (Dunnick *et al*., 1984a)

	Fertilisation rate				
Dose group (mg/kg bw/day)	**0**	**250**	**500**	**1000**	**2000**
Mating interval 1 (4 weeks)	85 %	75 %	88 %	85 %	85 %
Mating interval 2 (8 Weeks)	88 %	90 %	88 %	95 %	92 %
Mating interval 3 (12 weeks)	94 %	92 %	90 %	88 %	89 %
Average of mating intervals 1 - 3	89.0 %	85.7 %	88.7%	89.3 %	88.7 %
Mating interval recovery period (15 weeks)	90 %	-	-	92 %	95 %

A dose dependent decrease in the number of living implants per female was observed, which was statistically significant 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 conversely statistically significant increase in the number of dead implants 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. There was an overall significant increase in the percentage of dominant lethal mutations at 1000 mg/kg bw/day (mating interval 1 and 3) and 2000 mg/kg bw/day (all three mating intervals). Data relating to post-implantation loss for mating intervals 1 to 3 are reported in Table A8.

Table A8: Post-implantation loss in fertilised female mice in the rodent dominant lethal test with dimethyl methylphosphonate (Dunnick *et al.*, 1984a)

Dose group (mg/kg bw/day)	Mating interval	Living implants per female	Dead implants per female	% resorptions	% dominant lethal mutations[(a)]
0	Mating interval 1 (4 weeks)	11.9 ± 0.3	0.6 ± 0.2	5.3 ± 1.3	-
	Mating interval 2 (8 Weeks)	10.3 ± 0.5	1.1 ± 0.2	9.8 ± 2.1	-
	Mating interval 3 (12 weeks)	11.2 ± 0.4	0.8 ± 1.1	8.3 ± 1.5	-
	Average	**11.1**	**0.8**	**7.8**	**-**
250	Mating interval 1 (4 weeks)	12.2 ± 0.3	0.8 ± 0.2	5.8 ± 1.2	-3
	Mating interval 2 (8 Weeks)	11.4 ± 0.5	0.7 ± 0.1	6.5 ± 1.4	-10
	Mating interval 3 (12 weeks)	10.9 ± 0.5	0.8 ± 0.3	8.3 ± 2.9	3
	Average	**11.5**	**0.8**	**6.9**	**-3.3**
500	Mating interval 1 (4 weeks)	11.4 ± 0.4	1.1 ± 0.2*	9.6 ± 1.4*	4
	Mating interval 2 (8 Weeks)	11.2 ± 0.4	0.7 ±0.2	6.1 ± 1.6	-8
	Mating interval 3 (12 weeks)	11.1 ± 0.6	0.8 ± 0.2	6.6 ± 1.8	1
	Average	**11.2**	**0.9**	**7.4**	**-1**
1000	Mating interval 1 (4 weeks)	10.4 ± 0.5*	1.6 ± 0.3**	13.4 ± 2.2**	12*
	Mating interval 2 (8 Weeks)	10.5 ± 0.5	1.1 ± 0.2	10.1 ± 1.7	-2
	Mating interval 3 (12 weeks)	9.7 ± 0.5**	1.7 ± 0.3**	15.3 ± 2.7**	13**
	Average	**10.2**	**1.5**	**12.9**	**7.7**
2000	Mating interval 1 (4 weeks)	7.2 ± 0.6**	3.2 ± 0.4**	30.9 ± 4.2**	40**
	Mating interval 2 (8 Weeks)	6.5 ± 0.4**	3.7 ± 0.3**	34.6 ± 3.2**	37**
	Mating interval 3 (12 weeks)	6.3 ± 0.3**	4.7 ± 0.2 **	44.2 ± 2.3**	44**
	Average	**6.7**	**3.9**	**36.6**	**40.3**

*p < 0.05; **p<0.01

(a) calculated as 1 minus (average living implants in test group/average living implants in the control group) x 100

At the mating interval after the 15 week recovery period, no significant effect on the number of live or dead implants per female, the percentage resorptions or the percentage dominant lethal mutations was observed at 1000 or 2000 mg/kg bw/day when compared with the control. This indicates that there was some recovery in males following cessation of treatment. Data relating to post-implantation loss for mating interval after the 15 week recovery period are reported in Table A9.

Table A9: Post-implantation loss in fertilised female mice following 15 week recovery period for treated male mice in the dominant lethal test with dimethyl methylphosphonate (Dunnick *et al.*, 1984a)

Dose group (mg/kg bw/day)	Living implants per female	Dead implants per female	% resorptions	% dominant lethal mutations[(a)]
0	11.1 ± 0.4	0.4 ± 0.1	5.0 ± 1.6	-
1000	11.4 ± 0.3	0.5 ± 0.1	4.5 ± 1.1	-3
2000	11.1 ± 0.4	0.7 ± 0.1	6.2 ± 1.4	0

(a) calculated as 1 minus (average living implants in test group/average living implants in the control group) x 100

Under the conditions of the study, dimethyl methylphosphonate induced post implantation loss in mice indicative of dominant lethal mutations.

3.8.2.3 Rodent dominant lethal test with structural analogue

Study reference

Dunnick JK, Gupta BN, Harris MW and Lamb JC (1984b). Reproductive toxicity of dimethyl methyl phosphonate (DMMP) in the male Fischer 344 Rat. Toxicol. Appl. Pharmacol. 72: 379-387.

Detailed study summary and results

Test type

Published study similar to OECD 478: Rodent dominant lethal test. Non-GLP compliant study. The study deviated from the test guideline in that there was no concurrent positive control group and the number of corpora lutea were not counted. There was limited reporting of results, in particular histopathological evaluations.

Test substance

- *Name:* Dimethyl methylphosphonate. Substance is a structural analogue of the substance identified in CLH dossier.
- *Degree of purity:* > 99 %.
- *Impurities:* Not reported.
- *Batch number:* Not reported.

Test animals

- *Species/strain/sex:* Fischer 344 male and female rats.

- *No. of animals per sex per dose:* 20 males & 40 females per group.
- *Age and weight at the study initiation:*
    - Males: 42 days old on first day of dosing. Body weights at start of study were between 111g and 112 g.
    - Females: Age and weight at study initiation not reported. Females were reported to be 62 days old at mating.

Administration/exposure

- *Doses/concentration levels*: 0, 250, 500, 1000 and 2000 mg/kg bw/day dimethyl methylphosphonate.
- *Vehicle:* Water.
- *Details on test system and conditions, and details on route of administration, exposure:* Males were administered dimethyl propylphosphonate via oral gavage. Females were untreated.
- *Duration of treatment:* 5 days/ week for 90 days.
- *Positive control groups and treatment:* None.
- *Historical control data:* None reported.
- *Mating and mating intervals:* Animals were mated 1 male to 2 females. There was one mating interval at Days 84 to 88.
- *Assessment:*
    - Females were sacrificed 14 days after the middle of the mating interval and the uterine contents were assessed to determine the number of live and dead pups, and the percentage of resorptions.
    - Males were sacrificed after 90 days. Epididymal sperm samples were analysed and LH and FSH levels were assessed. Kidney, prostate, testes and epididymis weights were recorded. Histopathological examination was performed on kidney, prostate, coagulating gland, preputial gland, urinary bladder, ductus deferens, seminal vesicle, penis, testes, epididymis, urethral gland, bulbourethral gland, salivary gland, zymbal gland, tympanic bullae, pituitary and thymus.
- *Statistical methods:* Cochran – Armitage test and Fisher's exact test (sperm and pregnancy data); Jonck-heere's test and Mann-Whitney U test (all other parameters).

Results and discussion

In males, no mortality or clinical signs of toxicity were reported. The terminal body weight in males was statistically significantly reduced at 2000 mg/kg bw/day. The terminal body weights in males were 289 g, 284 g, 273 g, 276 g and 259 g in the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. Absolute organ weights were not reported. A statistically significant decrease in the "organ to body weight" ratio for epididymis was reported at 2000 mg/kg bw/day (1.4×10^3 compared with 1.5×10^3 in the control group). A statistically significant increase in the "organ to body weight" ratio for kidney was observed at 1000 mg/kg bw/day and above: 7.0×10^3, 7.1×10^3, 7.3×10^3, 7.8×10^3 and 8.3×10^3 for the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively. An increased incidence of kidney effects associated with regeneration, hyaline droplet generation, cytoplasmic hyaline bodies and cellular infiltrate into the interstitum was observed in all treatment groups. The incidence of kidney effects were 0/20, 5/20, 12/20, 8/20 and 15/20 in the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively.

A statistically significant decrease in the "organ to body weight" ratios for epididymis was reported at 2000 mg/kg bw/day (1.4×10^3 compared with 1.5×10^3 in the control group). Histopathological examination of testes found 18/20 males at 2000 mg/kg bw/day with lesions (compared with 0/20, 1/20, 0/20 and 0/20 in the 0, 250, 500 and 1000 mg/kg bw/day groups, respectively). These were reported to be lack of spermatogenesis, and degeneration, vacuolisation and necrosis of spermatogonial cells. In addition, hypospermatogenic tubules were reported to be reduced in diameter and lined mostly with Sertoli cells. Treatment related changes of the prostate were reported in 1/20 males at 1000 mg/kg bw/day and 4/20 males at 2000 mg/kg bw/day (compared with 0/20 in the remaining groups). These were reported to be multifocal infiltration of lymphocytes and plasma cells to the interstitum and dilation of acini cells containing cellular debris.

A statistically significant decrease in the percentage of motile sperm was observed from 1000 mg/kg bw/day. There was a dose dependent decrease in the mean sperm count, which was statistically significant at 2000 mg/kg bw/day. The incidence of sperm head abnormalities was also decreased at 2000 mg/kg bw/day. A summary of sperm evaluations are presented in Table A10.

Table A10: Analysis of sperm from male rats in the rodent dominant lethal test with dimethyl methylphosphonate (Dunnick *et al*., 1984b)

	Dose group (mg/kg bw/day)				
Evaluated parameter	**0**	**250**	**500**	**1000**	**2000**
Motile epididymal sperm	80.2 ± 2.7	80.5 ± 3.0	79.7 ± 3.6	71.5 ± 3.1*	35.8 ± 5.5**
Sperm count per g caudal epididymal tissue (x 10^6)	541.4 ± 25.1	515.2 ± 38.9	459.2 ± 35.2	432.2 ± 38.5	219.6 ± 34.0**
Sperm-head abnormalities	4.5 ± 0.3	5.6 ± 0.7	5.9 ± 0.5	6.9 ± 0.7	41.7 ± 5.1**

* $P < 0.05$; ** $P < 0.01$

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 pregnant females 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). The percentage of males with sperm positive females were 75 %, 85 %, 60 %, 50 % and 55 % in the 0, 250, 500, 1000 and 2000 mg/kg bw/day groups, respectively.

There was a statistically significant decrease in the percentage of pregnant females from 1000 mg/kg bw/day. The percentages 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). The reproductive parameters evaluated are summarised in Table A11.

Table A11: Analysis of reproductive function in rats in the rodent dominant lethal test with dimethyl methylphosphonate (Dunnick *et al*., 1984b)

Evaluated parameters	Dose group (mg/kg bw per day)				
	0	250	500	1000	2000
Number males with sperm positive females	15/20	17/20	12/20	10/20	11/20
Male fertility index	14/20	15/20	12/20	8/20	0/20**
Total number of pregnant females	20/40	19/40	17/40	11/40*	0/40**
Number live implants per litter	7.6 ± 0.7	7.8 ± 0.4	5.7 ± 0.6**	0.82 ± 0.5**	0**
Resorptions	6.1 %	14.9 %*	39.4 %**	79.1 %**	-

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

Under the conditions of the study, dimethyl methylphosphonate induced post implantation loss in rats indicative of dominant lethal mutations.

3.8.2.4 Mammalian spermatogonial chromosome aberration test

Study reference

Anonymous (1998a): Dimethyl propane phosphonate *in vivo* cytogenetic study of spermatogonia in the mouse to evaluate for induced clastogenic effects (Unpublished report).

Detailed study summary and results

Test type

Similar to OECD 483: mammalian spermatogonial chromosome aberration test. The study deviated from the test guideline in that the minimum number of animals in the high dose group was not in line with current guideline requirements, less than the required 200 metaphases were scored, the positive control group did not elicit an increase in chromosome aberrations and no table(s) of results were reported in the study report. GLP-compliant study.

Test substance

- *Name:* Dimethyl propylphosphonate. Identical to substance identified in CLH dossier.
- *Degree of purity:* > 99 %.
- *Impurities:* Not reported.
- *Batch number:* FHS 599/4.

Test animals

- *Species/strain/sex:* B6C3F1/BOM male mice.
- *No. of animals per sex per dose:* 3 males at 2000 mg/kg bw/day and 5 males at 0, 500 and 1000 mg/kg bw/day. 5 males in the positive control group.
- *Age and weight at the study initiation:* Following 13 weeks of treatment in the rodent dominant lethal test (Anonymous, 1995a, described in section 3.8.2.1) males were assigned to this study. Therefore, male mice were approximately 20 – 22 weeks old. No information on body weight.

Administration/exposure

- *Doses/concentration levels*: 0, 500, 1000, and 2000 mg/kg/day dimethyl propylphosphonate.
- *Vehicle:* Deionised water.
- *Details on test system and conditions, and details on route of administration, exposure:* Following 13 weeks of treatment in the rodent dominant lethal test (Anonymous, 1995a, described in section 3.8.2.1), 3 - 5 male B6C3F1/BOM mice from each group were selected and administered a further single dose via oral gavage of dimethyl propylphosphonate or the positive control.
- *Positive control groups and treatment:* 2000 mg/kg bw/day dimethyl methylphosphonate via oral gavage.
- *Assessment:* Colcemid was 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.
- *Criteria for scoring and number of cells analysed per animal:* 1000 cells per animal were examined for the mitotic index. 100 metaphases per animal were examined for structural chromosomal aberrations.
- *Statistical methods:* One-sided corrected Chi test.

Results and discussion

One male at 2000 mg/kg bw/day died after treatment with Colcemid but prior to sacrifice and was not assessed for chromosome aberrations. Table A12 below summarises the results. No increase in chromosomal aberrations in spermatogonial cells was observed in any of the dimethyl propylphosphonate groups or in the positive control group.

Table A12: Results from mammalian spermatogonial chromosome aberration test with dimethyl propylphosphonate (Anonymous, 1998a)

Dose (mg/kg bw/day)	**0**	**500**	**1000**	**2000**	**Positive control**
Number of animals assessed	5	5	5	2	5
Mitotic index	2.16 %	2.1 %	2.72 %	1.9 %	2.46 %
Number of metaphases evaluated per group	251	401	79	135	403
Number of chromosomal aberrations in spermatogonial cells	0	0	0	0	3 breaks 2 aberrant metaphases

The study report concluded that there was no evidence of a clastogenic effect of dimethyl propylphosphonate or the positive control in spermatogonia of mice. As the concurrent positive control did not elicit an increase in chromosome aberrations in spermatogonial cells, the dossier submitter considers the negative result observed in this study to be unreliable.

3.8.2.5 Mammalian erythrocyte micronucleus test

Study reference

Anonymous (1995b): Dimethyl propane phosphonate micronucleus test on the mouse (Unpublished report).

Detailed study summary and results

Test type

Similar to OECD 474: Mammalian erythrocyte micronucleus test. The study deviated from the test guideline in that the bone marrow was sampled at only one time point, the required 4000 polychromatic erythrocytes per animal were scored and the positive control did not elicit an increase in micronucleated immature erythrocytes. GLP-compliant study.

Test substance

- *Name:* Dimethyl propylphosphonate. Identical to substance identified in CLH dossier.
- *Degree of purity:* > 99 %.
- *Impurities:* Not reported.
- *Batch number:* FHS 599/4.

Test animals

- *Species/strain/sex:* B6C3F1/BOM male mice.
- *No. of animals per sex per dose:* 5 males per group.
- *Age and weight at the study initiation:* Following 13 weeks of treatment in the rodent dominant lethal test (Anonymous, 1995a, described in section 3.8.2.1) males were assigned to this study. Therefore, male mice were approximately 20 – 22 weeks old. No information on body weight.

Administration/exposure

- *Doses/concentration levels*: 0, 500, 1000, and 2000 mg/kg/day dimethyl propylphosphonate.
- *Vehicle:* Deionised water.
- *Details on test system and conditions, and details on route of administration, exposure:* Following 13 weeks treatment in the rodent dominant lethal test (Anonymous, 1995a, described in section 3.8.2.1), 5 male B6C3F1/BOM mice from each group selected and administered a further single dose via oral gavage of dimethyl propylphosphonate or the positive control.
- *Positive control groups and treatment:* 2000 mg/kg bw/day dimethyl methylphosphonate via oral gavage.
- *Historical control data:* None reported.
- *Sampling regime:* Males were euthanised 24 hours following treatment. Bone marrow was collected from the femur before staining and fixing.
- *Criteria for scoring and number of cells analysed per animal:* 2000 polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were scored for the presence of micronuclei and the number of NCE per 1000 PCE reported.
- *Statistical analysis:* Non-parametric Wilcoxon’s non-parametric rank sum test.

Results and discussion

Table A13 below summarises the results. No increase in micronucleated polychromatic erythrocytes was observed in any of the dimethyl propylphosphonate groups or in the positive control. No change in the ratio of normochromatic to polychromatic erythrocytes was observed in any of the dimethyl propylphosphonate groups or in the positive control. A statistically significant increase in micronucleated normochromatic erythrocytes was observed in the 1000 mg/kg bw/day dimethyl propylphosphonate group and in the positive control group. However, in the absence of dose related response in the dimethyl propylphosphonate treated groups, this increase is not considered biologically significant.

Table A13: Results from the mammalian erythrocyte micronucleus test with dimethyl propylphosphonate (Anonymous, 1995b)

Dose (mg/kg bw/day)	0	500	1000	2000	Positive control
Number NCE to PCE per 1000 PCEs	935 ± 266	832 ± 109	825 ± 106	1929 ± 1336	1064 ± 278
Micronucleated cell per 1000 NCE	1.3 ± 0.4	1.7 ± 1.0	2.4 ± 0.2*	1.7 ± 1.4	3.0 ± 1.1*
Micronucleated cell per 1000 PCE	2.5 ± 1.4	3.9 ± 0.9	3.1 ± 0.4	4.2 ± 2.0	4.3 ± 1.5

* = $p < 0.05$

The study report concluded that based on the results observed, there was no evidence of a clastogenic effect of dimethyl propylphosphonate or the positive control in mice. As the concurrent positive control did not elicit an increase in the frequency of micronucleated polychromatic erythrocytes, the dossier submitter considers the negative result observed in this study to be unreliable.

3.8.2.6 Mammalian bone marrow chromosome aberration test

Study reference

Anonymous (1996): Dimethyl propane phosphonate *in vivo* cytogenetic study of the bone marrow in the mouse to evaluate for induced clastogenic effects (Unpublished report).

Detailed study summary and results

Test type

Similar to OECD 475 mammalian bone marrow chromosome aberration test. The study deviated from the test guideline in that the minimum number of animals per dose group and number of dose groups was not in line with current guideline requirements, less than the required 200 metaphases per animal were analysed, the mitotic index was not reported and the positive control group did not elicit an increase in chromosome aberrations. GLP-compliant study.

Test substance

- *Name:* Dimethyl propylphosphonate. Identical to substance identified in CLH dossier.
- *Degree of purity:* > 99 %.
- *Impurities:* Not reported.

- *Batch number:* FHS 599/4.

Test animals

- *Species/strain/sex:* B6C3F1/BOM male mice.
- *No. of animals per sex per dose:* 4 males at 1000 mg/kg bw/day and 5 males at 0 and 500 mg/kg bw/day. 5 males in the positive control group.
- *Age and weight at the study initiation:* Following 13 weeks of treatment in the rodent dominant lethal test (Anonymous, 1995a, described in section 3.8.2.1) males were assigned to this study. Therefore, male mice were approximately 20 – 22 weeks old. No information on body weight.

Administration/exposure

- *Doses/concentration levels*: 0, 500 and 1000 mg/kg/day dimethyl propylphosphonate.
- *Vehicle:* Deionised water.
- *Details on test system and conditions, and details on route of administration, exposure:* Following 13 weeks of treatment in the rodent dominant lethal test (Anonymous, 1995a, described in section 3.8.2.1), 4 - 5 male B6C3F1/BOM mice from each group selected and administered two further doses via oral gavage of dimethyl propylphosphonate or the positive control.
- *Positive control groups and treatment:* 2000 mg/kg bw/day dimethyl methylphosphonate via oral gavage.
- *Historical control:* None reported.
- *Assessment:* Animals were sacrificed the day after the final treatment (exact time not provided in the study report). 2 hours before sacrifice, animals were administered Colcemid via i.p. injection. Bone marrow was extracted from the femur and 100 metaphases per animal were examined for structural chromosomal aberrations.
- *Criteria for scoring and number of cells analysed per animal:* 100 metaphases per animal were examined for structural chromosomal aberrations. Metaphases showing chromosome disintegration were also recorded.
- *Statistical analysis:* The one-sided correct chi test.

Results and discussion

Table A14 below summarises the results. No increase in the frequency of cells with structural chromosome aberrations (excluding gaps) was observed in the test groups or in the positive control group. There was a slight but not statistically significant increase in the frequency of cells with structural chromosome aberrations (including gaps) in the dimethyl propylphosphonate treated groups but not in the positive control group. However, the observed increase was not dose dependent and is therefore not considered biologically relevant.

Table A14: Results from bone marrow chromosome aberration test with dimethyl propylphosphonate (Anonymous, 1996)

Dose (mg/kg bw/day)	0	500	1000	Positive control
Evaluated metaphases	480	500	400	459
Gaps	1	8	3	0
Breaks	1	1	1	0
Fragments	0	1	2	0
Deletions	0	0	0	0
Exchanges	1	2	1	0
Metaphases with structural chromosome aberrations including gaps	3	9	6	0
Metaphases with structural chromosome aberrations excluding gaps	2	2	3	0
Metaphases with structural chromosome aberrations with exchanges	1	1	1	0

The study authors concluded that there was no evidence of a clastogenic effect of dimethyl propylphosphonate or the positive control in bone marrow of mice. As the concurrent positive control did not elicit an increase in structural chromosome aberrations, the dossier submitter considers the negative result observed in this study to be unreliable.

3.8.2.7 Alkaline elution assay

Study reference

Anonymous (1998b): Dimethyl propane phosphonate alkaline elution *in vivo* for the detection of induced DNA single strand breaks in mouse testes (Unpublished report).

Detailed study summary and results

Test type

Non-guideline DNA alkaline elution test. GLP compliant study.

- *Test design:* Male mice were treated with dimethyl propylphosphonate or the positive control. 24 hours after the final treatment, animals were sacrificed and a cell suspension of testicular cells was prepared from each animal. The cell suspensions from each animal were loaded onto polycarbonate filters, where the cells were lysed and protein digestion was performed for 1 hour. Filters were then washed with EDTA wash butter (pH 10) and the isolated DNA was eluted under alkaline conditions and collected in fractions overnight (TEAH buffer at pH 12.1). The DNA concentration in the collected fraction and filter fractions were then assessed using automatic fluorimetry. The total DNA was calculated by summing the DNA concentrations in the collected fraction and the filer fraction and the percentage of DNA on the filter in relation to the total DNA was calculated.

Test substance

- *Name:* Dimethyl propylphosphonate.

- *Degree of purity:* > 99 %.
- *Impurities:* Not reported.
- *Batch number:* FHS 599/4.

Test animals

- *Species/strain/sex:* B6C3F1/BOM male mice.
- *No. of animals per sex per dose:* 5 males per group.
- *Age and weight at the study initiation:* Following 13 weeks of treatment in the rodent dominant lethal test (Anonymous, 1995a, described in section 3.8.2.1) males were assigned to this study. Therefore, male mice were approximately 20 – 22 weeks old. No information on body weight.

Administration/exposure

- *Doses/concentration levels*: 0, 500 and 1000 mg/kg/day dimethyl propylphosphonate.
- *Vehicle:* Deionised water.
- *Details on test system and conditions, and details on route of administration, exposure:* Following 13 weeks of treatment in the rodent dominant lethal test (Anonymous, 1995a, described in section 3.8.2.1), 5 male B6C3F1/BOM mice from each group selected and administered a further single dose via oral gavage of dimethyl propylphosphonate or the positive control.
- *Positive control groups and treatment:* 2000 mg/kg bw/day dimethyl methylphosphonate via oral gavage.
- *Historical control:* None reported.
- *Assessment:* The following were calculated: the total DNA (sum of the DNA concentrations in the collected fraction and in the filter fraction), the percentage filter DNA (percentage DNA on the filter in relation to the total DNA), mean percentage filter DNA per animal (mean percentage filter DNA of all samples of one animal), mean percentage filter DNA per group (mean percentage filter DNA of all animals in a dose group), and C-T value (mean percentage filter DNA of vehicle control less mean percentage filter DNA of treated group).
- *Criteria for evaluating the results*:
    - *Positive*: A dose-dependent, significant and in parallel treated animals, reproducible increase in DNA single strand break induction is observed (C-T value >19 % and a C-T value of 15 %- 19 % for weak genotoxic potential).
    - *Negative*: None of the doses tested induces a biologically relevant and significant increase in DNA single strand breaks.
    - *Equivocal*: No dose-dependency but one dose induces an increase in single strand breaks in all animals.
- *Statistical analysis:* None.

Results and discussion

Table A15 below summarises the results. No significant increase in C-T value, designated by the study authors as a measure of the significance of DNA strand break induction, was observed in the dimethyl propylphosphonate treatment groups or the positive control.

Table A15: Results from alkaline elution of testicular DNA with dimethyl propylphosphonate (Anonymous, 1998b)

Dose (mg/kg bw/day)	0	500	1000	Positive control
Cell viability (% of control)	100 %	96.0 %	98.1 %	99.6 %
Mean C-T value	-	0	9.7	1.2

The study report concluded that there was no evidence that dimethyl propylphosphonate or the positive control induced an increase in DNA single strand breaks in the testes of mice. The dossier submitter notes that this is a non-guideline study where no historical control data was provided to demonstrate the sensitivity and accuracy of the test system to identify DNA single strand breaks. It is also noted that the positive control did not elicit an increase in DNA single strand breaks. Therefore, the dossier submitter considers the negative result observed in this study to be unreliable.

3.8.3 Human data

Not available.

3.8.3.1 Other data

3.8.3.2 Histopathological analysis of mouse testes and epididymides

Study reference

Anonymous (1995c): Dimethyl propane phosphonate histopathology of testes and epididymides of the mouse (Unpublished report).

Detailed study summary and results

Test type

Histopathological analysis of testes and epididymides only. No information on GLP compliance. Limited reporting of method and results.

Test substance

- *Name:* Dimethyl propylphosphonate (identical to substance identified in CLH dossier).
- *Degree of purity:* > 99 %.
- *Impurities:* Not reported.
- *Batch number:* FHS 599/4.

Test animals

- *Species/strain/sex:* B6C3F1/BOM male mice.
- *No. of animals per sex per dose:* 5 males per group.
- *Age and weight at the study initiation*: Male mice were approximately 20 – 22 weeks old. No information on body weight.

Study outline

- Following 13 weeks of treatment in the rodent dominant lethal test (Anonymous, 1995a, see section 3.8.2.1) males were assigned to the mammalian erythrocyte micronucleus test (Anonymous 1995b, see Section 3.8.2.3). At the end of the micronucleus test, the testes and epididymides from males treated with 0, 500, 1000 or 2000 mg/kg bw/day dimethyl propylphosphonate were fixed in Bouin's fluid, embedded in paraffin wax and stained. Sections were examined histopathologically. Testes and epididymides from animals of the positive control group (dimethyl methylphosphonate) were also examined.

Results and discussion

No increase in the incidence of testicular focal tubular atrophy was observed. The incidences were 4/5, 3/5, 2/5, 3/5 at 0, 500, 1000 and 2000 mg/kg bw/day, respectively. The incidence of testicular focal tubule atrophy was 2/5 in the positive control group.

An 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 was observed in the 2000 mg/kg bw/day group. The incidence of atypic cells and giant cells of the testes in the positive control group was 3/5 and 1/5, respectively. The study report notes that some of the atypic and giant cells observed were multinucleated (without providing further details). The study report notes that spermatogenesis appeared to be unaffected "in most of the tubules" and that the epididymis contained "plenty of sperms". The study authors considered the increase in the incidence of atypic and giant cells in the testes to be treatment related. No abnormalities were noted in the epididymis of the vehicle control, dimethyl propylphosphonate or positive control groups.

The dossier submitter considers that the increase in the incidence of atypic and giant cells in the testes of males treated with 2000 mg/kg bw/day dimethyl propylphosphonate may indicate the substance 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.

3.9 Carcinogenicity

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

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Pilot reproductive toxicity study

Study reference:

Anonymous (2012): Pilot study on reproduction / fertility toxicity after administration by gavage (pilot study for an OECD 408/422 study) (Unpublished report).

Detailed study summary and results:

Test type

Non-guideline pilot reproductive toxicity study. Non- GLP compliant study.

- *Test design:* The study was conducted as a pilot study for an OECD 408/422 study and therefore included smaller group sizes and fewer examinations than outlined in OECD 422. Male and female

rats were administered dimethyl propylphosphonate via oral gavage during a 2 week pre-mating period and a 2 week mating period. Females were treated during gestation to post natal day (PND) 4. Males were treated for a total of 44 days. Animals were mated 1 male:1 female. Vaginal smears were taken to determine time of insemination and gestation length. Pregnant females were allowed to litter. Females and offspring were subject to necropsy on PND 4.

Test substance

- *Name:* Dimethyl propylphosphonate. Identical to substance identified in CLH dossier.
- *Degree of purity:* 97 %.
- *Impurities:* Impurities not reported.
- *Batch number:* Q-1101.

Test animals

- *Species/strain/sex:* Rat Wistar (HsdRCCHan:Wist) male and female.
- *No. of animals per sex per dose:* 5 males and 5 females per dose.
- *Age and weight at the study initiation:* Age not reported. Body weights on study day 1 were 341 – 347 g (males) and 216 – 222 g (females).

Administration/exposure

- *Route of administration:* Oral (gavage).
- *Duration and frequency of test/exposure period:*
    - Males: 2 week pre-mating period and 2 week mating period, up to study day 44. Daily administration.
    - Females: 2 week pre-mating period, 2 week mating period, throughout gestation until PND 4. Daily administration.
- *Doses/concentration levels, rationale for dose level selection:* 0, 20, 100 and 500 mg/kg bw/day dimethyl propylphosphonate. The study report indicates that doses were selected based on the results of two 28-day repeated dose toxicity studies where dimethyl propylphosphonate was administered up to 1000 mg/kg bw/day. Evidence of hyaline droplet nephropathy in males was observed at 5 mg/kg bw/day and above. No significant effects were observed in females.
- *Control group and treatment:* The control group received corn oil with the same treatment schedule as the test groups.
- *Historical control data if available:* No data reported.
- *Vehicle:* Corn oil.
- *Test substance formulation, achieved concentration, stability and homogeneity of the preparation:*
    - Test substance formulations prepared once per week and were reported to be stable for 7 days. No stability or homogeneity data reported.
    - Achieved concentrations not reported.
    - Application volume was 5 ml/kg bw.

Description of test design:

- *Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):*
    - M/F ratio: 1 male: 1 female.
    - Length of cohabitation: 5 days.
    - Proof of pregnancy: Insemination established via vaginal smears on the morning after co-habitation or vaginal plug.
- *Premating exposure period for males and females:* 2 weeks.
- *Standardization of litters (yes/no and if yes, how and when):* No.
- *Parameters assessed for P:*
    - Clinical observations: Animals inspected twice per day for signs of morbidity, including evaluation of general state of health, behaviour, condition of fur and orifices, and for mortality.
    - Body weight: Recorded on study day 1 and daily thereafter.
    - Food and water consumption: Recorded weekly.
    - Oestrous cycle length and pattern: Not examined.
    - Sperm examination: Not examined.
    - Organs examined at necropsy: Uterus (number of implantation sites), ovaries (number of corpora lutea) and kidneys.
    - Reproductive parameters: Number of implantation sites, number of corpora lutea and number of pups delivered.
- *Parameters assessed F1:*
    - Pup parameters: Number of live and dead pups; sex of pups and body weight of pups recorded on PND 0 and 4.
    - Clinical observations: Clinical signs recorded on PND 0 and 4.
    - Necropsy: Macroscopic examination of pups with particular attention on reproductive organs and visible skeletal abnormalities.
- *Post exposure observation period:* None.

Results:

- *Actual dose received by dose level by sex if known:* 0, 20, 100 and 500 mg/kg bw/day.
- *Statistical treatment of results, where appropriate:* Analysis of Variance (ANOVA) and Dunnetts test; 2*N Chi^2 test and Fischers exact test with Bonferroni correction; 2*N Chi^2 test and Fischers exact test.

For P:

- *Number of animals at the start of the test and matings:* 5/sex/dose.
- *Time of death during the study and whether animals survived to termination:* No mortality observed.
- *Body weight data:*
    - Males: No effect on body weight was observed in males at any dose.

- 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 data during gestation is reported in Table A16 below.

Table A16: 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**

In order to further assess whether the effect on material body weight observed in the high dose group was due maternal toxicity or an intrauterine effect, the corrected maternal body weight changes were calculated in accordance with Annex I, 3.7.2.4.4 of CLP. These are reported in Table A17 below. No significant effect on the calculated mean corrected maternal body weight change was observed at any dose. Gravid uterine and placental weights were not available and thus not included in the corrected maternal body weight change calculations.

Table A17: Calculated corrected maternal body weight change from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose (mg/kg bw/day)	**0**					**20**					**100**					**500**				
Animal number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Initial bw (g) (Study day 1)	223	227	220	226	217	223	216	216	227	214	211	215	221	224	216	219	219	214	209	222
Mean initial bw (g) (study day 1)	222.6					219.2					217.4					216.6				
Terminal bw (g) (GD 21/22)	377	394	420	267	391	394	394	408	392	284	379	382	382	364	370	270	263	320	363	314
Mean terminal bw (g) (GD 21/22)	369.8					374.4					375.4					306.0				
Maternal bw change* (g)	154	167	200	41	174	171	178	192	165	70	168	167	161	140	154	51	44	106	154	92
Mean maternal bw change* (g)	147.2					155.2					158.0					89.4				
Total pup wt. (g)	77.1	90.4	104.3	0	74.6	84.7	86.2	78	84.2	0	73.5	76.4	74.2	52	78.5	0	0	5.3	37.2	0
Mean pup wt. (g)	69.28					66.62					70.92					8.5				
Corrected maternal bw change** (g)	76.9	76.6	95.7	41	99.4	86.3	91.8	114	80.8	70	94.5	90.6	86.8	88	75.5	51	44	100.7	116.8	92
Mean corrected maternal bw change** (g)	77.92					88.58					87.08					80.9				

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

- *Food and water consumption:*
    - Males: Mean food consumption was increased in males at 500 mg/kg bw during week 5 of the study (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. There was no effect on water consumption.
    - Females: 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). There was no effect on water consumption.
- *Absolute and relative organ weight data for the parental animals:* Not reported.
- *Histopathological findings: nature and severity:* Histopathological examination was limited to kidneys, uterus (number of implantations) and ovaries (number of corpora lutea). At 500 mg/kg bw/day, pelvic dilation of 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 this dose.

  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 at 0, 20, 100 and 500 mg/kg bw/day, respectively. Renal tubular swelling and/or vacuolation was also observed from 20 mg/kg bw/day (5/5 at each dose compared with 0/5 in the control). There was an increased incidence of hyaline droplets at 100 mg/kg bw/day (4/5) and 500 mg/kg bw/day (5/5) compared with the control (0/5). Pelvic dilation with urothelial degeneration was reported in 1/5 males at 20 mg/kg bw/day.
- *Toxic response data including indices of mating, fertility, gestation, birth, viability and lactation; indicate the numbers used in calculating the indices:*
    - There was no effect on mating or gestation indices.
    - The number of females with implantations was 4/5, 4/5, 5/5 and 3/5 in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively. This corresponded with a biologically significant decrease in the fertility index in females in the high dose group. The fertility indices were reported as 80 %, 80 %, 100 % and 60 % in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
    - There was a decrease in live born pups in the high dose group, which corresponded, with a biologically significant decrease in the live birth index. The live birth indices were reported as 100 %, 100 %, 100 % and 62.5 % in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
    - The pup viability index was 0 in the high dose group due to no pups surviving beyond PND 1. The viability indices were reported as 100 %, 100 %, 98.46 % and 0 % in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
    - Lactation indices were not reported.
- *Clinical observations:* No remarkable clinical observations reported.
- *Haematological and clinical biochemistry findings if available:* Not reported.
- *Effects on sperm:* Not reported.

- *Number of P females cycling normally and cycle length:* Not reported.
- *Duration of gestation:* No effect on gestation length was observed. The mean gestation lengths were reported to be 22.50, 22.25, 22.20 and 22.67 days for the 0, 20, 100 and 500 mg/kg bw/day groups, respectively.
- *Precoital interval (number of days until mating and number of oestrous periods until mating):*
    - No effect on number of mating days to GD 0 was observed. The mean time to insemination was reported to be 2.0, 1.8, 3.0 and 2.2 days for the 0, 20, 100 and 500 mg/kg bw/day groups, respectively.
    - The number of oestrus periods until mating was not reported.
- *Number of implantations, corpora lutea, litter size:*
    - There was a biologically significant decrease in the total number of implantation sites in the high dose group: The mean number of implantation sites were 56, 58, 65, 33 in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
    - There was no effect on the number of corpora lutea.
    - There was a statistically significant decrease in the mean litter size in the high dose group. The mean litter sizes were 13.25, 13.5, 12.0 and 5.0 at 0, 20, 100 and 200 mg/kg bw/day, respectively.
    - There was a statistically significant decrease in the number of pups (live and dead) at birth in the high dose group. The total number of pups were reported to be 53, 54, 60 and 12 in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
- *Number of pre- and post-implantation loss:*
    - No information on pre-implantation loss reported.
    - A significant increase in post implantation loss was observed at the high dose group. The incidences were reported as 3, 4, 5 and 21 for 0, 20, 100 and 200 mg/kg bw/day groups, respectively. When the values were adjusted on a per litter basis, there was also a statistically significant increase in post implantation loss in the high dose group. The post implantation loss on a per litter basis was 0.75, 1.0, 1.0 and 7 for the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
- *Number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead foetuses:*
    - No information on number of abortions reported.
    - 1 female at 500 mg/kg bw/day was reported to have had stillborn pups. No other groups reported stillborn pups.
    - No information on the number of resorptions and/or dead foetuses reported.
- *Number of live births:* The number of live born pups was reduced in the high dose group. The number of live born pups was 53, 54, 60 and 10 in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively. There was a resulting biologically significant decrease in the live birth index at 500 mg/kg bw/day (62.5 % compared with 100 % in the control).
- *Data on functional observations:* Not reported.

For F1 pups/litters (per dose):

- *Mean number of live pups (litter size):*
    - The mean number of live pups per litter on PND 0 was statistically significantly reduced in the high dose group. The mean litter sizes were reported as 13.25, 13.5, 12.0 and 5 in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
    - The mean number of live pups per litter on PND 4 was 13.25, 13.50, 11.80 and 0 in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
- *Sex ratio:* The percentage of male pups was significantly decreased in the high dose group. The percentages of male pups on PND 0 were reported to be 66.14, 44.64, 43.08 and 14.29 in the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
- *Viability index (pups surviving 4 days/total births):* No pups in the high dose group survived beyond PND 1. The viability index at PND 4 was reported to be 100 %, 100 %, 98.46 % and 0 % for the 0, 20, 100 and 200 mg/kg bw/day groups, respectively.
- *Mean litter or pup weight by sex and with sexes combined:* A slight decrease in pup weight was observed in the high dose group on PND 0. No pups from this group survived to PND 4. Pup weights are summarised in Table A18.

Table A18: Summary of pup weights from a pilot reproductive toxicity study with dimethyl propylphosphonate (Anonymous, 2012)

Dose mg/kg bw/day	Pup weight on PND 0 (g)			Pup weights on PND 4 (g)		
	Males	Females	Males & Females	Males	Females	Males & Females
0	6.7	6.21	6.55	11.34	10.62	11.11
20	6.37	6.10	6.21	10.71	10.35	10.50
100	6.13	6.03	6.05	11.11	11.17	11.13
500	5.66	5.26	5.33	-	-	-

- *Clinical observations:* No remarkable clinical observations noted in pups up to 100 mg/kg bw/day during PND 0 - 4. At 500 mg/kg bw/day, no pup survived to PND 1.
- *Necropsy observations:* The number of pups subject to necropsy were 53, 54, 59 and 3 in the 0, 20, 100 and 500 mg/kg bw/day groups, respectively.
    - 2/3 pups at 500 mg/kg bw/day had no milk in their stomach.
    - 1/59 pups at 100 mg/kg bw/day had hydronephrosis of the left kidney.

Discussion:

The study 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 reproductive parameters. However, despite this, a significant effect on the fertility index, the post implantation loss, 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.

3.11 Specific target organ toxicity – single exposure

Not evaluated as part of this dossier.

3.12 Specific target organ toxicity – repeated exposure

Not evaluated as part of this dossier.

3.13 Aspiration hazard

Not evaluated as part of this dossier.

4 ENVIRONMENTAL HAZARDS

Not evaluated as part of this dossier.

5 REFERENCES

Anonymous (1993a). Dimethylpropanphosphonat Salmonella / microsome test. (Unpublished report).

Anonymous (1993b). Dimethylpropanphosphonate mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay in vitro. (Unpublished report).

Anonymous (1995a). Dimethyl propane phosphonate dominant lethal test on the male mouse using subchronic treatment. (Unpublished report)

Anonymous (1995b): Dimethyl propane phosphonate micronucleus test on the mouse. (Unpublished report)

Anonymous (1995c): Dimethyl propane phosphonate histopathology of testes and epididymides of the mouse. (Unpublished report)

Anonymous (1996): Dimethyl propane phosphonate in vivo cytogenetic study of the bone marrow in the mouse to evaluate for induced clastogenic effects. (Unpublished report)

Anonymous (1998a): Dimethyl propane phosphonate in vivo cytogenetic study of spermatogonia in the mouse to evaluate for induced clastogenic effects. (Unpublished report)

Anonymous (1998b): Dimethyl propane phosphonate alkaline elution in vivo for the detection of induced DNA single strand breaks in mouse testes. (Unpublished report)

Anonymous (2012). Pilot study on reproduction / fertility toxicity after administration by gavage (pilot study for an OECD 408/422 study). (Unpublished report).

Dunnick JK, Solleveld HA, Harris, MW, Chapin R and Lamb JC (1984a). Dimethyl methyl phosphonate induction of dominant lethal mutations in male mice. Mutat. Res.**138**: 213-218.

Dunnick JK, Gupta BN, Harris MW and Lamb JC (1984b). Reproductive toxicity of dimethyl methyl phosphonate (DMMP) in the male Fischer 344 Rat. Toxicol. Appl. Pharmacol. **72:** 379-387.