Committee for Risk Assessment

RAC

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

Adopted

16 September 2021

16 September 2021

CLH-O-0000007021-89-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Dimethyl propylphosphonate**

EC Number: **242-555-3**

CAS Number: **18755-43-6**

The proposal was submitted by **Ireland** and received by RAC on **6 August 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Ireland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **24 August 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 October 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nathalie Printemps**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	-	-	-
RAC opinion	TBD	Dimethyl propylphosphonate	242-555-3	18755-43-6	Muta. 1B Repr. 1B	H340 H360Df	GHS08 Dgr	H340 H360Df	-	-	-
Resulting Annex VI entry if agreed by COM	TBD	Dimethyl propylphosphonate	242-555-3	18755-43-6	Muta. 1B Repr. 1B	H340 H360Df	GHS08 Dgr	H340 H360Df	-	-	-

GROUNDS FOR ADOPTION OF THE OPINION

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).

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 when 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 that 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**.

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 females 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, which are not considered to be secondary to maternal toxicity. Therefore, RAC considers that overall data on DMPP fulfills the criteria and warrants for classification as **Repr. 1B, H360D**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).