A review of the toxicological information available for dicyclopentadiene (DCPD) pertinent to the assessment of DCPD's potential to cause endocrine disruption

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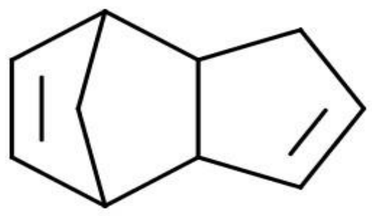
Introduction

Dicyclopentadiene (3a,4,7,7a-tetrahydro-4,7-methanoindene, EC no. 201-052-9; CAS no. 77-73-6), abbreviated **DCPD**, is an olefinic hydrocarbon chemical with the molecular formula $C_{10}H_{12}$.

At room temperature, commercial DCPD is a clear colourless liquid with a camphor-like odour. The substance is manufactured and/or imported in Europe at volumes >1,000 tons per year and is used as an intermediate in the manufacturing of chemicals and polymers.

DCPD



Concerns related to foetotoxicity observed in multi-generation and prenatal developmental toxicity studies in rats and rabbits led the Registrants part of the Lower Olefins and Aromatics (LOA) REACH consortium to self-classify DCPD as a **Category 2 reproductive toxicant** (suspected human reproductive toxicant) according to the EU CLP Regulation (EC 1272/2008) criteria. The proposal was based on effects including reduced pup body weight, increased pup mortality and decreased pup survival, occurring at oral doses equal to or below those that produced significant toxicity in adult female animals.

To **elucidate whether the observed foetotoxic effects may be triggered by an endocrine mode of action**, CEFIC's Lower Olefins Sector Group (LOSG), composed of the main DCPD interested petrochemical companies, reviewed all ED-relevant scientific evidence identified on DCPD.

In this regard, OECD[1] and EFSA/ECHA[2] principles as well as the scientific criteria laid down in Regulation (EU) 2018/605 for the assessment of endocrine disrupting (ED) properties of chemical substances were followed by identifying, collating and assessing the existing information pertaining to the potential endocrine activity and adversity of DCPD. Existing *in vitro* and *in vivo* information was complemented with additional structure-activity modelling using ECHA-recommended (Q)SAR software tools. Lines of evidence were assessed following a weight of evidence (WoE) approach.

Toxicological information available for DCPD pertinent to the assessment of an endocrine mode of action

In vivo: Regulatory studies

DCPD has been **evaluated for repeated dose toxicity as well as developmental and reproductive toxicity ('DART') endpoints in a range of reliable in vivo assays**:

- Repeated dose toxicity testing in rats and dogs (oral route)
- Repeated dose toxicity testing in mice, rats and dogs (inhalation route)
- DART toxicity, i.e. teratology study, multigeneration study, prenatal development study in rats and/or rabbits (oral route)

Overall, the studies do not show clear teratogenic effects at doses that are not maternally toxic. Rather, they show some indication of foetotoxic effects. For example, spontaneous abortions occurred in rabbits of one litter at 100 mg DCPD/kg bw/day in the absence of a statistically-significant reduction in maternal body weight. Also, available rat pre-natal developmental toxicity and multigeneration studies with DCPD suggest that it produced some foetotoxic effects including reduced pup body weight, increased pup mortality and/or decreased pup survival at doses equivalent to or below those that produced clear signs of maternal toxicity.

However, **none of the studies revealed any findings providing evidence for an ED-mediated mode of action involved in the observed foetoxicity**. Solely the OECD 422 study revealed effects in the adrenals, which may allow postulating a disturbance of the hypothalamic-pituitary-adrenal axis. However, on their own, the effects observed, an increase of fatty droplets in the fascicular zone of the adrenals, cannot explain the foetotoxicity.

In silico: (Q)SAR modelling

A structure activity screen of **DCPD** and its main hydroxylation metabolite **tricyclo[5.2.1.02,6]dec-3-ene-8,9-diol** was conducted using EFSA/ECHA guidance (2018)[2] recommended (Q)SAR tools to determine functional groups associated structural alerts for reproductive and developmental toxicity (DART) endpoints:

- **OECD Toolbox v. 4.3.**[3]

Profiler	Information	DCPD	Metabolite
Oestrogen Receptor Binding (v.2.2)	Method relevant for reproductive toxicity endpoints in fish and mammals.	Non-binder, without OH or NH2 group	Non-binder, with impaired OH or NH2 group
rtER Expert System ver.1 USEPA (v.1.0)	Identifies potential to bind rainbow trout oestrogen receptor	Multicyclic hydrocarbons special rules (not relevant as DCPC does not fulfil the criterion of similarity with tamoxifen)	No alert found
Retinoic Acid Receptor Binding (v.1.1)	Method relevant for developmental toxicity; identifies potential to bind retinoic acid receptor.	Not possible to classify according to these rules[1]	Not possible to classify according to these rules
DART scheme v.1.3	Decision tree to identify chemicals with structural features associated with DART toxicants	No known precedent for reproductive and developmental toxic potential	No known precedent for reproductive and developmental toxic potential

- **Endocrine Disruptome**[4]
  DCPD and its main metabolite present only a medium probability to bind the AR receptor as antagonist and a low probability to bind to all other human nuclear receptors.
- **Danish (Q)SAR models**[5]
  Neither DCPD nor its main metabolite were predicted to bind and activate the oestrogen receptor (ER) α, to be an antagonist of the androgen receptor (AR), to inhibit thyroperoxidase (TPO), to have very low binding affinity for the thyroid receptor (TR) and not to bind to the pregnane X receptor (PXR).
- **VEGA platform**[6]
  Based on the two models of VEGA, DCPD or its main metabolite are neither predicted to bind to ER nor to induce ER-mediated effects.

In conclusion, based on the selected DART-sensitive profilers, DCPD and tricyclo[5.2.1.02,6]dec-3-ene-8,9-diol were predicted negative for DART endpoints.

In vitro: US EPA TOX21 programme and ToxCast

The ToxCast programme of the US EPA, which is designed to prioritise chemicals based on the results of **high-throughput screening (HTS)**, currently includes 89 ED specific *in vitro* assays. These assays address the E (oestrogen), A (androgen), T (thyroid) and S (steroidogenesis) modalities (i.e., E=23; A=15; T=13; S=26) and are also used as part of the US EPA's Endocrine Disruptor Screening Programme (EDSP21). The ToxCast programme contains additional assays that are associated with direct ED relevant receptors, adverse outcome pathways (AOPs) (http://actor.epa.gov/dashboard/).

DCPD was tested under the US EPA EDSP21 and ToxCast programmes. The results suggest that the substance **does not interact with any of the tested oestrogen, androgen or thyroid receptors *in vitro***. DCPD was also inactive on other nuclear receptors (PPARs), except for the NVS_NR_hPPARg assay (see below), and for aromatase inhibition.

Species/ Model/ Modality	Study System	Assay Result
Peroxisome proliferator-activated receptor gamma	ATG_PPARg_TRANS_up	Inactive
	NVS_NR_hPPARg	Active
	OT_PPARg_PPARgSRC1_0480	Inactive
	OT_PPARg_PPARgSRC1_1440	Inactive
	Tox21_PPARg_BLA_Agonist_ratio	Inactive

Considering that DCPD was inactive in 13 cell-based PPAR assays (i.e. 6PPARg, 3PPARa and 3 PPARd), all linked to the same AOP, including Attagene, its activity in the NVS_NR_hPPARg assay can be considered to be due to over-sensitivity. DCPD is therefore considered to be inactive also for the PPAR/PPARg pathway.

Conclusion

A substantial amount of *in silico*, *in vitro* and *in vivo* information of relevance to an endocrine assessment was identified for DCPD. This includes publicly available scientific data, newly developed (Q)SAR modelling information using ECHA-recommended software tools and *in vivo* studies in rats, rabbits and/or dogs. Considering all information, there is currently no evidence to suggest that the developmental effects observed with DCPD in rat and rabbit studies occur via an endocrine mode of action.

[1] *OECD, 2012. OECD Work related to endocrine disrupters. https://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm;* [2] *EFSA/ECHA, 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009;* [3] OECD, 2017. The OECD QSAR toolbox for grouping chemicals into categories, version 3.4., http://toolbox.oasis-lmc.org/?section=archive and OECD, 2019. The OECD QSAR toolbox for grouping chemicals into categories, version 4.3., http://toolbox.oasis-lmc.org/?section=download; [4] Kolsek *et al.* (2014). Endocrine Disruptome - An open source prediction tool for assessing endocrine disruption potential through nuclear receptor binding. J. Chem. Inf. Model. 54:1254-1267*;* [5] Danish (Q)SAR Database, 2019. http://qsar.food.dtu.dk/*;* [6] Benfenati E, Manganaro A and Gini G (2013). VEGA-QSAR: AI inside a platform for predictive toxicology. Proceedings of the workshop 'Popularize Artificial Intelligence 2013', vol 18.