Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Dimethyl propylphosphonate

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

CLH-O-0000007021-89-01/F

Adopted

16 September 2021

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIMETHYL PROPYLPHOSPHONATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: Dimethyl propylphosphonate
EC number: 242-555-3
CAS number: 18755-43-6
Dossier submitter: Ireland

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2020	Germany		MemberState	1
Comment received				
We support the proposed classification as Muta. 1B based on positive results with the sub-stance in a rodent dominant-lethal test, i.e. an in vivo heritable germ cell mutagenicity test in mammals, conducted in accordance with OECD 478 and GLP. In the CLH report it is stated that the study authors of the rodent dominant-lethal test con-cluded that there was a clear indication of a mutagenic effect of dimethyl propylphospho-nate under the conditions of the study. The dossier submitter considers that the study was well conducted and reliable and that the clear increase in pre- and post-implantation-loss in untreated females mated with dimethyl propylphosphonate-treated males is indicative of a treatment-related effect. The dossier submitter acknowledges that the lack of performed statistical analysis could be considered as a limitation of the study. The DE CA supports the interpretation of the dossier submitter that a clear biologically significant response was ob-served in the dimethyl propylphosphonate-treated groups and that under the conditions of this study, dimethyl propylphosphonate induced dominant lethal mutations in mice. Thus, the DE CA supports that a Muta. 1B classification is warranted for the substance.				
Dossier Submitter's Response				
The IE CA would like to thank the DE CA for their comments and support.				
RAC's response				
Thank you for your comment. RAC agrees with the DS's proposal.				

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2020	Sweden		MemberState	2

Comment received

We note that the available in vitro mutagenicity tests of DMPP for gene mutations (OECD TG 471 and OECD TG 476) are negative (although no cytotoxicity was evident at any dose) and that no micronucleus or chromosomal aberration tests are available in in vitro or in vivo (somatic cells) to confirm the mechanism of mutagenicity. The in vivo somatic genotoxicity studies (all negative) of DMPP are not considered reliable and therefore cannot be taken into account for classification purposes. However, there was a germ cell in vivo test performed according to OECD TG 473 available that was considered reliable. The rodent dominant lethal test, designed to detect DLs as a result of gross chromosomal aberrations (but not excluding gene mutations) of DMPP shows dose dependent increase in the rate of post implantation loss per fertilised female from 500 mg/kg bw/day (24.5%, 55.0 % and 82.6 % at 500, 1000 and 2000 mg/kg bw/day vs 5.6 % in control). At 2000 mg/kg bw/day there was high general toxicity reported as 12/20 males died prior to study termination and from 500 mg/kg bw/day the males displayed severe clinical signs (incidence, time points and duration not clear from the report and no Annex I with study details is available). In this study, also decreased fertilisation rate and increased pre-implantation loss per fertilized female at 2000 mg/kg bw/day were observed. Dominant-lethal mutations cause early embryonic death before or around the time of implantation. Thus, based on available data it is not possible to conclude if the observed pre-implantation loss is due to dominant lethal effects or not. Furthermore, in the OECD 408/422 reproductive toxicity screening pilot study in rats, increases incidence of pre-implantation loss was also reported at the highest dose tested (500 mg/kg bw/day). Provided as supporting evidence, there were two studies of limited reporting with the structurally similar substance, dimethyl methylphosphonate, investigating dominant lethal effects in mice and rats. We agree that this information could be used as supporting information, however, the justification for this read-across (other than structural similarity) is not described in the CLH-report. In these studies, similar to the DL assay on DMPP, high doses of DMMP up to 2000 mg/kg bw were administered. Increased incidences of post-implantation loss were reported from 250 mg/kg bw/day in the rat study and from 1000 mg/kg bw/day in the mouse study. Decreased fertility at 1000 and 2000 mg/kg bw/day (no females pregnant at 2000 mg/kg bw/day) and increased pre-implantation loss at 2000 mg/kg bw/day were reported in the rat study. Moreover, relative epididymis weight at 2000 mg/kg bw/day was decreased, lack of spermatogenesis, and degeneration, vacuolisation and necrosis of spermatogonial cells in testes were observed in 18/20 males at 2000 mg/kg bw/day (0/20 in control); increased incidence of sperm head abnormalities seen at 2000 mg/kg bw/day.
The relationship between chromosomal aberrations, the histopathological effect on testis and morphology of sperms is unclear, however, it cannot be excluded based on available data that the observed effects of DMPP/DMMP on sperm parameters are linked to the mutagenic effect.
Overall, we concur with the dossier submitter that the criteria for classification in Muta. 1B are met due to positive results from in vivo heritable germ cell mutagenicity tests in mammals.

Dossier Submitter's Response

The IE CA would like to thank the SE CA for their comments and support. Please find below our responses to the specific observations made.

- We agree that the available *in vitro* data with DMPP investigates only gene mutation and that the available *in vivo* somatic cell studies with DMPP are unreliable. Therefore, we agree that based on this data, no conclusion on the mechanism of mutagenicity can be drawn. However, the available *in vivo* germ cell study (dominant lethal test) with DMPP is considered to be reliable. As indicated in the SE CA comment, the dominant lethal test is designed to detect dominant lethal mutations as a result of gross chromosomal aberrations, but that gene mutations cannot be excluded. In this study, a clear dose dependent increase in pre- and post-implantation loss was observed in untreated females mated with DMPP treated males. Therefore, we consider that this study provides evidence of an effect on male germ cells, although no conclusion regarding whether this is due to gene mutation or chromosome aberration can be drawn. However, we note that CLP Annex I, 3.5 does not specify that the mechanism of mutagenicity needs to be established to support classification as a germ cell mutagen.

- With respect to the toxicity observed in males in the rodent dominant lethal study with DMPP, the study report notes that that the clinical signs of toxicity observed in the 1000 and 2000 mg/kg bw/day groups (apathy, semi-anaesthetised state, reduced reflexes, recumbency and difficulty breathing) were observed for at least 8 hours after each application. The incidence of these findings per dose group was not reported. As reported in the CLH proposal (and the accompanying Annex I with the study details), of the 12/20 males that died prior to study termination, 5/20 males died prior to the first mating interval at 5 weeks, 3/20 males died prior to the second mating interval at 9 weeks and 4/20 males died prior to the third mating interval at 13 weeks.

- The SE CA comment notes that a decrease in fertilisation rate was observed at 2000 mg/kg bw/day. As stated in the CLH report, due to the high mortality and clinical signs of toxicity observed in males at 2000 mg/kg bw/day we consider that it cannot be excluded that the lower fertilisation rates observed in this group may be attributed to systemic toxicity of DMPP to males rather than a specific genotoxic effect. Due to the lower fertilisation rate in this group, the data related to pre- and post-implantation loss for all groups were presented "per fertilised female". Therefore, we consider the effect on pre- and post-implantation loss to be independent of the reduced fertility rate and thus it cannot be excluded that it is due to dominant lethal mutations.
  We agree that the dominant lethal test is designed to detect dominant lethal mutations which are 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 due only to a dominant lethal effect. The comment states that there was an increase in pre-implantation loss at the highest dose (500 mg/kg bw/day) in the pilot reproductive toxicity study with DMPP. However, no information on pre-implantation loss was reported in the study report for this study.

- As indicated in the CLH report, the dominant lethal tests on the structurally similar substance, dimethyl methylphosphonate (DMMP), are included as supporting information only and no read-across is proposed. DMMP was selected as the positive control in the dominant lethal test with DMPP by the study authors as a "class specific positive control" and thus the dominant lethal tests with DMMP were included in the CLH proposal as supporting information only. We consider that the results of the dominant lethal test with DMPP to be sufficient to support classification as Muta. 1B. Full details of both dominant lethal tests with DMMP are included in Annex I to the CLH report.

We have included below an overview of the available data for both DMPP and DMMP from their respective REACH registration dossiers.

DMPP and DMMP are organophosphorus substances. They are structurally similar, differing only in the alkyl chain length (methyl in DMMP and propyl in DMPP). Both substances have similar molecular weights (152 for DMPP and 124 for DMMP), log Kow (0.5 for DMPP and -0.61 for DMMP) and water solubilities (> 900 g/l for DMPP and > 100 g/L for DMMP).

Neither substance is acutely toxic, both are irritating to eyes but not to skin. Neither substance is classified for skin sensitisation.

Both DMPP and DMMP have similar target organs in repeated dose toxicity studies, although there appears to be differences in potency. The longest duration study with DMPP is a 28-day repeated dose toxicity study, where the liver (an increase in organ weight and hepatocellular hypertrophy at ≥ 40 mg/kg bw/day) and kidney (an increased incidence of renal surface changes, tubular degeneration and dilation and an increase in basophilic tubules in males at ≥ 5 mg/kg bw/day) were identified as target organs. A decrease in grip strength in females was also reported at 1000 mg/kg bw/day. In a 28-day repeated dose toxicity study with DMMP, liver (an increase in organ weight at 1790 mg/kg bw/day) and kidney (an increase in organ weight and evidence of protein resorption droplets in the proximal convoluted tubule in males at 535 mg/kg bw/day) were also identified as target organs. In 90-day repeated dose toxicity studies with DMMP, hepatocellular hypertrophy was observed at ≥ 195 mg/kg bw/day, renal tubule regeneration at ≥ 65 mg/kg bw/day, nephrosis and hyaline droplet degeneration at ≥ 250 mg/kg bw/day and testicular atrophy at ≥ 250 mg/kg bw/day.

The available *in vitro* genotoxicity data for DMPP is limited: a negative Ames test and a negative *in vitro* gene mutation in mammalian cells are reported. For DMMP, both positive and negative Ames tests are reported. DMMP was positive in an *in vitro* gene mutation test in mammalian cells and negative in an *in vitro* chromosome aberration test. A positive *in vitro* sister chromatid exchange assay in mammalian cells with DMMP is also reported. No reliable somatic cell genotoxicity data are available for DMPP and no somatic cell genotoxicity data are reported for DMMP. Positive dominant lethal tests in mice with both DMPP and DMMP are available. DMMP was also positive in a dominant lethal test in rats.

With respect to developmental toxicity, a prenatal developmental toxicity study with DMMP is available where evidence of developmental delay (decrease in skeletal maturation) at ≥ 1000 mg/kg bw/day was observed. This effect appeared to occur in the absence of marked maternal toxicity. No prenatal developmental toxicity study is available for DMPP. However, in a pilot reproductive toxicity study with DMPP, a decrease in live birth index, mean litter size and percentage of male pups was observed at 500 mg/kg bw/day, with no pups surviving beyond post-natal day 1 at this dose.

With respect to effects on fertility, in the pilot reproductive toxicity study with DMPP, there was a decrease in the fertility index, the number of implantation sites, and number of pups at birth at 500 mg/kg bw/day. At this dose there was also an increase in prenatal loss. No study investigating effects on fertility (other than the dominant lethal tests) are reported for DMMP.

Based on the above, the dossier submitter considers DMPP to be structurally and toxicologically similar to DMMP, and thus the dominant lethal tests with DMMP can be used as supporting information in the CLH proposal.

RAC's response

Thank you for your comment. RAC agrees with the DS's proposal and response.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2020	Germany		MemberState	3

Comment received

Fertility:

We support the proposed classification as Repr. 1B for fertility based on an available pilot reproductive toxicity study with dimethyl propylphosphonate. The study provides clear evidence of an effect on sexual function and fertility not considered to be a secondary non-specific consequence to other toxic effects.

In the pilot non-GLP-compliant reproductive toxicity study (Anonymous, 2012), the effects considered indicative of interference with sexual function and fertility were observed at 500 mg/kg bw/day:
• a decrease in the fertility index (60 % vs. 80 % in the Ctrl),
• a biologically significant decrease in the number of implantation sites (total: 33 vs. 56 in the Ctrl),
• a significant increase in the post-implantation loss per litter (7 vs. 0.75 in the Ctrl, $p < 0.01$) and
• a significant decrease in the total number of pups born (12 vs. 53 in the Ctrl, $p < 0.01$).
At the same dose, no marked systemic toxicity in dams was observed. In contrast, no mortality and increased food consumption were reported. After correction for pup weight, no changes in BW of dams of the highest dose was observed. The DE CA supports the dossier submitter's view that despite some limitations the study provides clear evidence of an effect on sexual function and fertility.
Moreover, we also support that the increase in the incidences of pre- and post-implantation loss observed in the dominant lethal test with dimethyl propylphosphonate (Anonymous, 1995a), can be considered as additional indication of an effect on sexual function and fertility. In this study, post-implantation loss was increased from 500 mg/kg bw/day (dead implants per fertilized female: 3.0 vs. 0.8 in the Ctrl) and pre-implantation-loss from 1000 mg/kg bw/day (1.5 vs. 0.9 in the Ctrl). While females were not treated in this test, changes in mating behaviour of treated males could be caused by general toxicity as evident from clinical signs of apathy, semi-anaesthetised state and difficulty breathing in males from 1000 mg/kg bw/day and high mortality rate at 2000 mg/kg bw/day.

Effects on development:

We support the proposed classification as Repr. 1B for developmental effects based on the pilot reproductive toxicity study with dimethyl propylphosphonate. In the study, a significant decrease in the number of live-born pups and live-birth index was observed (Anonymous, 2012).
The live-birth index was reduced from 100 % in the control to 62.5 % in the 500 mg/kg bw/day dose. Furthermore, no pups at 500 mg/kg bw/day survived beyond PND 1 and thus the viability index at PND 4 at 500 mg/kg bw/day was 0 %.
We support the conclusion of the dossier submitter that the observed effects on development are not secondary to maternal toxicity.
Further, we acknowledge that the effect on post-implantation loss in the dominant lethal

test provides supporting evidence for an effect on development.

We are of the opinion that the classification of the substance as Repr. 1B, H360FD is warranted.

Dossier Submitter's Response

The IE CA would like to thank the DE CA for their comments and support.

RAC's response

Thank you for your comment. RAC agrees to classify the observed effects on development and fertility. 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. Based on the severe developmental effect, classification of DMPP as Repr. 1B is considered appropriate. Considering the observed effects on fertility and the limitations of the study (e.g. low sensitivity, few animals), the data on fertility are inconclusive to decide on category 1B. Therefore, RAC considers that Repr. Cat. 2, H361f is more appropriate for DMPP.

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2020	Sweden		MemberState	4

Comment received

Adverse effects on fertility and sexual function
The pre-implantation losses observed in the DL test of DMPP and in the pilot reproductive toxicity screening study of DMPP are considered more appropriate to address under the classification of germ cell mutagenicity, based on the results of DL test indicating mutations in germ cells. This is in accordance with CLP Annex I, 3.7.1.1. Moreover, the decrease in fertility index (however unclear if due to pre-implantation loss of fertilized ova or or loss of unfertilized ova) and the histopathological effects on testes and sperm parameters, as reasoned under the comments to mutagenicity, could also be due to the mutagenic effects in germ cells. Therefore, it could be discussed whether also classification for adverse effects on fertility is warranted or not, in addition to the Muta. 1B classification. Furthermore, considering high general toxicity (mortality and severe clinical conditions) of males in the DL test of DMPP at the same dose levels as the observed adverse effects on fertility, category 1B is appears not appropriate.

Adverse effects on the development of the offspring
Similarly to the comments on fertility, we do not think that classification for adverse effects on the development of the offspring is warranted since the observed effects (increased post-implantation loss, decreased %male pups and decreased viability index up to PND 1) are more appropriately addressed under the classification of germ cell mutagenicity, in line with Annex I, 3.7.1.1.

Dossier Submitter's Response

We would like to thank the SE CA for their comments. We agree that there may be some overlap between the effects observed in the dominant lethal test and those observed in the pilot reproductive toxicity study. Therefore, we can appreciate the point made by the SE CA that in accordance with CLP Annex I, 3.7.1.1, it may be more appropriate to address the effects observed in the pilot reproductive toxicity study under germ cell mutagenicity. In preparing the CLH proposal, we had considered this point and concluded that a separate classification for toxicity to reproduction is warranted. The aims of the

dominant lethal test and the pilot reproductive toxicity study are different. In particular, the pilot reproductive toxicity study was not designed to confirm that the effects observed were solely due to a dominant lethal effect in male rats. Therefore, as it cannot be excluded that other mechanisms of action were responsible for the effects observed in the pilot reproductive toxicity study, we consider that a separate classification for toxicity to reproduction is warranted.

Please find below our responses to the specific observations made.

Adverse effects on fertility and sexual function:

- The pilot reproductive toxicity study report states that the fertility index was calculated as the number of pregnant females/number of sperm positive females. No information on pre-implantation loss or loss of unfertilised ova is reported.

- No histopathological examination of males was performed in the pilot reproductive toxicity study. As indicated in the SE CA comment, a histopathological examination of the testes and epididymides was performed as part of the follow up *in vivo* mammalian erythrocyte micronucleus test (Anonymous, 1995c) reported in the germ cell mutagenicity section of the CLH report. 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 of males was reported in the high dose group (2000 mg/kg bw/day). The study report notes that spermatogenesis was "apparently unaffected in most of the tubules and that the epididymides contained plenty of sperm". No further details are provided in the study report. As indicated in the CLH report, as only a limited histopathological examination was performed on a small number of animals, we consider that no firm conclusions can be drawn from this data. However, it may indicate that DMPP reaches the testes.

- We agree that in the dominant lethal test with DMPP, mortality and clinical signs of toxicity were observed in male mice at ≥ 1000 mg/kg bw/day. At the lower dose of 500 mg/kg bw/day, no mortality or clinical signs of toxicity were observed. The pilot reproductive toxicity study was conducted in rats, with the highest dose set at 500 mg/kg bw/day. In this study, no mortality or clinical signs of toxicity were reported in the test animals and no effect on body weight was reported in males at any dose. The effect on fertility observed in the pilot reproductive toxicity study in rats occurred at doses lower than those in the dominant lethal test which caused the high general toxicity. Therefore, we do not consider the effects observed to be secondary to general toxicity and thus classification in category 1B is appropriate.

Adverse effect on development

- As indicated above, we agree that there may be some overlap between the effects observed in the dominant lethal test and those observed in the pilot reproductive toxicity study. We consider that the effects observed on the number of pups born, the number of dead pups, the mean litter size, the viability of pups on PND 4 and the percentage of male pups are indicative of an effect on development. These effects occurred in the absence of maternal toxicity and therefore we consider that classification in category 1B is appropriate.

RAC's response

Thank you for your comment. See response to comment 3.