CONSIDERATIONS OF ALTERNATIVE METHODS ON TESTING PROPOSALS IN YOUR REGISTRATION

Please complete this form and provide information for each of the points below.

If you have more than one testing proposal, please copy and paste the three bullet points within the same document and complete the details as appropriate for each testing proposal.

This document will be published on ECHA website along with the third party consultation on the testing proposal(s).

Public substance name: Dichloromethylbenzene
EC Number: 249-854-8
CAS Number: 29797-40-8

Date of considerations: 8 June 2017

- **Hazard endpoint for which vertebrate testing was proposed:**

  Reproductive toxicity (extended one-generation reproductive toxicity study) with the registered substance in the range:
  - **Cohort 1A (Reproductive toxicity);**
  - **Cohort 1B (Reproductive toxicity without extension to mate the Cohort 1B animals to produce the F2 generation).**

- **Considerations that the general adaptation possibilities of Annex XI of the REACH Regulation were not adequate to generate the necessary information**

  - available GLP studies

There is no Reproduction/Developmental Screening Test or EOGRTS available for dichloromethylbenzene (Isomer mixture). Two studies according OECD 422 (Combined repeated Dose Toxicity Study with the reproduction/ Developmental Toxicity Screening test) are available for two isomers; see paragraph on WoE.

  - available non-GLP studies

There are no non-GLP fertility studies available.

  - historical human data

There are no historical human data available.

  - (Q)SAR

No data available; there is no QSAR model available which is accepted by ECHA for the endpoint fertility.

  - *in vitro* methods

No data available; there is no in vitro method available which is accepted by ECHA for the endpoint fertility.

  - weight of evidence

There is no Reproduction/ Developmental Screening Test or EOGRTS available for dichloromethylbenzene (Isomer mixture). Two studies according OECD 422 (Combined repeated Dose Toxicity Study with the reproduction/ Developmental Toxicity Screening test) are available for two isomers.

2,4-Dichloro-1-methylbenzene EC: 202-445-8 CAS: 95-73-8 (CIPC 1994):
Rats received 0 (vehicle), 12.5, 79, 500 mg/kg/day test substance via gavage. Salivation was observed in males and females receiving 12.5 mg/kg/day or more just after the administration. Body weight gain was reduced in 500 mg/kg females in the pregnancy and lactation periods. Food consumption was decreased in the 500 mg/kg group on day 2 of administration in males and on day 10 of administration and day 3 of pregnancy in females.

On the hematological and blood chemical analyses, the 500 mg/kg males exhibited decreased numbers of platelets and increased cholinesterase values. Increases in relative organ weights were seen for the livers of males and females, and kidneys of males at 500 mg/kg. On gross necropsy, dark brown discoloration of the liver was observed in 9/12 males receiving 500 mg/kg.

On microscopic examination, hypertrophy of hepatocytes in the centrilobular zones of the livers was observed in all 500 mg/kg males and in 2/12 79 mg/kg males. Atrophy and regeneration of kidney tubular epithelium, and dilatation of tubules, were seen in 2/12 males and in 1/12 females given 500 mg/kg, and in 1/12 79 mg/kg males. Occurrence of hyaline droplets and eosinophilic body deposition in tubular epithelia was increased in males receiving 79 mg/kg or more. The LOEL for repeated dose toxicity was found to be 12.5 mg/kg/day.

Only at 500 mg/kg bw/day, the highest dose tested, in combination with maternal toxicity a lowered fertility index was seen. Missing vaginal plugs or a sparseness of sperms in the vagina were rated in 6/7 copulated and nongestated rats. Male and female offspring of the 500 mg/kg group exhibited slightly lowered body weights on days 1 and 4 of lactation. The test substance did not show any effects of delivery of maternal behavior, as well as viability, clinical signs, and gross necropsy findings for offspring.

1,3 Dichloro-2-methylbenzene EC: 204-269-7 CAS: 118-69-4 (CIPC 2002):
Rats received 0 (vehicle), 30, 100, 300 and 1000 mg/kg/day test substance via gavage.

General toxicity: based on centrilobular hypertrophy of hepatocytes observed in females and males of the 300 and 1000 mg/kg/day groups. Decrease of body weight gain starting at 300 mg/kg/day in males only.

Reproductive toxicity: No adverse effects at any dose level were noted for copulation, fertility or delivery.

Reproductive and offspring toxicity: in the short English abstracts the authors indicate that numbers of dead pups were increased and viability on postnatal day 4 was decreased in the 300 and 1000 mg/kg groups (statistical significance not indicated). Pup weights on postnatal day 4 tended to be decreased in the 1000 mg/kg group. No morphological abnormalities were found in pups of any treated group.

Repeated dose toxicity studies with dichloromethylbenzene did not show any effect on reproductive organs in males or females.
In a subacute oral toxicity study according to OECD Guideline 407, 5 male and 5 female Wistar-rats per group received 0, 20, 100 or 500 mg/kg/day per gavage on 7 days per week for 4 weeks. No substance-related effects on sexual organs could be observed; the following reproductive organs were investigated: testes, epididymis, ovaries, oviduct, prostate and seminal vesicles (Bomhard, 1993).

A sub-chronic oral toxicity study was conducted in the rat according to OECD TG 408

following an ECHA decision on a compliance check. The test item, formulated in polyethylene glycol 400, was administered daily at least 90 days by oral gavage administration to SPF-bred Wistar rats. One control group and three treated groups were tested, each consisting of 10 males and 10 females. The dose levels for this 90-day oral gavage study were selected to be 0, 20, 100, 400 mg/kg/day for males and 0, 100, 400 and 800 for females. The lowest dose level of 20 mg/kg/day for males is based on the anticipated kidney effects (alpha 2u globulin nephropathy). The highest dose level of 800 mg/kg/day is based on the anticipated low systemic toxicity of dichloromethylbenzene. No effect (weight and histopathology) was observed on any reproductive organ investigated (Beerens-Heijnen, 2016).

Recent literature (Mangelsdorf et al 2003, Ulbrich & Palmer 1995, Janer et al 2007, Dent 2007, Sanbuissho et al 2009) concluded that in rodents histopathological examinations in repeated dose toxicity studies of reproductive tissues are of high value and high sensitivity for evaluation of reproductive toxicity in males and females. Data evaluation of 117 substances or substance classes by Ulbrich & Palmer (1995) revealed that histopathology and organ weight analysis provide the best general purpose means of detecting substances with potential to affect male fertility, particularly those related to effects on spermatogenesis. The data evaluation by Mangelsdorf et al (2003) revealed that the most sensitive endpoint for detecting adverse effects of chemicals on male reproduction is the histopathology of the testis. Sanbuissho et al (2009) revealed that comprehensive histopathological examination of the female reproduction organs is a good tool to assess female reproduction function. Pathological findings of ovarian toxicity (decreases in follicles, increases in atretic follicles, increases in currently formed corpora lutea, etc.) reflected the female fertility parameter (irregular estrous cycle, pre-implantation loss). The data analysis by Dent (2007) revealed that subchronic toxicity studies are suitable to predict effects on rodent fertility.

In conclusion there is no evidence of a specific reproductive potential in the early studies with 2,4-Dichloro-1-methylbenzene (CIPC 1994) and 1,3-Dichloro-2-methylbenzene (CIPC 2002) since offspring observations are reported at maternal toxic doses and the observations are not consistent between two OECD TG 422 studies. Repeated dose toxicity studies with dichloromethylbenzene did not show any effect on reproductive organs in males or females.

Overall there is no evidence of a specific reproductive potential of dichloromethylbenzene. Further testing on this substance should be of low priority taking also animal welfare considerations into account.

- grouping and read-across

There is no Reproduction/ Developmental Screening Test or EOGRTS available for dichloromethylbenuzene (Isomer mix). Two studies according OECD 422 (Combined repeated Dose Toxicity Study with the reproduction/ Developmental Toxicity Screening test) are available for two isomers.

- substance-tailored exposure driven testing

Not applicable

- [approaches in addition to above [if applicable]

Not applicable

- other reasons [if applicable]

Not applicable

- **Considerations that the specific adaptation possibilities of Annexes VI to X (and column 2 thereof) were not applicable**:

According to Annex X an extended One-Generation Reproductive Toxicity study is a standard information requirement. The available data reported above show no evidence of a specific reproductive potential of dichloromethylbenzene. Further testing on this substance should be of low priority taking also animal welfare considerations into account.

References:

C.G.M. Beerens-Heijnen 2016: REPEATED DOSE 90-DAY ORAL TOXICITY STUDY WITH DICHLOROMETHYLBENZENE BY DAILY GAVAGE IN THE RAT (study report), Report send to Lanxess. Testing laboratory: WIL Research Europe B.V., Report no: WIL Research Project 508051; Draft. Owner company; LANXESS Deutschland GmbH, Report date:

Bomhard E 1993: Dichlortoluol-Isomerengemisch; Untersuchungen zur subacuten Toxizität an Wistar-Ratten (Verabreichung via Magensonde über ca. 4 Wochen) (study report), BAYER AG, Fachbereich Toxikologie, Friedrich-Ebert-Str. 217-333, D-5600 Wuppertal 1, Germany. Testing laboratory: BAYER AG, Fachbereich Toxikologie, Friedrich-Ebert-Str. 217-333, D-5600 Wuppertal 1, Germany, Report no: 22273. Owner company; Lanxess, Report date: May 25, 1993

Chemicals Investigation Promoting Committee (CIPC) 1994: 2,4-Dichlorotoluene, CAS No. 9573-8 (publication), Chemicals Investigation Promoting Committee. Testing laboratory: Office of Environmental Chemicals Safety Environmental Health Bureau, Ministry of Health & Welfare, Japan, Report date:

Chemicals Investigation Promoting Council (CIPC) 2002: Toxicity Testing Reports of Environmental Chemicals, Vol.9, 2002; 2,6-Dichlorotoluene (publication), Chemicals Investigation Promoting Council. Testing laboratory: Office of Environmental Chemicals Safety, Evaluation and Licensing Div., Pharmaceutical and Medical Safety Bureau, Ministry of Health, Labour & Welfare, Japan, Report no: ISSN 1340-3842. Report date:

Dent, 2007: Strength and limitations of using repeated-dose toxicity studies to predict effects on fertility. Regulatory Toxicology and Pharmacology 48, 241-258

Janer et al., 2007: A retrospective analysis of the added value of the rat two-generation reproductive toxicity study versus the rat subchronic toxicity study. Reproductive Toxicology 24, 103-113

Mangelsdorf. et al., 2003: Some aspects relating to the evaluation of the effects of chemicals on male fertility. Regulatory toxicology and Pharmacology 36, 69-98

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Ulbrich & Palmer, 1995: Detection of effects on male reproduction – a literature survey. J am. College of Toxicology 14, 293-327