CENTER FOR HORMONFORSTYRRENDE STOFFER

# Prioritisation of Endocrine Disruptors for Regulation

CeHoS-5.3

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# Abbreviations

| | |
|---|---|
| 4-CHBP | 4-chloro-4-hydroxybenzophenone |
| 4DHBP: | 4,4'-dihydroxybenzophenone |
| 4-MPB: | 4-methylbenzophenone |
| 4-OHBP: | 4-hydroxybenzophenone |
| 4-PhBP: | 4-phenylbenzophenone |
| ARN: | Assessment of Regulatory Needs |
| BP: | Benzophenone |
| BP-1: | Benzophenone-1; 2,4-dihydroxybenzophenone |
| BP-2: | Benzophenone-2; 2,2',4,4'-tetrahydroxybenzophenone |
| BP-3: | Benzophenone-3; 2-hydroxy-4-methoxybenzophenone |
| BP-4: | Benzophenone-4 |
| BP-5: | Benzophenone-5 |
| BP-6: | Benzophenone-6; 2,2'-dihydroxy-4,4'-dimethoxybenzophenone |
| BP-8: | Benzophenone-8; 2,2'-dihydroxy-4-methoxybenzophenone |
| BPR: | Biocidal Products Regulation |
| CAS: | Chemical Abstracts Service |
| CeHoS: | Centre on Endocrine Disrupters |
| CLP: | Classification, Labelling and Packaging Regulation |
| DHHB: | hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate |
| EAS-: | (anti)Estrogenic-, (anti)Androgenic- and Steroidal- |
| EATS-: | (anti)Estrogenic-, (anti)Androgenic-, Thyroid- and Steroidal- |
| D4: | Octamethylcyclotetrasiloxane |
| D5: | Cyclopentasiloxane |
| DTU: | Technical University of Denmark |
| ECHA: | European Chemicals Agency |
| ED: | Endocrine Disruptor |
| EFSA: | European Food Safety Authority |
| ENV: | Environment |
| EOGRTS: | Extended One Generation Reproductive Toxicity Study |
| EU: | European Union |
| EU COM: | European Commission |
| GHS: | Global Harmonised System |
| HH: | Human Health |
| MoA: | Mode of Action |
| Mut: | Mutagenicity |
| POP: | Persistent Organic Pollutants |
| PPPR: | Plant protection products Regulation |
| (Q)SAR: | (Quantitative) Structure-Activity Relationships |
| REACH: | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| SCCS: | Scientific Committee on Consumer Safety |
| SDU: | University of Southern Denmark |
| SPIN: | Substances in Preparations in the Nordic Countries |
| SVHC: | Substances of Very High Concern |
| T: | Thyroid |
| WHO: | World Health Organization |
| WoE: | Weight of Evidence |

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# Dansk resumé

I EU er det højt prioriteret at minimere eksponering af mennesker og miljø for hormonforstyrrende stoffer (ED). Medlemslandene kan tage initiativ til at identificere stoffer som ED under REACH eller (fra 2023) ved klassificering i CLP. 40-60.000 stoffer anvendes kommercielt, og der findes flere lister over mistænkte ED-stoffer. Der er derfor et stort behov for prioritering af hvilke stoffer der er mest relevante at spille ind i disse regulatoriske processer. Det overordnede mål med dette projekt var at identificere ED-stoffer af høj prioritet for regulering under REACH eller CLP. Det danske Center for Hormonforstyrrende Stoffer udviklede i 2018 lister over ED og mistænkte ED-stoffer til brug for myndighederne (Hass et al., 2018). Oplysninger om mere end 8000 stoffer blev samlet, filtreret og vurderet for at finde stoffer med ED-egenskaber. I den proces blev en "prioriteret basisliste" på 171 stoffer udviklet. I dette projekt blev denne liste udvidet til en opdateret prioriteret basisliste på 192 stoffer. Efter anvendelse af en række opstillede eksklusionskriterier, var der 97 fokusstoffer tilbage. En foreløbig litteraturscreening af 10 af de 97 fokusstoffer viste, at der for 9 ud af 10 fokusstoffer fandtes nogen information om endokrin aktivitet og/eller skadelig effekt. 5 ud af de 10 fokusstoffer blev vurderet til at være af større potentiel interesse baseret på *in vitro*- og gnaverstudier, mens 1 ud af de 10 fokusstoffer blev anset for at være af større potentiel interesse baseret på *in vitro*- og fiske-/paddestudier. Derfor blev de fleste af de 97 fokusstoffer vurderet til at være af potentiel regulatorisk interesse – med mulighed for prioritering til enten ED-identificering eller data-genereringsprocesser. Ud af de 97 fokusstoffer blev der desuden identificeret 5 stofgrupper. Baseret på nationale prioriteter blev der foretaget en foreløbig screening for ED-effekter af en gruppe af 12 benzophenoner. Et ”heatmap” over ED-effekter af benzophenonerne illustrerede datatilgængelighed på tværs af gruppen, herunder EAS-relateret og Thyreoidea-relateret ED-potentiale for nogle af stofferne. “Heatmappet” kan eventuelt bruges i fremtidige analyser af benzophenonernes ED-effekter baseret på gruppering og anvendelse af data på tværs af arter. Dette projekt kunne følges op af en yderligere vurdering af de 9 fokusstoffer, som havde information om endokrin aktivitet og/eller skadelig effekt, som led i prioritering til fremtidig datagenerering eller ED-identificering. Desuden kunne de resterende 87 fokusstoffer også screenes for ED-effekter. Der kunne også følges op med yderligere vurdering af benzophenonernes ED-potentiale baseret på gruppering, og der kunne tilføjes flere stoffer til den opdaterede prioriterede basisliste.
I et større perspektiv påviste dette projekt en omfattende mangel på ED-relevante data og et stort behov for opdatering af standardinformationskravene i REACH til at inkludere ED-relevant information for så mange stoffer som muligt. Desuden blev behovet for at forbedre anvendelsen af tilgængelige data, ved for eksempel at vurdere grupper af stoffer og anvende data på tværs af arter, understreget.

# English abstract

In the EU, it is highly prioritised to minimise exposure of humans and the environment to the most hazardous substances, including Endocrine Disruptors (EDs). The EU Member States can take the initiative to identify substances as EDs under REACH or (from 2023) by harmonised classification in CLP. 40-60.000 substances are in global commerce and several lists of suspected endocrine disruptors exists. There is therefore a high need for prioritisation of which substances to move forward for these regulatory processes. The overall aim of this project was to identify EDs of high priority for regulatory action under REACH or CLP. In 2018, the Danish Centre on Endocrine Disruptors developed lists of EDs and suspected EDs for use by the authorities (Hass et al., 2018). Information about more than 8000 substances was compiled, filtered, and evaluated to find substances with ED properties. In that process, a "priority basis list" of 171 substances was developed.

In this project, this list was expanded to an updated priority basis list of 192 substances. After applying a series of established exclusion criteria, 97 focus substances remained. A preliminary literature screening of 10 of the 97 focus substances showed that for 9 out of 10 selected focus substances, some information was available on endocrine activity and/or adversity. 5 out of the 10 focus substances were considered to be of higher potential interest based on in vitro and rodent studies, while 1 out of the 10 focus substances were considered to be of higher potential interest based on in vitro and fish/amphibian studies. Therefore, most of the 97 focus substances were judged to be of potential regulatory interest – and could possibly be considered for either ED identification or data generation processes. Out of the 97 focus substances, 5 groups of substances were also identified. Based on national priorities, a preliminary screening for ED effects of a group of 12 benzophenones was conducted. A "heatmap" of ED related effects of the benzophenones illustrated data availability across the group, including EAS-related and Thyroid-related ED-potential for some of the substances. The "heatmap" could pave the way for further analysis of the ED-potential of benzophenones based on grouping and cross-species considerations.

This project could be followed up by further evaluation of the 9 focus substances with some information on ED potential, to analyse their priority for future data generation or ED identification process. Furthermore, the remaining 87 focus substances could be screened for ED-relevant effects. The ED potential of benzophenones could be explored based on a grouping approach, and it could be considered to add more substances to the updated prioritised basis list.

In a bigger perspective, this project highlighted a comprehensive lack of ED-relevant data, which calls for updated standard information requirements under REACH to include ED relevant information for as many substances as possible. The need to maximize the use of available data was also emphasised, for example by assessing groups of substances and applying cross-species extrapolations.

# Introduction

40-60.000 chemical substances are in global commerce (WHO 2022), and in the EU more than 26.000 substances are registered under REACH (ECHA 2023). New substances are continuously being developed, and in 2017 the chemicals industry was the largest manufacturing industry in the world, with the production of chemicals foreseen to double from 2017 to 2030 (WHO 2022). Humans are inadvertently exposed to chemical substances through air, drinking water and food, and through consumer products such as cosmetics, clothes, paints, furniture, and kitchenware (WHO 2022). Human biomonitoring studies in the EU, show a growing number of chemical substances in human blood and urine, and combined prenatal exposure to several substances have been associated with unwanted health effects such as reduced fetal growth and lower birth rates (EU COM, 2020). Chemical substances also find their way to the environment, either directly from production plants, or indirectly through wastewater or in the waste phase. In 2020, 84% of Europeans were worried about the impact of chemical substances on their health, and 90% of Europeans were worried about the impact on the environment (EU COM, 2020).

In the EU, it is highly prioritised to minimise exposure of humans and the environment to the most hazardous substances, including endocrine disruptors (EDs). There are various regulatory paths to restrict the use of EDs, including identification of substances as EDs in the Plant Protection Product Regulation (PPPR), the Biocidal Product Regulation (BPR), under REACH as Substances of Very High Concern (SVHC), and from 2023 also under the Classification, Labelling and Packaging (CLP) Regulation.

It is up to the Member States to take the initiative to identify substances as ED SVHCs under REACH or with harmonised classifications under the CLP. The Member States can also initiate substance evaluation processes under REACH, which can lead to requests for additional testing to clarify specific concerns, including for endocrine disruption. With the vast number of substances to which humans and the environment are exposed, and numerous lists of suspected endocrine disruptors in the public domain, there is a high need for prioritisation of which substances to move forward for these regulatory processes.

In 2018, the Danish Centre on Endocrine Disruptors, CeHoS, developed lists of EDs and suspected EDs for use by the authorities (Hass et al., 2018). Information about more than 8000 substances were compiled, filtered, and evaluated to find substances with ED properties. For only a small subset of those 8000 substances, the literature was screened to search for information on ED properties, and for only 13 of those, a more thorough evaluation was conducted. It was therefore in this project considered possible that additional substances of high regulatory interest could be identified by continuation of the work conducted in Hass et al., 2018.

Regulatory activities, regulatory and scientific evaluations, and development of lists of suspected EDs had been continuously ongoing from 2018 to 2023. Additional substances were

therefore included in this project, in our search for substances of high priority for regulatory action.

## Aim

The overall aim of this project was to identify Endocrine Disruptors (EDs) of high priority for regulatory action under REACH or CLP.

To achieve this, the work conducted in Hass et al., 2018 was continued, since it was considered possible that additional substances of high regulatory interest could be identified on that basis.

New lists and developments were furthermore considered to include additional substances suspected of having ED properties.

## Results

### Updated prioritised basis list (192 substances)

In 2018, CeHoS compiled more than 8000 substances based on existing lists from ECHA and publicly available lists of suspected EDs. Through filtering steps, including integration of information about hazard and exposure from ECHA's registration database, (Q)SAR predictions and exposure estimates from the Nordic SPIN database, a "prioritized basis list" of 171 substances was developed (Hass et al., 2018) (see figure 1).

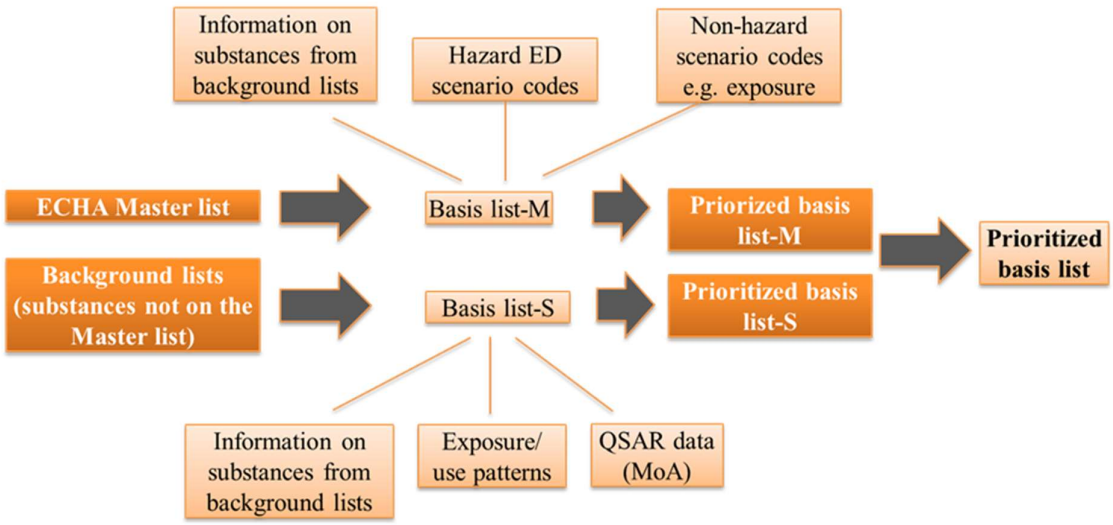


**Figure 1** | Schematic overview of the filtering of substances conducted by CeHoS in 2018 (Hass et al., 2018).

From this "prioritized basis list", 52 substances were selected for literature screening by the steering group. After the literature screening, 13 substances were chosen for a more detailed evaluation of ED properties (12 were evaluated for effects relevant for human health, and 3 were evaluated for effects relevant for the environment).

Some activities were undertaken in 2019 and 2021 as follow-up on Hass et al., 2018. In 2019, CeHoS-partners at DTU National Food Institute (DTU) conducted a project for The Danish Consumer Council, in which the 52 substances selected for literature screening in Hass et al.,

2018 were screened for information on endocrine activity and ED-related adversity with relevance for human health (main focus on studies in vitro and in vivo in rodents). In 2021, CeHoS-partners at the University of Southern Denmark (SDU) screened 98 substances from the "prioritized basis list" for literature relevant for ED in the environment (main focus on studies in vitro and in vivo in fish, amphibians, and birds). Thus, a relatively large number of substances from the "prioritized basis list" had not been screened for ED-relevant literature (119 for relevance for human health and 73 for relevance for the environment). It was therefore in this project considered likely that more substances of regulatory interest could potentially be identified from that list. It was thus decided to continue the work conducted in Hass et al., 2018, and use the "prioritized basis list" and the follow up work conducted in 2019 and 2021 as a starting point.

Regulatory activities, regulatory and scientific evaluations, and development of lists of suspected EDs had been continuously ongoing from 2018 to 2023. Further inclusion of additional substances was therefore explored by screening of publicly available lists of suspected EDs. The screening was conducted by searching the web with the search terms "lists of endocrine disruptors." Information about the identified lists were collated in a tabular format (annex 1). The overall conclusion of this screening was that some of the lists used in Hass et al., 2018 had been revised or were on the way to be phased out, and new lists had been developed and were publicly available. It was not evaluated in detail whether the revisions, updates and changes affected the number or identity of substances on the background lists in Hass et al., 2018. It was however concluded that some of the lists could add some substances which were not included in 2018. This was for example the case for the EU Commission priority list of 28 potential endocrine disruptors to be evaluated by the Scientific Committee on Consumer Safety (SCCS).

Based on the screening, it was decided to generate a non-exhaustive updated prioritised basis list (annex 2), including:

A. Substances on the "prioritized basis list" (171 substances) from Hass et al., 2018.
B. Substances on the EU Commission priority list of 28 potential endocrine disruptors to be evaluated by SCCS (https://single-market-economy.ec.europa.eu/sectors/cosmetics/cosmetic-products-specific-topics/endocrine-disruptors_en ).

7 substances were found on both lists (triclocarban, homosalate, butylparaben, ethylhexyl methoxycinnamate/OMC, benzophenone-2/BP-2, salicylic acid, deltamethrin), and doublets were deleted. The final number of substances on the updated prioritised basis list was therefore 192 substances.

It is important to note that this is not an exhaustive list. Additional publicly available lists of substances which could be included were identified as part of this project (annex 1) and other possible criteria for inclusion of substances were developed (1-3 below).
However, after gathering the updated prioritised basis list based on A. and B. above and applying the agreed exclusion criteria (described in the following section), a surprisingly

large number (97) of focus substances were identified. The following additional inclusion criteria (1-3 below) were therefore not applied in this project, solely due to lack of resources. In future prioritisation projects, these (and other) additional inclusion criteria could however be considered, for expanding the updated prioritised basis list:

1. Substances identified as possible endocrine disruptors in ECHA reports on Assessment of Regulatory Needs (ARNs).
2. Specific substances which seem similar enough to identified endocrine disruptors to be identified as endocrine disruptors based on read-across or grouping.
3. Substances found to induce effects relevant for endocrine disruption in the ECHA-review of conducted Extended One Generation Reproductive Toxicity Studies (EOGRTS).

### 97 focus substances

The aim of this project was to identify EDs of high priority for regulation under REACH or CLP. It was decided that such substances should not be strictly regulated, they should not be identified as endocrine disruptors, they should not previously have been evaluated by CeHoS, some data on endocrine activity and/or adversity of the substance should be available, their use should be relevant for regulation under REACH and they should not be under evaluation or have finalised evaluation in REACH processes.
It was furthermore decided that within this project, thorough evaluation of substances would primarily be prioritised if data was judged to be available to possibly identify them as ED for both human health and the environment, since this would draw on the cross-species experience in the project group. This would, however, not exclude a more thorough evaluation of any of the substances for ED-potential related to only human health, only the environment or both at a later time point.

In the prioritisation of focus substances, the following exclusion criteria were applied:

1. The substance was strictly regulated.
    a. The substance was classified as a mutagen in category 1A (Mut 1A) or 1B (Mut 1B) in GHS/CLP. This criterion would only exclude substances for prioritisation as relevant for human health. It should be noted that no other classifications were used as exclusion criteria. Classifications for Reproductive Toxicity or Carcinogenicity would for example not lead to exclusion. This was chosen since it was still unknown how classified EDs would be regulated in downstream regulations, and whether an ED classification in some cases would lead to stricter regulations than for e.g. Reproductive Toxicity or Carcinogenicity.
    b. The substance was included in the POP regulation.

2. The substance was identified or proposed as an endocrine disruptor in the EU.
    a. The substance was identified or proposed as a substance of very high concern (SVHC) under REACH based on endocrine disruption or as an endocrine disruptor under the biocidal products regulation (BPR). If the identification was done only for human health or only for the environment, the substance was only excluded for that part.

3. The substance had previously been evaluated for endocrine disruption by CeHoS. If the evaluation was conducted only for human health or only for the environment, the substance was only excluded for further evaluation for that part.

4. There was a lack of data on endocrine disruptive properties.
    a. The substance had been screened by CeHoS, DTU or SDU for studies relevant for evaluation of endocrine disruption, and it had been evaluated that the substance was not prioritised for further scrutiny due to lack of data in one of the following:
        i. CeHoS 2018: Searches conducted in Hass et al., 2018.
        ii. DTU 2019: Searches conducted in a project for The Danish Consumer Council, in which the 52 substances selected for literature screening in Hass et al., 2018 were screened for information regarding endocrine activity and adversity with relevance for human health (mainly in vitro studies and in vivo studies in rodents).
        iii. SDU 2021: Searches conducted in a screening of 98 substances from the "prioritized basis list" from Hass et al., 2018 for literature relevant for ED in the environment (mainly in vitro and in vivo in fish, amphibians, and birds).

5. The use of the substance was judged not to be highly relevant for regulation under REACH or CLP.
    a. The substance was not registered under REACH. However, if the substance was on the list of substances often found in cosmetics, developed by The Danish Consumer Council (see appendix 1), the lack of registration under REACH would not lead to exclusion.
    b. The substance was registered under REACH, but only as an intermediate. This exclusion criterion was only used for human health.
    c. The substance was registered under REACH, but it was stated in the REACH registration dossier that it was currently not used in the EU.

6. The substance was under evaluation or new data was under development.
    a. The substance was undergoing evaluation under REACH or the biocidal products regulation (BPR) and endocrine disruption was one of the evaluated concerns.
    b. New data on the substance were known to be under development, and the data were not expected to be published during the period of this project.

7. The substance had been evaluated for endocrine disruption under REACH with an unresolved concern for ED, a conclusion on follow-up on ED or a conclusion on no follow up on ED.

The exclusion criteria were applied using a stepwise approach, with consideration of relevance for human health and relevance for the environment separately for each substance. Some substances were assigned more than one exclusion criterion, but in general, the search for exclusion criteria was halted if exclusion criteria were identified with relevance for both human health and the environment. Using this approach, 97 focus substances remained included, with some focus substances only included for human health relevance and others only included for environmental relevance (table 1). 95 substances were excluded from further evaluation since they were excluded for both human health and environmental relevance (annex 3). Overall, the main reasons for exclusion from further focus was lack of ED-relevant

information and lack of relevant use (figure 2). Lack of ED-relevant data was especially prevalent for environmentally relevant effects, i.e. from studies in fish, amphibians, and birds.

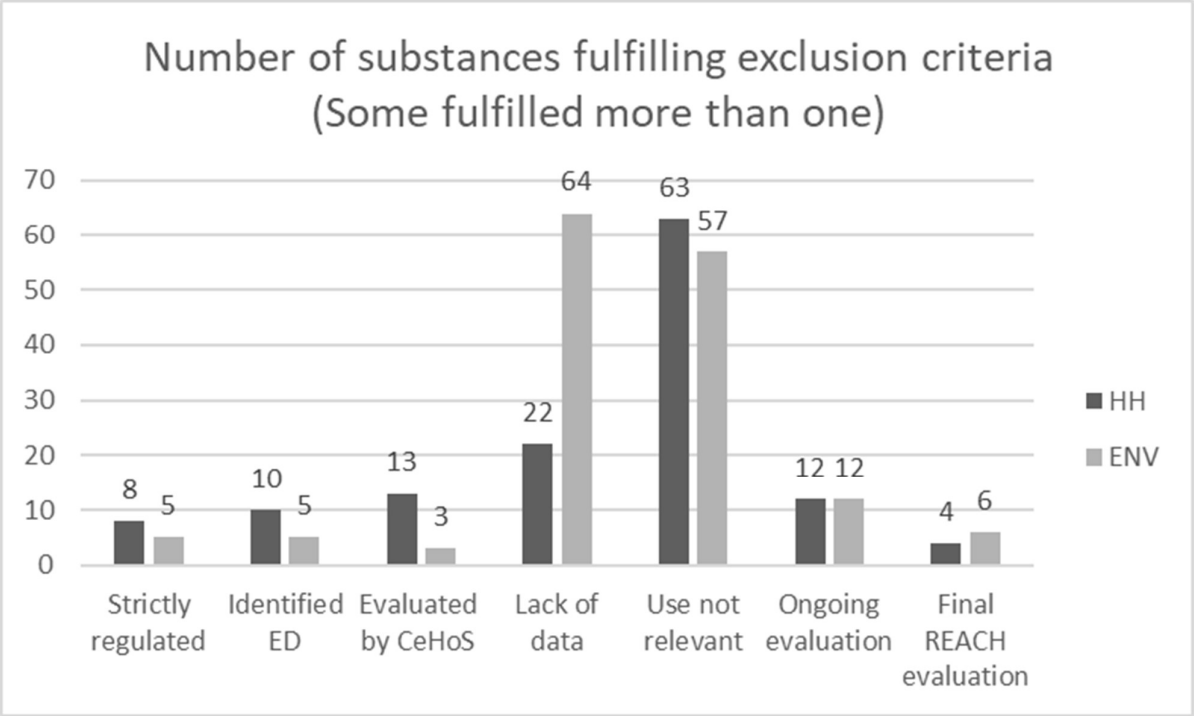


**Figure 2** | Number of substances from the updated prioritised basis list fulfilling exclusion criteria for either human health relevance (HH, dark grey) or environmental relevance (ENV, Light grey). Some substances fulfilled more than one exclusion criterion.

After applying the exclusion criteria, 97 focus substances remained (table 1). The list of focus substances consisted of a sub-group relevant for human health (75) and a sub-group relevant for the environment (51). 29 of the focus substances were included as relevant for both human health and the environment, as illustrated in figure 3.

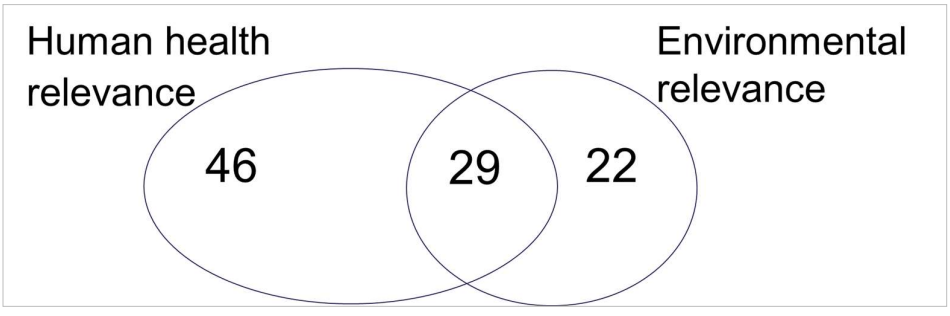


**Figure 3** | 97 focus substances divided in sub-groups.

**Table 1** | 97 focus substances.
Some focus substances were included for both human health and environmental relevance, some were included for only human health relevance and others were included for only environmental relevance. In the table, "+HH" indicates inclusion for human health relevance and "+ENV" indicates inclusion for environmental relevance. For substances included for only human health or only environmental relevance, exclusion criteria for the excluded part were noted.

| CAS no | Name | Included for human health relevance (+HH) or Exclusion criteria | Included for environmental relevance (+ENV) or Exclusion criteria |
|---|---|---|---|
| 15356-60-2 | (+)-menthol | +HH | +ENV |
| 42594-17-2 | (Octahydro-4,7-methano-1H-indenediyl)bis(methylene) diacrylate | +HH | Lack of ED relevant data (SDU 2021) |
| 110-25-8 | (Z)-N-methyl-N-(1-oxo-9-octadecenyl)glycine | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | +ENV |
| 23847-08-7 | 1,1'-dithiobis[hexahydro-2H-azepin-2-one] | +HH | Lack of ED relevant data (SDU 2021) |
| 87-66-1 | 1,2,3-trihydroxybenzene | +HH | +ENV |
| 95-63-6 | 1,2,4-trimethylbenzene | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | +ENV |
| 68515-49-1 | 1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich | +HH | +ENV |
| 106-93-4 | 1,2-dibromoethane | +HH | +ENV |
| 26780-96-1 | 1,2-Dihydro-2,2,4-trimethylquinoline, oligomers | +HH | Lack of ED relevant data (SDU 2021) |
| 40601-76-1 | 1,3,5-tris[[4-tert-butyl-3-hydroxy-2,6-xylyl]methyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione | +HH | Lack of ED relevant data (SDU 2021) |
| 68953-84-4 | 1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs. | +HH | Lack of ED relevant data (SDU 2021) |
| 1569-02-4 | 1-ethoxypropan-2-ol | +HH | Lack of ED relevant data (SDU 2021) |
| 80-54-6 | 2-(4-tert-butylbenzyl)propionaldehyde | Evaluated as suspected ED by CEHOS (2018) | +ENV |
| 142-18-7 | 2,3-dihydroxypropyl laurate | +HH | Lack of ED relevant data (SDU 2021) |

| CAS no | Name | Included for human health relevance (+HH) or Exclusion criteria | Included for environmental relevance (+ENV) or Exclusion criteria |
|---|---|---|---|
| 1948-33-0 | 2-tert-butylhydroquinone | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | +ENV |
| 48145-04-6 | 2-phenoxyethyl acrylate | +HH | Lack of ED relevant data (SDU 2021) |
| 6386-73-8 | 3,3',5-tribromobisphenol A | Lack of ED relevant data (CEHOS 2018 + DTU2019) | +ENV |
| 19780-11-1 | 3-(2-dodecenyl)succinic anhydride | +HH | Lack of ED relevant data (SDU 2021) |
| 14901-07-6 | 4-(2,6,6-trimethylcyclohex-1-ene-1-yl)-but-3-ene-2-one | +HH | Lack of ED relevant data (SDU 2021) |
| 599-64-4 | 4-(a,a-dimethylbenzyl)phenol | +HH | +ENV |
| 101-14-4 | 4,4'-methylenebis[2-chloroaniline] | +HH | Lack of ED relevant data (SDU 2021) |
| 101-77-9 | 4,4'-methylenedianiline | +HH | +ENV |
| 123-30-8 | 4-aminophenol | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | +ENV |
| 123-07-9 | 4-ethylphenol | +HH | Lack of ED relevant data (SDU 2021) |
| 98-52-2 | 4-tert-butylcyclohexanol | +HH | Lack of ED relevant data (SDU 2021) |
| 107-13-1 | Acrylonitrile | +HH | Lack of ED relevant data (SDU 2021) |
| 5421-46-5 | Ammonium mercaptoacetate | +HH | Lack of ED relevant data (SDU 2021) |
| 67774-74-7 | Benzene, C10-13-alkyl derivs. | +HH | Lack of ED relevant data (SDU 2021) |
| 131-56-6 | Benzophenone-1/BP-1, | +HH | +ENV |
| 131-55-5 | Benzophenone-2 (BP-2) (2,2',4,4'-tetrahydroxybenzophenone) | +HH | +ENV |
| 4065-45-6 | Benzophenone-4/BP-4, | +HH | +ENV |

| CAS no | Name | Included for human health relevance (+HH) or Exclusion criteria | Included for environmental relevance (+ENV) or Exclusion criteria |
|---|---|---|---|
| 6628-37-1 | Benzophenone-5/BP-5, | +HH | +ENV |
| 85-68-7 | Benzyl butyl phthalate | SVHC for ED (HH) | +ENV |
| 118-58-1 | Benzyl salicylate, | +HH | +ENV |
| 103-24-2 | Bis(2-ethylhexyl) azelate | +HH | +ENV |
| 142-16-5 | Bis(2-ethylhexyl) maleate | +HH | Lack of ED relevant data (SDU 2021) |
| 56-35-9 | Bis(tributyltin) oxide | REACH registration, only for intermediate uses | +ENV |
| 107-88-0 | Butane-1,3-diol | +HH | +ENV |
| 94-26-8 | Butylparaben | SVHC for ED (HH) | +ENV |
| 59-50-7 | Chlorocresol | +HH | +ENV |
| 104-54-1 | Cinnamyl alcohol | +HH | +ENV |
| 1319-77-3 | Cresol | +HH | Lack of ED relevant data (SDU 2021) |
| 10016-20-3 | Cyclohexapentylose | +HH | +ENV |
| 69430-24-6 | Cyclomethicone | +HH | +ENV |
| 541-02-6 | Cyclopentasiloxane/decamethylcyclopentasiloxane/D5 | +HH | +ENV |
| 94361-06-5 | Cyproconazole | +HH | +ENV |
| 131-17-9 | Diallyl phthalate | +HH | Lack of ED relevant data (SDU 2021) |
| 84-74-2 | Dibutyl phthalate | SVHC for ED (HH) | +ENV |
| 683-18-1 | Dibutyltin dichloride | +HH | +ENV |
| 97-23-4 | Dichlorophen | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | +ENV |
| 101-83-7 | Dicyclohexylamine | +HH | Lack of ED relevant data (SDU 2021) |

| CAS no | Name | Included for human health relevance (+HH) or Exclusion criteria | Included for environmental relevance (+ENV) or Exclusion criteria |
|---|---|---|---|
| 84-69-5 | Diisobutyl phthalate | SVHC for ED (HH) | +ENV |
| (26761-40-0) / 68515-49-1 | Di-isodecyl phthalate (DIDP), 1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich | +HH | +ENV |
| 26544-38-7 | Dihydro-3-(tetrapropenyl)furan-2,5-dione | +HH | Lack of ED relevant data (SDU 2021) |
| 74-31-7 | Diphenyl-p-phenylenediamine | +HH | Lack of ED relevant data (SDU 2021) |
| 3648-20-2 | Diundecyl phtalate, DuDP, branched and linear | +HH | Lack of ED relevant data (SDU 2021) |
| 124-22-1 | Dodecylamine | +HH | Lack of ED relevant data (SDU 2021) |
| 16423-68-0 | Erythrosine B, FD&C Red No. 3 | +HH | Lack of ED relevant data (SDU 2021) |
| 97-53-0 | Eugenol | +HH | +ENV |
| 122-14-5 | Fenitrothion | Evaluated as ED by CEHOS (2018) | +ENV |
| 76674-21-0 | Flutriafol | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | +ENV |
| 91744-09-1 | Glycerides, C16-18 and C18-unsatd. mono- | +HH | Lack of ED relevant data (SDU 2021) |
| 99607-70-2 | Heptan-2-yl [(5-chloroquinolin-8-yl)oxy]acetate | +HH | Lack of ED relevant data (SDU 2021) |
| 118-56-9 | Homosalate | Lack of ED relevant data (CEHOS 2018 + DTU 2019) + New data under development | +ENV |
| 4247-02-3 | Isobutyl paraben | SVHC for ED (HH). Evaluated as ED by CEHOS (2018) | +ENV |
| 501-30-4 | Kojic acid, 5-hydroxy-2-hydroxymethyl-4-pyrone | +HH | +ENV |

| CAS no | Name | Included for human health relevance (+HH) or Exclusion criteria | Included for environmental relevance (+ENV) or Exclusion criteria |
|---|---|---|---|
| 108-67-8 | Mesitylene | +HH | Lack of ED relevant data (SDU 2021) |
| 68-11-1 | Mercaptoacetic acid | +HH | Lack of ED relevant data (SDU 2021). |
| 99-97-8 | N,N-dimethyl-p-toluidine | +HH | Lack of ED relevant data (SDU 2021) |
| 39236-46-9 | N,N''-methylenebis[N'-[3-(hydroxymethyl)-2,5-dioxoimidazolidin-4-yl]urea] | +HH | Lack of ED relevant data (SDU 2021) |
| 1338-24-5 | Naphthenic acids | +HH | +ENV |
| 106-25-2 | Nerol | +HH | Lack of ED relevant data (SDU 2021) |
| 7718-54-9 | Nickel dichloride | +HH | +ENV |
| 556-67-2 | Octamethylcyclotetrasiloxane (D4) | Evaluated as ED by CEHOS (2018) | +ENV |
| 66063-05-6 | Pencycuron | +HH | Lack of ED relevant data (SDU 2021) |
| 78-11-5 | Pentaerithrityl tetranitrate | +HH | Lack of ED relevant data (SDU 2021) |
| 87-22-9 | Phenethyl salicylate | +HH | Lack of ED relevant data (SDU 2021) |
| 108-95-2 | Phenol | +HH | +ENV |
| 118-55-8 | Phenyl salicylate | REACH registration, only for intermediate uses | +ENV |
| 3811-04-9 | Potassium chlorate | +HH | Lack of ED relevant data (SDU 2021) |
| 3164-85-0 | Potassium 2-ethylhexanoate | +HH | Lack of ED relevant data (SDU 2021) |
| 94581-15-4 | Resin acids and Rosin acids, fumarated, esters with pentaerythritol | +HH | +ENV |
| 108-46-3 | Resorcinol | Proposed SVHC for ED (HH) | +ENV |

| CAS no | Name | Included for human health relevance (+HH) or Exclusion criteria | Included for environmental relevance (+ENV) or Exclusion criteria |
|---|---|---|---|
| 69-72-7 | Salicylic acid | Evaluated as ED by CEHOS (2018) | +ENV |
| 7758-19-2 | Sodium chlorite | +HH | Lack of ED relevant data (SDU 2021) |
| 15708-41-5 | Sodium feredetate | +HH | Lack of ED relevant data (SDU 2021) |
| 9005-64-5 | Sorbitan monolaurate, ethoxylated | +HH | Lack of ED relevant data (SDU 2021) |
| 1461-25-2 | Tetrabutyltin | REACH registration, only for intermediate uses | +ENV |
| 97-74-5 | Tetramethylthiuram monosulphide | +HH | +ENV |
| 16470-24-9 | Tetrasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(4-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate] | +HH | Lack of ED relevant data (SDU 2021) |
| 3380-35-4 | Triclosan | +HH | +ENV |
| 2031-67-6 | Triethoxy(methyl)silane | +HH | Lack of ED relevant data (SDU 2021) |
| 2943-75-1 | Triethoxyoctylsilane | +HH | Lack of ED relevant data (SDU 2021) |
| 3319-31-1 | Tris(2-ethylhexyl) benzene-1,2,4-tricarboxylate | +HH | Lack of ED relevant data (SDU 2021) + REACH evaluation ongoing |
| 1067-53-4 | Tris(2-methoxyethoxy)vinylsilane | +HH | Lack of ED relevant data (SDU 2021) |
| 1330-78-5 | Tris(methylphenyl) phosphate | Evaluated as ED by CEHOS (2018) | +ENV |
| 14324-55-1 | Zinc bis(diethyldithiocarbamate) | +HH | Lack of ED relevant data (SDU 2021) |

### Preliminary literature screening of 10 selected focus substances

Due to limited time and resources, it was not possible to screen the literature for all the 97 focus substances. Instead, a subsample of 10 out of the 97 focus substances were selected for a preliminary literature screening. The criteria for selection of the 10 focus substances were based on an interest in this project to focus on substances of relevance for both the environment and human health. 29 of the 97 focus substances were prioritised for both the environment and human health. From those 29 substances, the project group selected 10 in agreement with the Danish EPA. This number of selected substances mainly reflected resource considerations, and the specific 10 substances were mainly chosen based on national regulatory interests and priorities.

The aim of the literature screening was to investigate whether information was available on endocrine activity and ED-related adversity respectively, with focus on the EATS modalities.

The following methodology was used:

1. Information was retrieved from ECHA's publicly available database of registered substances by entering the CAS no. (ECHA 2023). Available synonyms were noted together with information on tonnage level.
2. ECHA registration dossiers were screened for effects relevant for endocrine disruption. The section on reproductive toxicity was always screened, and in some cases also the section on repeated dose toxicity.
3. Search strings were developed based on CAS numbers and synonyms.
4. The open literature was searched using "Web of Science (all database mode)"
5. Retrieved abstracts were screened for information relevant for endocrine disruption.
6. Relevant information was summarised and compiled in a tabular format.

Since only a preliminary screening of the literature was conducted, simple search strings were developed, and it could not be excluded that some relevant studies were not identified. Further, the studies were not systematically evaluated for reliability or quality, and no weight of evidence analysis was applied. When effects in animal studies were identified, they were noted without a thorough evaluation of other signs of systemic or general toxicity, effects on body weights or other observations that might affect the findings. The screenings with search strings, summary of retrieved information and data availability on endocrine activity and adversity for the 10 substances were collated in annex 4. The overall summaries of the retrieved data were compiled in table 2.

**Table 2** | Preliminary screening of ED relevant information for 10 selected focus substances.

| CAS | Name | Some info on endocrine activity/adversity* (No info and no effect not noted) | |
|---|---|---|---|
| | | Human health | Environment |
| 87-66-1 | 1,2,3-trihydroxybenzene | **EAS:** Endocrine activity | |
| | | **T:** Adversity | |
| 101-77-9 | 4,4'-methylenedianiline | **EAS:** Endocrine activity | **EAS:** Endocrine activity and adversity** |
| | | **T:** Endocrine activity and adversity** | **T:** Endocrine activity |

| | | | |
|---|---|---|---|
| 59-50-7 | Chlorocresol | **EAS:** Endocrine activity and adversity** | **EAS:** Endocrine activity |
| | | **T:** Endocrine activity | **T:** Endocrine activity |
| 10016-20-3 | Cyclohexapentylose | **EAS:** Endocrine activity | **EAS:** Endocrine activity |
| 94361-06-5 | Cyproconazole | **EAS:** Endocrine activity and adversity** | **EAS:** Endocrine activity |
| | | **T:** Adversity | **T:** Endocrine activity and adversity |
| 97-53-0 | Eugenol | **EAS:** Endocrine activity and adversity | |
| 501-30-4 | kojic acid | **T:** Endocrine activity and adversity** | |
| 89-78-1/ 15356-60-2 | menthol and (+)-menthol | **EAS:** Endocrine activity and adversity** | |
| 1338-24-5 | naphthenic acids | **EAS:** Endocrine activity and adversity | **EAS:** Endocrine activity |
| | | **T:** Adversity | **T:** Adversity |
| 108-95-2 | Phenol | **EAS:** Endocrine activity and adversity | **EAS:** Endocrine activity |
| | | | **T:** Endocrine activity |

* Studies were not evaluated for reliability or quality, and no weight of evidence analysis was applied. When effects in animal studies were identified, they were noted without a systematic evaluation of other signs of systemic or general toxicity, effects on body weights or other observations that might affect the findings. No thorough evaluation of link between endocrine activity and adversity was conducted. No available information and no effect observed was generally not noted.

**Cases where a link between the compiled endocrine activity and adversity information seemed strongest.

In table 2, "EAS" refers to endpoints usually considered relevant for the sex hormone system, and "T" refers to endpoints usually considered relevant for the thyroid hormone system. It should, however, be noted that cross-talk between "EAS" and "T" modalities may occur. "Human health" refers to endpoints usually considered relevant for human health, and "Environment" refers to endpoints usually considered relevant for the environment. However, cross-species extrapolations may be relevant and should be considered when possible, including use of rodent data in environmentally related ED evaluations.

The preliminary literature screening showed that for 9 out of 10 selected focus substances, some information was available on endocrine activity and/or adversity. Furthermore, for "Human health" 5 out of the 10, and for "Environment" 1 out of 10, selected focus substances were judged to be of higher potential interest, since information on both endocrine activity and adversity was available, which was evaluated to be potentially linked. It must, however, be noted that no thorough evaluation of study reliability, no weight of evidence analyses and no mode of action analyses with an evaluation of the biological plausibility of a link between endocrine activity and adversity was conducted in this project. Therefore, the level of evidence would need to be evaluated further for each substance before final prioritisation could be made.

As initially decided, thorough evaluation of substances within this project would be prioritised only if data was judged to be available to possibly identify the substances as ED for both human health and the environment, drawing on the cross-species experience in the project group. As illustrated in table 2, for none of the substances, information on endocrine activity and

adversity for both human health and the environment was available. Therefore, no further thorough evaluation was conducted within this project.

It would, however, be relevant to consider further evaluation of 9 of the 10 selected focus substances for which some information was available on endocrine activity and/or adversity. This could be either for data generation or ED identification purposes. The application of cross-species considerations could be explored in cases where information on endocrine activity and adversity was present for e.g. "Human health", and only information on endocrine activity was present for "Environment" (or vice versa).

In the next step, it was decided to look further into groups of substances, since the restriction of groups of substances was highlighted as a political priority in the EU for minimising exposure to the most harmful substances, including endocrine disruptors (EU COM, 2020; EU COM, 2022).

### 5 groups of focus substances

From the list of 97 focus substances, the following groups or pairs were identified:

- Salicylic acid and salicylates (salicylic acid and phenyl-, phenethyl- and benzyl-salicylate)
- Benzophenones (Benzophenone-1, -2, -4, and -5)
- Cyclic siloxanes (D4, D5)
- Phthalates (Diundecyl-, Dibutyl-, Diisobutyl-, Benzyl butyl-, Diallyl-, Diisodecyl-)
- Parabens (Butyl- and isobutyl)

### Preliminary literature screening of a group of 12 benzophenones

Based on national priorities, it was decided to conduct a preliminary literature screening for ED relevant information on a group of benzophenones. The group included benzophenone and benzophenone-3, which both had been evaluated by Denmark in REACH substance evaluations. The following benzophenones were included in the group:

- BP, Benzophenone, CAS 119-61-9
- 4-MBP, 4-methylbenzophenone, CAS 134-84-9
- 4-OHBP, 4-hydroxybenzophenone, CAS 1137-42-4
- 4DHBP, 4,4'-dihydroxybenzophenone, CAS 611-99-4
- BP-2, 2,2',4,4'-tetrahydroxybenzophenone, CAS 131-55-5
- BP-1, 2,4-dihydroxybenzophenone, CAS 131-56-6
- BP-3, 2-hydroxy-4-methoxybenzophenone, CAS 131-57-7
- BP-8, 2,2'-dihydroxy-4-methoxybenzophenone, CAS 131-53-3
- BP-6, 2,2'-dihydroxy-4,4'-dimethoxybenzophenone, CAS 131-54-4
- 4-PhBP, 4-phenylbenzophenone, CAS 2128-93-0
- 4-CHBP, 4-chloro-4-hydroxybenzophenone, CAS 42019-78-3
- DHHB, hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate, CAS 302776-68-7

A preliminary literature screening was conducted. The aim of the literature screening was to investigate whether information was available on endocrine activity and ED-related adversity respectively, with focus on the EATS modalities. Information about metabolism that could be

useful in a grouping context and information about other ED-related (non-EATS) effects were noted if it was identified in the preliminary screening.

The following methodology was used:

1. Information was retrieved from ECHA's publicly available database of registered substances by entering the CAS no. (ECHA 2023). Tonnage level was noted.
2. ECHA registration dossiers were screened for effects relevant for endocrine disruption in the sections on reproductive toxicity and repeated dose toxicity.
3. The open literature was searched using "SciFinder" (CAS), using the CAS number and a filter for "Endocrine*". This was a change in methodology from the use of individual search strings based on synonyms used to search the "Web of Science", as applied when searching for literature for the 10 individual focus substances. The use of SciFinder led to a much more targeted search, leaving out publications investigating substances with e.g. part of the substance name in the nomenclature.
4. Retrieved abstracts were screened for information relevant for endocrine disruption.
5. Relevant information was extracted, using the effect categories laid out in the ECHA/EFSA guidance on identification of endocrine disruptors (ECHA/EFSA, 2018) as a starting point.

Since only a preliminary screening of the literature was conducted based on CAS number search in SciFinder, it cannot be excluded that some relevant studies were not identified. Further, the studies were not evaluated for reliability or quality, and no weight of evidence analysis was applied. When effects in animal studies were identified, they were noted without a thorough evaluation of other signs of systemic or general toxicity, effects on body weights or other observations that might affect the findings. The screenings with search strings and extracted information for the 12 benzophenones were collated in annex 5.

Overall summaries of the extracted information were compiled in table 3 and 4, which can be regarded as "heatmaps" of ED-related information on the group of 12 benzophenones.

**Table 3** | "Heatmap" of EAS-relevant information on the group of 12 benzophenones

| CAS No. / Name (abbreviation) | 119-61-9 | 134-84-9 | 1137-42-4 | 611-99-4 | 131-55-5 | 131-56-6 | 131-57-7 | 131-53-3 | 131-54-4 | 2128-93-0 | 42019-78-3 | 302776-68-7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BP | 4-MBP | 4-HBP | 4-DHBP | BP-2 | BP-1 | BP-3 | BP-8 | BP-6 | 4-PhBP | 4-CHBP | DHHB |
| **EAS RELEVANT** | | | | | | | | | | | | |
| **In vitro** | | | | | | | | | | | | |
| Estrogenecity (E) | 4Y, 4N | 1Y | 9Y | 6Y | 11Y | 8Y | 8Y | 1Y | 1Y,2N | | 4Y | |
| Estrogen-like changes in gene expression, motility etc. | | | | | 1Y | 4Y | 2Y | | | | | |
| Anti-E | | | | | | | 1Y | | | | | |
| Androgenecity | | | | | 1Y | | | | | | | |
| Anti-A | 1Y,1N | | 3Y | 2Y | 5Y | 4Y | 5Y,1N | 1Y | 1Y,1N | | 1Y | |
| Steroid synthesis changes | | | | | 2Y | 1Y | | | | | | |
| **Rodents** | | | | | | | | | | | | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Estradiol level (EAS) | 1N | | | | 1Y | | | | | | | |
| Follicle stimulating hormone level (EAS) | 1N | | | | | | | | | | | |
| Luteinising hormone (LH) level (EAS) | 1N | | | | | | | | | | | |
| Testosterone level (EAS) | 1N | | | | 2Y | | 2Y | | | | | |
| Uterotrophic (E) | 3Y, 3N | | 3Y | 4Y | 6Y | 7Y | 1Y, 6N | 1 N | 1Y,1N | | | 1N |
| Uterotrophic (anti-E) | | | 1Y | 1Y | 1Y | 1Y,1N | 2N | | 2N | | | |
| Hershberger (A and anti-A) | | | | | | | 1N | | | | | 1N |
| **Other in vivo mechanistic** | | | | | | | | | | | | |
| Other mechanistic (E) | | | | | 5Y | | 5Y | 1 Y | | | | |
| Other mechanistic (A/anti-A) | | | | | | 1Y | | | | | | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Age at puberty (EAS) | 1N | | | | | | 1N | | | | | |
| Anogenital distance | 1N | | | | 1N | | 1N | | | | | |
| Epididymis weight/histopathology (EAS) | 2Y,1N | | | | | | | | | | | |
| Estrous cyclicity (EAS) | 2N | | | | | | 4Y,1N | | | | | |
| Genital anomalies (EAS) | | | | | 1Y | | | | | | | |
| Mammay gland histopathology (EAS) | | | | | | | 2Y | | | | | |
| Nipple development (EAS) | | | | | | | 1N | | | | | |
| Ovary weight/histopathology (EAS) | 2N | | | | | | | | | | | |
| Prostate weight/histopathology (EAS) | 2N | | | | | | | | | | | |
| Seminal vesicle weight/histopathology(EAS) | 2N | | | | | | | | | | | |
| Testis weight/histopathology (EAS) | 2Y,1N | | | | | | | | | | | 1Y |

| CAS No. / Name (abbreviation) | 119-61-9 BP | 134-84-9 4-MBP | 1137-42-4 4-HBP | 611-99-4 4-DHBP | 131-55-5 BP-2 | 131-56-6 BP-1 | 131-57-7 BP-3 | 131-53-3 BP-8 | 131-54-4 BP-6 | 2128-93-0 4-PhBP | 42019-78-3 4-CHBP | 302776-68-7 DHHB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sperm parameters (EAS) | 2N | | | | 1Y | | 5Y,2N | | | | | |
| Uterus weight/histopathology (EAS) | 2N | | | | | | | | | | | |
| **Sensitive to, but not diagnostic of, EATS (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Fetal development | | | | | | | 3N | | | | | |
| Litter size | | | | | | | | | | 1Y | | |
| Implantation loss | | | | | | | 1Y | | | 1Y | | |
| Learning and memory in offspring | | | | | | | 1Y | | | | | |
| Tumor types | | | | | | | 2Y | | | | | |
| **Fish** | | | | | | | | | | | | |
| **Other in vivo mechanistic** | | | | | | | | | | | | |
| Effects on gene transcription | | | | | | | 5Y | 1Y | | | | |
| Estrogenecity (E) | 1N | | | | | | | | | | | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Vitellogenin | | | 1N | | 3Y | 2Y | 4Y | | | | | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Secondary sex characteristics | | | | | 2Y | | 2Y | | | | | |
| Sex ratio | | | | | | | 2Y | | | | | |
| **Sensitive to, but not diagnostic of, EATS (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Other activities | | | | | | | 7Y | | | | | |
| Other adverse | | | | | 1Y | | 1Y | | | | | |
| **Amphibians** | | | | | | | | | | | | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Vitellogenin | | | | | 1Y | | | | | | | |
| Sex ratio | | | | | 1Y | | | | | | | |
| Effects on testis | | | | | | | 1Y | | | | | |
| **Other ED-related effects** | | | | | | | | | | | | |
| Motility of human sperm cells | | | | | | | 1Y | | | | | |
| Decreased PR transcription | | | | | | | 1Y | | | | | |
| PPAR-gamma agonism | | | | | | | 1Y | | | | | |
| ERR-gamma | | | 1Y | 1Y | | 1Y | | | | | | |
| AhR binding | | | | | | | 1Y | | 1Y | | | |
| Ex vivo neurotox | | | | | | | 1Y | | | | | |

**Table 4** | "Heatmap" of Thyroid-relevant information on the group of 12 benzophenones

| CAS No. / Name (abbreviation) | 119-61-9 | 134-84-9 | 1137-42-4 | 611-99-4 | 131-55-5 | 131-56-6 | 131-57-7 | 131-53-3 | 131-54-4 | 2128-93-0 | 42019-78-3 | 302776-68-7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BP | 4-MBP | 4-HBP | 4-DHBP | BP-2 | BP-1 | BP-3 | BP-8 | BP-6 | 4-PhBP | 4-CHBP | DHHB |
| **T RELEVANT** | | | | | | | | | | | | |
| **In vitro** | | | | | | | | | | | | |
| TPO activation | | | 1N | | | 1Y | 1N | 1Y | | | | |
| TPO inhibition | 1Y | | 1N | | 2Y | | 2N | | | | | |
| TTR binding | | | 1Y | 1Y | 2Y | 1Y | 1Y | 1Y | | | | |
| TR agonist | | | | | 2Y | | 2Y | | | | | |
| Altered gene expression | 1Y | | | | 1Y | 1Y | 1Y | 1Y | | | | |
| Inhibition of Th synthesis | | | | | | | 1N | | | | | |
| **Rodents** | | | | | | | | | | | | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| T3 and/or T4 level | | | 1Y | | 4Y | | 2Y,1N | | | | | |
| Thyroid stimulating hormone level (TSH) | | | 1Y | | 2Y,1N | | 1Y, 1N | | | | | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Thyroid weight/histopathology | 1Y,2N | | | | | | 1N | | | 1Y | | |
| **Sensitive to, but not diagnostic of, EATS (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Fetal development | | | | | | | 3N | | | | | |
| Litter/pup weight | | | | | | | 1Y | | | | | |
| Learning and memory in offspring | | | | | | | 1Y | | | | | |
| Tumor types | | | | | | | 2Y | | | | | |
| **Fish** | | | | | | | | | | | | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | | | | | | | | | | | | |
| Thyroid hormones | | | | | | 1Y | 2Y | 1Y | | | | |

### *Data availability for benzophenones*

The heatmaps showed that more information was available for some benzophenones than for others, with most data extracted on BP, BP-2, and BP-3. It was also evident that more in vitro and in vivo mechanistic information was available than information about ED-related adversity. The many blank cells indicated lack of data on the substances, which was especially prevalent for "EATS-mediated effects" in rodents, fish, and amphibians. It should however be noted that we did not systematically note absence of effects on ED-relevant endpoints investigated in OECD TG studies included in the REACH registration dossiers. Absence of effects may therefore in some cases be reflected as absence of data in the heatmaps, especially for in vivo endpoints in rodents.

In annex 5, the REACH registration tonnage was noted for each substance together with a list of the studies included in the repeated dose toxicity and reproductive toxicity sections of the REACH registration dossier. In general, data availability in the dossiers varied with tonnage levels, as expected. However, BP-1 and 4-MBP were both registered under REACH in 100-1000t/a, yet very little toxicity information was available in the dossiers. For BP-1, an OECD Toolbox grouping approach was applied for repeated dose toxicity, whereas a reference to a CeHoS SIN List report from 2012 was used for reproductive toxicity. For 4-MBP, read-across to benzophenone was applied for both repeated dose toxicity and reproductive toxicity. Especially for BP-1, which in the heatmaps showed a clear mechanistic estrogenic, anti-androgenic and thyroid-disrupting profile, it would be valuable to have more repeated dose and reproductive toxicity studies available for evaluation of ED-related adversity.

### *EAS-related findings, benzophenones*

In general, it seemed that the benzophenones showed estrogenic and anti-androgenic properties in vitro, with a subgroup (BP-1, BP-2, 4-OHBP and 4DHBP) showing more potent estrogenic effects in rodent screening studies, than the rest of the group. There was a scarcity of information on EAS-relevant "EATS-mediated effects" in rodents, and when available (BP, BP-2, BP-3), there was some data indicating effects and some indicating no effects. The substance with the clearest profile on ED-related adversity was BP-2, but also here only a limited number of studies were available. In fish and amphibians, most data were available on BP-2 and BP-3. Both substances induced in vivo mechanistic effects in fish and EATS-mediated effects in fish and amphibians.

All in all, most EAS-related data was identified for BP-2 and BP-3, and especially the fish and amphibian data indicated ED potential, whereas further analyses of the rodent data would be needed to provide a biologically plausible link between the endocrine activity and the adversity observed.

### *T-related findings, benzophenones*

It seemed that the benzophenones had the potential to interfere with the thyroid hormone system in vitro. Some of the substances affected the levels of thyroid hormones in rodents (4-OHBP and BP-2, possibly also BP-3) and in fish (BP-1, BP-3, BP-8), whereas generally there was a lack of information on "EATS-mediated effects" in rodents and fish.

# Discussion

In this project we searched for EDs of high priority for regulatory action in the EU under REACH or CLP. With 40-60.000 substances in global commerce, more than 26.000 substances registered under REACH, and numerous lists of suspected endocrine disruptors in the public domain, there are many ways to do so. We chose to build on the compilation of data and filtering of substances conducted by CeHoS in 2018 (Hass et al., 2018).

*Information availability bias: ED potential not excluded for >8000 non-prioritised substances*

One major limitation of the methodology applied was that lack of data generally led to exclusion of substances, leading to clear information availability bias.

In all the prioritisation steps conducted in Hass et al., 2018 and in this project, lack of information led to exclusion of substances:

- In Hass et al., 2018 more than 8000 substances were filtered down to 171. Lack of data indicating endocrine activity or ED-related adversity (including effects observed and reported in ECHA's registration dossiers and positive QSAR predictions for ED relevant endpoints) or exposure potential (data collected in the Nordic SPIN database) led to exclusion.
- 28 substances prioritised by the EU Commission for evaluation for ED potential by the SCCS were in this project included in the updated prioritised basis list of 192 substances. When the EU Commission prioritised these substances for evaluation, it was based on some information of ED relevance, and lack of such data would lead to exclusion.
- The exclusion criteria applied to select focus substances from the updated prioritised basis list in this project were developed with the specific aim to identify REACH relevant substances that could potentially be identified as endocrine disruptors under REACH or CLP. Regulatory identification of substances as EDs requires robust evidence for ED related adversity and mode of action. Substances without available studies investigating ED properties were therefore excluded at this step.

All in all, this induces a bias as focus was directed at data rich substances, and it was noted that ED potential cannot be excluded for all the substances not included in this project due to lack of data.

*Selection of focus substances was context dependent*

The exclusion criteria applied to select focus substances from the updated prioritised basis list were developed for the specific aim to identify REACH relevant substances that could potentially be identified as endocrine disruptors under REACH or CLP.

Apart from the need for robust information for identification, as discussed above, this also implied that the use, regulation, and status in REACH process were applied as exclusion criteria, i.e.:

-The substances were registered under REACH.

-The substances were not already strictly regulated.

-The substances were not undergoing a compliance check or substance evaluation under REACH, which could lead to generation of data relevant for ED evaluation.

These exclusion criteria were very context dependent, and it was noted that ED potential cannot be excluded for the substances on the updated prioritised basis list that were not moved forward to the list of focus substances because the use or regulatory status was judged to be irrelevant for the aim of this project.

### *Additional substances with possible ED potential*

In the framework of this project, the updated prioritised basis list is non-exhaustive and could be expanded by including additional substances. This could for example be substances identified as potential EDs in ECHA's Assessments of Regulatory Needs (ARNs), substances similar to substances already identified as EDs or substances inducing ED related effects in EOGRTSs conducted after data generation process under REACH. The >8000 substances compiled and filtered in Hass et al., (2018) could also be revisited and re-filtered with updated criteria. Most substances were excluded from prioritisation in that project due to lack of ED-related hazard data or exposure data, which could be updated by including e.g. hazard data generated in the US ToxCast and/or Tox21 programmes.
In a broader perspective, the entire starting point for the prioritisation could be reconsidered. One alternative starting point could be the exposome, including untargeted analyses from human biomonitoring and environmental monitoring studies.

### *Lack of ED relevant information*

A general lack of ED relevant information was evident from the massive exclusion of data poor substances in all prioritisation steps, as described above. In the filtering of the updated prioritised basis list in this project, it became clear that the lack of ED-relevant information was more severe for environmentally relevant information than for human health relevant information. 64 substances were excluded due to lack of ED-related information relevant for the environment, whereas 22 substances were excluded due to lack of ED-related information relevant for human health in this step. The same pattern was observed in the preliminary screening of 10 of the focus substances, where no environmentally relevant ED information was available for 4 out of 10 substances, whereas some human health relevant ED information was available for all 10 substances. This finding implies that there is a need for data generation, especially on environmentally relevant ED endpoints, and that the available data should be used to the greatest extent possible, e.g. by use of cross-species extrapolation wherever possible and relevant, including use of rodent data in environmentally related ED evaluations. The need for data generation calls upon an update of the REACH standard information requirements to include ED-relevant information for as many registered substances as possible.

### *97 substances of potential regulatory interest*

Most of the 97 focus substances compiled in this project are judged to be of potential regulatory interest – and could possibly be considered for either ED identification or data generation processes. The preliminary literature screening of 10 of the 97 focus substances showed that for 9 out of 10 selected focus substances, some information was available on endocrine activity and/or adversity. 5 out of 10 focus substances were considered to be of higher potential interest based on in vitro and rodent studies, while 1 out of 10 was considered

to be of higher potential interest based on in vitro and fish/amphibian studies with both endocrine activity and adversity information, which was evaluated to be potentially linked. It must, however, be noted that no thorough evaluation of study reliability, no weight of evidence analyses and no mode of action analyses with an evaluation of the biological plausibility of a link between endocrine activity and adversity was conducted in this project. Therefore, the level of evidence would need to be evaluated further for each substance before final prioritisations could be made.

The criteria for selection of the 10 focus substances for preliminary screening was based on an interest in this project to focus on substances of relevance for both the environment and human health. 29 of the 97 focus substances were prioritised for both the environment and human health. From those 29 substances, the project group selected 10 in agreement with the Danish EPA. This number of selected substances mainly reflected resource considerations, and the specific 10 substances were mainly chosen based on national interests and priorities. Thus, there is a likely high potential for regulatory interest of most of the 87 focus substances not selected for preliminary screening in this project.

*Preliminary screening methodology developed*

A preliminary screening methodology was developed in this project. It was applied to 10 individual substances first, and subsequently to a group of benzophenones. Small modifications were introduced when the benzophenones were screened. The search methodology was changed from using Web of Science to using SciFinder CAS, which led to a more targeted approach. Further, the way the results were presented and integrated was modified for the benzophenones to provide an overview of a group of substances. The preliminary screening methodology developed was pragmatic compared to the current consensus and guidelines for identification of endocrine disruptors. It provided a quick overview of information, which could be used for prioritisation purposes, and it sought to pave the way for cross-species extrapolations where possible. A limitation of the methodology was that it did not encompass more thorough evaluation steps, e.g. of study reliability, weight of evidence analyses or evaluation of biological plausibility of a link between endocrine activity and adversity. Further evaluation of the specific substances would therefore be needed before decisions about regulatory actions could be taken.

*Grouping of benzophenones*

A “heatmap” of ED-relevant effects was created to provide an overview and support grouping considerations. The heatmap did not lead to any firm conclusions, but it illustrated data availability across the group, including EAS-related and Thyroid-related ED potential for some of the substances. The next steps in a grouping approach could be to analyse structural similarities between the substances, how they are related by metabolism, and whether this is similar between species (rodents, fish, amphibians). It could also be investigated how different structural elements affect the different endocrine activities of the substances. On this basis, it may be possible to draw conclusions about how to build (a) reliable group(s) of benzophenones and for which effects it is possible to read across between substances.

Furthermore, the application of cross-species considerations could be elaborated, in order to maximise the use of available data.

### *Cross-species considerations*

Hazard assessment of endocrine disruptors has historically been developed separately for human health relevance, mainly based on rodent studies, and environmental relevance, mainly based on fish and amphibian studies. We have in this project sought to integrate knowledge and expertise from assessors with backgrounds in human health and environmental ED assessment, respectively. There are, however, some limitations in the methodology we have used. Some exclusion criteria and data searches were continuations of previous projects, and the separated thinking of human health and environmental relevance was often continued in data extractions and summarisation of data. It is therefore also inherent in tables and wording throughout this project. We have tried to highlight the need for cross-species considerations where relevant, and in future projects and follow up work, it is highly recommended to apply cross-species extrapolations to the widest possible extent.

# Conclusion and recommendations

In this project, 97 focus substances were identified. Most of the 97 focus substances were judged to be of potential regulatory interest – and could possibly be considered for either ED identification or data generation processes. A preliminary literature screening of 10 of the 97 focus substances showed that for 9 out of the 10 selected focus substances, some information was available on endocrine activity and/or adversity. 5 out of the 10 focus substances were considered to be of higher potential interest based on in vitro and rodent studies, while 1 out of the 10 was considered to be of higher potential interest based on in vitro and fish/amphibian studies. It should be noted that the preliminary screening did not include any evaluation of reliability of studies, WoE assessments or evaluation of a link between endocrine activity and adversity. Further evaluation of the substances would therefore be warranted before final prioritisations could be made.

We did a preliminary screening of a group of benzophenones and created a "heatmap" of ED-related effects, which illustrated data availability across the group, including EAS-related and T-related ED potential for some of the substances. The heatmap also paved the way for further analysis of the ED potential of benzophenones based on grouping and cross-species considerations.

We identified a general lack of ED relevant information. In the filtering of the updated prioritised basis list in this project, it became clear that the lack of ED-relevant information was more severe for environmentally relevant information than for human health relevant information. This finding implies that there is a need for data generation, especially on environmentally relevant ED endpoints, and that the available data should be used to the greatest extent possible, e.g. by use of cross-species extrapolation wherever possible and relevant, including use of rodent data in environmentally related ED evaluations.
The need for data generation calls upon an update of the REACH standard information requirements to include ED-relevant information for as many registered substances as possible.

## *Recommendations*

**Recommended follow up activities:**

- The ED potential of benzophenones could be further explored based on a grouping approach, including e.g., structural similarities, structural alerts, metabolism considerations across species and possibilities for cross-species extrapolations.

- The 9 out of 10 preliminarily screened focus substances with information on endocrine activity and/or adversity could be further evaluated to analyse their priority for ED identification or data generation processes.

- The remaining 87 focus substances could be preliminary screened for literature as single substances or in groups where possible. The developed preliminary screening methodology could be applied.

- More substances could be added to the updated prioritised basis list, for example substances identified as potential EDs in ECHA's Assessments of Regulatory Needs (ARNs), substances similar to substances already identified as EDs or substances inducing ED related effects in EOGRTSs conducted in data generation process under REACH.

- The >8000 substances compiled and filtered in Hass et al., (2018) could be revisited and re-filtered with updated criteria, since most substances were excluded from prioritisation in that project due to lack of data.

**Recommendations in a broader perspective:**

- A comprehensive lack of ED-relevant data was highlighted, especially on environmentally relevant ED endpoints. This lack of data calls for updated standard information requirements under REACH to include ED relevant information for as many substances as possible.

- The entire starting point for the prioritisation could be reconsidered. One alternative starting point could be the exposome, including untargeted analyses from human biomonitoring and environmental monitoring studies.

- Furthermore, the need to maximize the use of available data is emphasized, for example by assessing groups of substances and applying cross-species extrapolations.

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# Annex 1 Review of lists of suspected endocrine disruptors (EDs)

**Purpose:**
The purpose was to investigate whether lists used to generate the background lists in the CEHOS ED list project from 2018 had been updated since the background lists were generated in September 2016.

**Method:**
Revisions and updates were identified by visiting the relevant web pages. In addition, a search for new ED lists was performed in June 2022 by searching the web for "lists of endocrine disruptors". Detailed information was collated in table 1 and 2.

**Conclusion:**
Some of the lists used in the original project had been revised or were on the way to be phased out, and new lists had been developed and are publicly available.

We did not evaluate in all details whether the revisions, updates and changes affected the number or identity of substances on the original background lists.

However, it was noted that some work, such as the ECHA assessments of regulatory needs (ARNs)[1], might add some substances which were not included in the CEHOS project from 2018 (including a sub-group of bisphenols, see table 2).

It was also noted that other reports or grouping initiatives (e.g., on PFASs) were available in the public domain.

[1] https://echa.europa.eu/assessment-regulatory-needs

# Annex 1 – Review of lists of suspected endocrine disruptors (EDs)

**Table 1** | Lists included in Hass et al., 2018 and information on ED relevant updates.

| Institution | List | ED relevant updates (Yes/No) |
|---|---|---|
| DK CEHOS | Assessment of Danish Criteria for Identification of Endocrine Disruptors (CeHoS reports)<br><br>(Hass et al. 2012a, 2012b) | No |
| European Commission | The Priority List of Chemicals | No<br><br>No longer available |
| DK Consumer Council | The DK Consumer Council list (32 substances)<br><br>(DK Consumer Council, 2016) | Yes<br>Had been substituted with a list of 42 suspected EDs in cosmetics – see table 2 |
| ChemSec | The Substitute it now! (SIN) list (ChemSec, 2022) | No<br>Numerous updates, but none with suspected EDs |
| European Trade Union Confederation | Trade Union Priority List for Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Authorisation (European Trade Union Confederation, 2010) | No<br><br>In 2010 a Trade Union Priority list v.2.0 was published, which included "suspected endocrine disruptors listed in the Community Strategy ". Since the ED list project from 2018 already included substances from the EU COM Community Strategy, this would not add any new substances. |
| TEDX | The List of potential Endocrine Disruptors | No<br>No longer available |
| WHO | State of the science of Endocrine Disrupting Chemicals (WHO, 2012) | No<br><br>Not updated since 2016 |

## Annex 1 – Review of lists of suspected endocrine disruptors (EDs)

**Table 2** | Updated/revised/new ED lists identified in June 2022. Evaluation of whether the lists included additional substances compared to those compiled in Hass et al., 2018.

| Institution | List | Evaluation of whether the list includes additional substances compared to those compiled in the ED list project 2018 |
|---|---|---|
| European Commission | 28 suspected EDs in cosmetics to be evaluated by the Scientific Committee for Consumer Safety, SCCS (EU COM, 2022) | *Possibly additional substances*<br><br>***1st call for data:***<br><br>Benzophenone-3,<br>kojic acid,<br>4-methylbenzylidene camphor,<br>propylparaben,<br>triclosan,<br>resorcinol,<br>octocrylene,<br>triclocarban,<br>butylated hydroxytoluene (BHT),<br>benzophenone,<br>homosalate,<br>benzyl salicylate,<br>genistein<br>daidzein<br><br>***Future calls for data:***<br>Butylparaben,<br>tert-butylhydroxyanisole/Butylated hydroxyanisole/BHA,<br>ethylhexyl methoxycinnamate(EHMC)/ octylmethoxycinnamate (OMC)/ octinoxate,<br>benzophenone-1/BP-1,<br>benzophenone-2/BP-2,<br>benzophenone-4/BP-4,<br>benzophenone-5/BP-5,<br>methylparaben,<br>cyclopentasiloxane/ decamethylcyclopentasiloxane/D5,<br>Cyclomethicone,<br>salicylic acid,<br>butylphenyl methyl propanol/BMHCA,<br>triphenyl phosphate,<br>deltamethrin |

## Annex 1 – Review of lists of suspected endocrine disruptors (EDs)

| Institution | List | Evaluation of whether the list includes additional substances compared to those compiled in the ED list project 2018 | |
|---|---|---|---|
| Danish Consumer Council | 42 endocrine disruptors found in personal care products (Danish Consumer Council, 2022). | *Possibly additional substances*<br><br>***Found in more products (18):***<br>Benzophenone-1<br>Benzophenone-3<br>Benzophenone-4<br>Benzyl salicylate<br>BHT or Butylated Hydroxytoluene<br>Butylparaben<br>Butylphenyl metylpropional<br>Cyclopentasiloxane<br>Cyclomethicone (may contain cyclopentasiloxane)<br>Ethylhexyl Methoxycinnamate<br>Ethylparaben<br>Ethyl salicylate<br>Homosalate<br>Methylparaben<br>Propylparaben<br>Octocrylene<br>Resorcinol<br>Salicylic acid | ***Found in fewer products:***<br><br>Benzophenone<br>Benzophenone-2<br>Benzophenone-5<br>BHA or Tert.-Butylhydroxyanisole<br>Cyclotetrasiloxane<br>Diethyl Phthalate (DEP)<br>Genistein<br>Isoamyl P-methoxycinnamate<br>Koji acid<br>MEK (Butanone)<br>Octoxynol<br>Triclosan<br>Triphenyl phosphate<br><br>***Not found in products:***<br><br>4-Hydroxybenzoic Acid<br>4-Methylbenzylidene Camphor<br>Acetyl Hexamethyl Tetralin<br>Dihydroxybiphenyl<br>Deltamethrin<br>Hydroxycinnamic Acid<br>Hexamethylindanopyran<br>Nitrophenol<br>Resmethrin<br>Styrene<br>T-butyl methyl ether (MTBE) |
| UNEP | Worldwide initiatives to identify | Possibly additional substances | |

## Annex 1 – Review of lists of suspected endocrine disruptors (EDs)

| Institution | List | Evaluation of whether the list includes additional substances compared to those compiled in the ED list project 2018 |
|---|---|---|
| | endocrine disrupting chemicals (EDCs) and potential EDCs (UNEP 2017) | The listed initiatives from e.g., Australia, US and Japan could include some suspected EDs, not included in the ED list project from 2018. |
| ECHA ED | Endocrine disruptor assessment list (ECHA, 2022a) | *Possibly additional substances*<br><br>Lists of substances undergoing an ED assessment that have been brought for discussion to ECHA's ED Expert Group. |
| ECHA ARNs | Assessments of regulatory needs (ARNs) (ECHA, 2022b)<br><br>Including ARN on bisphenols<br><br>(ECHA, 2021) | *Additional substances*<br><br>Substances with possible ED properties included in some ARNs. One example is 34 bisphenols identified as suspected EDs after an evaluation of 148 bisphenols as a group:<br><br>EC/List No. 201-245-8 (BPA), 201-025-1 (BPB), 401-720-1 (4,4'-Isobutylethylidenediphenol), 216-036-7 (BPAF) and its 8 salts (278-305-5; 425-060-9; 443-330-4; 468-740-0; 469-080-6; 479-100-5; 943-265-6; 947-368- 7), 201-250-5 (BPS), 201-240-0 (BPC), 204-279-1 (TBMD), 201-618-5 (6,6'-di-tert-butyl-4,4'-butylidenedi-mcresol), 242-895-2, 248-607-1, 405-520-5 (D8), 217-121-1 (DAB), 227-033-5 (TMBPA), 210-658-2 (BPF), 411- 570-9, 277-962-5 (contains BPS, 500-086-4 (contains BPA), 500-263-6 (contains BPA), 500-607-5 (contains BPA), 701-362-9, 904-653-0 (contains BPA), 908-912-9 (contains BPF), 926-571-4 (contains BPA), 931-252-8 (contains BPA), 941-992-3 (contains BPS), 943-503-9 (contains BPA). |

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# Annex 2 Updated prioritised basis list (192 substances)

**Table 1** | Updated prioritised basis list (192 substances)

| CAS# | Name |
|---|---|
| 15356-60-2 | (+)-menthol |
| 79-77-6 | (E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one |
| 42594-17-2 | (octahydro-4,7-methano-1H-indenediyl)bis(methylene) diacrylate |
| 110-25-8 | (Z)-N-methyl-N-(1-oxo-9-octadecenyl)glycine |
| 87-66-1 | 1 , 2 , 3-trihydroxybenzene |
| 23847-08-7 | 1,1'-dithiobis[hexahydro-2H-azepin-2-one] |
| 95-63-6 | 1,2,4-trimethylbenzene |
| 68515-49-1 | 1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich |
| 106-93-4 | 1,2-dibromoethane |
| 26780-96-1 | 1,2-Dihydro-2,2,4-trimethylquinoline, oligomers |
| 2451-62-9 | 1,3,5-tris(oxiranylmethyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione |
| 40601-76-1 | 1,3,5-tris[[4-tert-butyl-3-hydroxy-2,6-xylyl]methyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione |
| 68953-84-4 | 1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs. |
| 1569-02-4 | 1-ethoxypropan-2-ol |
| 6846-50-0 | 1-isopropyl-2,2-dimethyltrimethylene diisobutyrate |
| 95-38-5 | 2-(2-heptadec-8-enyl-2-imidazolin-1-yl)ethanol |
| 80-54-6 | 2-(4-tert-butylbenzyl)propionaldehyde |
| 80-54-6 | 2-(4-tert-butylbenzyl)propionaldehyde/butylphenyl methylpropanol/BMHCA/lysmeral |
| 142-18-7 | 2,3-dihydroxypropyl laurate |
| 123-17-1 | 2,6,8-trimethylnonan-4-ol |
| 111-76-2 | 2-butoxyethanol |
| 48145-04-6 | 2-phenoxyethyl acrylate |
| 1948-33-0 | 2-tert-butylhydroquinone |
| 6386-73-8 | 3 , 3' , 5-tribromobisphenol A |
| 19780-11-1 | 3-(2-dodecenyl)succinic anhydride |
| 56-18-8 | 3,3'-iminodi(propylamine) |
| 15087-24-8 | 3-benzylidene camphor (3-BC) |
| 14901-07-6 | 4-(2,6,6-trimethylcyclohex-1-ene-1-yl)-but-3-ene-2-one |
| 599-64-4 | 4-(a,a-dimethylbenzyl)phenol |
| 101-14-4 | 4,4'-methylenebis[2-chloroaniline] |
| 101-77-9 | 4,4'-methylenedianiline |
| 123-30-8 | 4-aminophenol |
| 1131-60-8 | 4-Cyclohexylphenol |
| 104-43-8 | 4-dodecylphenol |
| 123-07-9 | 4-ethylphenol |
| 36861-47-9 | 4-methylbenzylidene camphor, 4-MBC |
| 98-52-2 | 4-tert-butylcyclohexanol |
| 446-72-0 | 5,7-dihydroxy-3-(4-hydroxyphenyl)-4-benzopyrone; genistein |
| 486-66-8 | 7-hydroxy-3-(4-hydroxyphenyl)-4-benzopyrone; daidzein |
| 79-06-1 | Acrylamide |

# Annex 2 – Updated prioritised basis list (192 substances)

| CAS# | Name |
|---|---|
| 107-13-1 | Acrylonitrile |
| 68155-27-1 | Amines, C12-18-alkyl |
| 5421-46-5 | Ammonium mercaptoacetate |
| 520-36-5 | Apigenin |
| 67774-74-7 | Benzene, C10-13-alkyl derivs. |
| 108-98-5 | Benzenethiol |
| 56-55-3 | Benzo(a)anthracene |
| 50-32-8 | Benzo(a)pyrene |
| 205-99-2 | Benzo[b]fluoranthene |
| 192-97-2 | Benzo[e]pyrene |
| 205-82-3 | Benzo[j]fluoranthene |
| 207-08-9 | Benzo[k]fluoranthene |
| 119-61-9 | Benzophenone |
| 131-56-6 | Benzophenone-1/BP-1 |
| 131-55-5 | Benzophenone-2/BP-2 |
| 131-57-7 | Benzophenone-3/BP-3, oxybenzone |
| 4065-45-6 | Benzophenone-4/BP-4 |
| 6628-37-1 | Benzophenone-5/BP-5 |
| 85-68-7 | Benzyl butyl phthalate |
| 118-58-1 | Benzyl salicylate |
| 82657-04-3 | Bifenthrin |
| 584-79-2 | Bioallethrin = d- trans allethrin |
| 103-24-2 | Bis(2-ethylhexyl) azelate |
| 142-16-5 | Bis(2-ethylhexyl) maleate |
| 56-35-9 | Bis(tributyltin) oxide |
| 1675-54-3 | Bisphenol A diglycid ether (BADGE) |
| 3253-39-2 | Bisphenol A dimethacrylate |
| 1478-61-1 | Bisphenol AF |
| 107-88-0 | Butane-1,3-diol |
| 128-37-0 | Butylated hydroxytoluene (BHT) |
| 94-26-8 | Butylparaben |
| 59-50-7 | Chlorocresol |
| 2921-88-2 | Chlorpyrifos |
| 218-01-9 | Chrysene |
| 104-54-1 | Cinnamyl alcohol |
| 130-26-7 | Clioquinol |
| 1319-77-3 | Cresol |
| 10016-20-3 | Cyclohexapentylose |
| 69430-24-6 | Cyclomethicone |
| 541-02-6 | Cyclopentasiloxane/decamethylcyclopentasiloxane/D5 |
| 91465-08-6 | Cyhalothrin |
| 52315-07-8 | Cypermethrin |
| 94361-06-5 | Cyproconazole |
| 27554-26-3 | Di-´´isoalkyl´´ phthalates * |
| 131-17-9 | Diallyl phthalate |
| 25376-45-8 | Diaminotoluene |
| 192-65-4 | Dibenzo[a , e]pyrene |

# Annex 2 – Updated prioritised basis list (192 substances)

| CAS# | Name |
|---|---|
| 53-70-3 | Dibenzo[a , h]anthracene |
| 189-64-0 | Dibenzo[a , h]pyrene |
| 189-55-9 | Dibenzo[a , i]pyrene |
| 191-30-0 | Dibenzo[a , l]pyrene |
| 84-74-2 | Dibutyl phthalate |
| 1067-33-0 | Dibutyltin di(acetate) |
| 683-18-1 | Dibutyltin dichloride |
| 97-23-4 | Dichlorophen |
| 84-61-7 | Dicyclohexyl phthalate |
| 101-83-7 | Dicyclohexylamine |
| 119446-68-3 | Difenoconazole |
| 84-75-3 | Dihexyl phthalate (DHP) |
| 26544-38-7 | Dihydro-3-(tetrapropenyl)furan-2,5-dione |
| 84-69-5 | Diisobutyl phthalate |
| (26761-40-0)<br>68515-49-1 | Di-isodecyl phthalate (DIDP), 1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich |
| 131-18-0 | Di-n-pentylphthalate (DPP) |
| 117-84-0 | Dioctyl phthalate |
| 74-31-7 | Diphenyl-p-phenylenediamine |
| 3648-20-2 | Diundecyl phtalate, DuDP, branched and linear |
| 124-22-1 | Dodecylamine |
| 16423-68-0 | Erythrosine B, FD&C Red No. 3 |
| 66230-04-4 | Esfenvalerate |
| 27986-36-3 | Ethanol, 2-(nonylphenoxy)- |
| 80844-07-1 | Ethofenprox |
| 5466-77-3/<br>83834-59-7 | Ethylhexyl methoxycinnamate(EHMC)/octyl methoxycinnamate (OMC)/octinoxate |
| 97-53-0 | Eugenol |
| 122-14-5 | Fenitrothion |
| 51630-58-1 | Fenvalerate |
| 76674-21-0 | Flutriafol |
| 91744-09-1 | Glycerides, C16-18 and C18-unsatd. mono- |
| 9036-19-5 | Glycols, polyethylene, mono((1,1,3,3-tet = Poly(oxy-1,2-ethanediyl), .alpha.-[(1,1,3,3-tetramethylbutyl) phenyl]-.omega.-hydroxy- |
| 99607-70-2 | Heptan-2-yl [(5-chloroquinolin-8-yl)oxy]acetate |
| 520-33-2 | Hesperetin |
| 25637-99-4 | Hexabromocyclododecane |
| 70-30-4 | Hexachlorophene |
| 6259-76-3 | Hexyl salicylate |
| 118-56-9 | Homosalate |
| 35554-44-0 | Imazalil |
| 193-39-5 | Indeno[1 , 2 , 3-cd]pyrene |
| 4247-02-3 | Isobutyl paraben |
| 501-30-4 | Kojic acid |
| 115-95-7 | Linalyl acetate |
| 68-11-1 | Mercaptoacetic acid |
| 108-67-8 | Mesitylene |

# Annex 2 – Updated prioritised basis list (192 substances)

| CAS# | Name |
|---|---|
| 2032-65-7 | Methiocarb |
| 119-36-8 | Methyl salicylate |
| 99-76-3 | Methylparaben |
| 298-00-0 | Methylparathion |
| 99-97-8 | N,N-dimethyl-p-toluidine |
| 39236-46-9 | N,N''-methylenebis[N'-[3-(hydroxymethyl)-2,5-dioxoimidazolidin-4-yl]urea] |
| 142-59-6 | Nabam |
| 1338-24-5 | Naphthenic acids |
| 106-25-2 | Nerol |
| 7718-54-9 | Nickel dichloride |
| 97-78-9 | N-lauroylsarcosine |
| 556-67-2 | Octamethylcyclotetrasiloxane (D4) |
| 6197-30-4 | Octocrilene |
| 66063-05-6 | Pencycuron |
| 78-11-5 | Pentaerithrityl tetranitrate |
| 52645-53-1 | Permethrin |
| 85-01-8 | Phenanthrene |
| 87-22-9 | Phenethyl salicylate |
| 108-95-2 | Phenol |
| 143-74-8 | Phenol red |
| 121158-58-5 | Phenol, dodecyl-, branched |
| 68937-41-7 | Phenol, isopropylated, phosphate (3:1) |
| 118-55-8 | Phenyl salicylate |
| 2310-17-0 | Phosalone |
| 80-26-2 | P-menth-1-en-8-yl acetate |
| 9014-90-8 | Poly(oxy-1,2-ethanediyl) |
| 68987-90-6 | Poly(oxy-1,2-ethanediyl), alpha-(octylphenyl)-omega-hydroxy-branched |
| 3164-85-0 | Potassium 2-ethylhexanoate |
| 3811-04-9 | Potassium chlorate |
| 67747-09-5 | Prochloraz |
| 60207-90-1 | Propiconazole |
| 94-13-3 | Propylparaben, propyl 4-hydroxybenzoate |
| 121-29-9 | Pyrethrin |
| 117-39-5 | Quercetin |
| 94581-15-4 | Resin acids and Rosin acids, fumarated, esters with pentaerythritol |
| 108-46-3 | Resorcinol |
| 69-72-7 | Salicylic acid |
| 7758-19-2 | Sodium chlorite |
| 15708-41-5 | Sodium feredetate |
| 9005-64-5 | Sorbitan monolaurate, ethoxylated |
| 9005-65-6 | Sorbitan monooleate, ethoxylated |
| 85586-07-8 | Sulfuric acid, mono-C12-14-alkyl esters, sodium salts |
| 26002-80-2 | Sumithrin |
| 107534-96-3 | Tebuconazole |
| 25013-16-5 | Tert-butylhydroxyanisole/Butylated hydroxyanisole/tert-butyl-4-methoxyphenol/BHA |
| 1461-25-2 | Tetrabutyltin |
| 97-74-5 | Tetramethylthiuram monosulphide |

# Annex 2 – Updated prioritised basis list (192 substances)

| CAS# | Name |
|---|---|
| 16470-24-9 | Tetrasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(4-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate] |
| 57018-04-9 | Tolclofos-methyl |
| 731-27-1 | Tolylfluanid |
| 101-20-2 | Triclocarban |
| 3380-34-5 | Triclosan |
| 2031-67-6 | Triethoxy(methyl)silane |
| 2943-75-1 | Triethoxyoctylsilane |
| 115-86-6 | Triphenyl phosphate |
| 3319-31-1 | Tris(2-ethylhexyl) benzene-1,2,4-tricarboxylate |
| 1067-53-4 | Tris(2-methoxyethoxy)vinylsilane |
| 1330-78-5 | Tris(methylphenyl) phosphate |
| 13674-87-8 | Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP) |
| 14324-55-1 | Zinc bis(diethyldithiocarbamate) |
| 52918-63-5 | α-cyano-3-phenoxybenzyl [1R-[1α(S*),3α]]-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate/deltamethrin |

# Annex 3 95 excluded substances

**Table 1** | 95 excluded substances with applied exclusion criteria for human health (HH) and the environment (ENV).

| CAS no | Name | Exclusion criteria (HH) | Exclusion criteria (ENV) |
|---|---|---|---|
| 79-77-6 | (E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Lack of ED relevant data (CEHOS 2018 + SDU 2021) |
| 2451-62-9 | 1,3,5-tris(oxiranylmethyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione | Strictly regulated (CLP Mut 1B) | Lack of ED relevant data (SDU 2021) |
| 6846-50-0 | 1-isopropyl-2,2-dimethyltrimethylene diisobutyrate | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Lack of ED relevant data (CEHOS 2018) |
| 95-38-5 | 2-(2-heptadec-8-enyl-2-imidazolin-1-yl)ethanol | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Lack of ED relevant data (CEHOS 2018 + SDU 2021) |
| 80-54-6 | 2-(4-tert-butylbenzyl)propionaldehyde/butylphenyl methylpropanol/BMHCA/lysmeral | REACH evaluation concluded in 2022 with unresolved ED concern | REACH evaluation concluded in 2022 with unresolved ED concern |
| 123-17-1 | 2,6,8-trimethylnonan-4-ol | REACH registration, only for intermediate uses | Lack of ED relevant data (SDU 2021) + REACH registration, only for intermediate uses |
| 111-76-2 | 2-butoxyethanol | Lack of ED relevant data (CEHOS 2018 + DTU 2019). | Lack of ED relevant data (SDU 2021) |
| 56-18-8 | 3,3'-iminodi(propylamine) | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Lack of ED relevant data (CEHOS 2018 + SDU 2021) |
| 15087-24-8 | 3-benzylidene camphor (3-BC) | Not registered under REACH<br>+<br>Evaluated as suspected ED by CEHOS (2012 + 2018) | SVHC for ED (ENV) |
| 1131-60-8 | 4-Cyclohexylphenol | REACH registration, only for intermediate uses | Lack of ED relevant data (SDU 2021) |
| 104-43-8 | 4-dodecylphenol | Not registered under REACH | Not registered under REACH |

## Annex 3 - 95 excluded substances

| CAS no | Name | Exclusion criteria (HH) | Exclusion criteria (ENV) |
|---|---|---|---|
| 36861-47-9 | 4-methylbenzylidene camphor, 4-MBC | SVHC for ED (HH) | Not registered under REACH + REACH evaluation concluded in 2023 with unresolved ED concern |
| 446-72-0 | 5,7-dihydroxy-3-(4-hydroxyphenyl)-4-benzopyrone; genistein | Not registered under REACH | Not registered under REACH |
| 486-66-8 | 7-hydroxy-3-(4-hydroxyphenyl)-4-benzopyrone; daidzein | Not registered under REACH | Not registered under REACH |
| 79-06-1 | Acrylamide | Strictly regulated (CLP Mut 1B + SVHC for Mut) | Lack of ED relevant data (CEHOS 2018 + SDU 2021) |
| 68155-27-1 | Amines, C12-18-alkyl | Not registered under REACH | Not registered under REACH |
| 520-36-5 | Apigenin | Not registered under REACH | Not registered under REACH |
| 108-98-5 | Benzenethiol | REACH registration, only for intermediate uses | Lack of ED relevant data (SDU 2021) |
| 56-55-3 | Benzo(a)anthracene | Not registered under REACH | Not registered under REACH |
| 50-32-8 | Benzo(a)pyrene | Strictly regulated (CLP Mut 1B + SVHC for Mut) + Regulated as POP + Not registered under REACH | Strictly regulated as POP + Not registered under REACH |
| 205-99-2 | Benzo[b]fluoranthene | Strictly regulated as POP + Not registered under REACH | Not registered under REACH (2023). Included in the POP Regulation. |
| 192-97-2 | Benzo[e]pyrene | Not registered under REACH | Not registered under REACH |
| 205-82-3 | Benzo[j]fluoranthene | Not registered under REACH | Not registered under REACH |
| 207-08-9 | Benzo[k]fluoranthene | Strictly regulated as POP + Not registered under REACH | Strictly regulated as POP + Not registered under REACH |
| 119-61-9 | Benzophenone | REACH evaluation concluded in 2018 with unresolved ED concern | REACH evaluation concluded in 2018 with unresolved ED concern |
| 131-57-7 | Benzophenone-3, oxybenzone | REACH evaluation ongoing | REACH evaluation ongoing |
| 82657-04-3 | Bifenthrin | Not registered under REACH | Not registered under REACH + Evaluated as suspected ED (ENV) by CEHOS (2018) |

## Annex 3 - 95 excluded substances

| CAS no | Name | Exclusion criteria (HH) | Exclusion criteria (ENV) |
|---|---|---|---|
| 584-79-2 | Bioallethrin = d- trans allethrin | Not registered under REACH | Not registered under REACH |
| 1675-54-3 | Bisphenol A diglycid ether (BADGE) | REACH evaluation concluded in 2021 with unresolved ED concern | REACH evaluation concluded in 2021 with unresolved ED concern |
| 3253-39-2 | Bisphenol A dimethacrylate | Not registered under REACH | Not registered under REACH |
| 1478-61-1 | Bisphenol AF | Evaluated as ED (HH) by CEHOS (2018) | Evaluated as ED (ENV) by CEHOS (2018) |
| 128-37-0 | Butylated hydroxytoluene (BHT) | REACH evaluation ongoing | REACH evaluation ongoing |
| 2921-88-2 | Chlorpyrifos | Not registered under REACH | Not registered under REACH |
| 218-01-9 | Chrysene | Not registered under REACH | Not registered under REACH |
| 130-26-7 | Clioquinol | Registered under REACH but currently no use in the EU | Registered under REACH but currently no use in the EU + Lack of ED relevant data (SDU 2021) |
| 91465-08-6 | Cyhalothrin | Not registered under REACH | Not registered under REACH |
| 52315-07-8 | Cypermethrin | Not registered under REACH | Not registered under REACH |
| 27554-26-3 | Di-´´isoalkyl´´ phthalates * | Not registered under REACH | Not registered under REACH + Lack of ED relevant data (SDU 2021) |
| 25376-45-8 | Diaminotoluene | REACH registration, only for intermediate uses | Lack of ED relevant data (SDU 2021) |
| 192-65-4 | Dibenzo[a , e]pyrene | Not registered under REACH | Not registered under REACH |
| 53-70-3 | Dibenzo[a , h]anthracene | Not registered under REACH | Not registered under REACH |
| 189-64-0 | Dibenzo[a , h]pyrene | Not registered under REACH | Not registered under REACH |
| 189-55-9 | Dibenzo[a , i]pyrene | Not registered under REACH | Not registered under REACH |
| 191-30-0 | Dibenzo[a , l]pyrene | Not registered under REACH | Not registered under REACH |
| 1067-33-0 | Dibutyltin di(acetate) | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Lack of ED relevant data (CEHOS 2018 + SDU 2021) |
| 84-61-7 | Dicyclohexyl phthalate | SVHC for ED (HH) | REACH evaluation ongoing |
| 119446-68-3 | Difenoconazole | Not registered under REACH | Not registered under REACH |

## Annex 3 - 95 excluded substances

| CAS no | Name | Exclusion criteria (HH) | Exclusion criteria (ENV) |
|---|---|---|---|
| 84-75-3 | Dihexyl phthalate (DHP) | Not registered under REACH | Not registered under REACH |
| 131-18-0 | Di-n-pentylphthalate (DPP) | Not registered under REACH + Evaluated as ED (HH) by CEHOS (2018) | Not registered under REACH |
| 117-84-0 | Dioctyl phthalate | Not registered under REACH | Not registered under REACH |
| 66230-04-4 | Esfenvalerate | Not registered under REACH | Not registered under REACH |
| 27986-36-3 | Ethanol, 2-(nonylphenoxy)- | Not registered under REACH | SVHC for ED (ENV) + Not registered under REACH. |
| 80844-07-1 | Ethofenprox | BPR evaluation ongoing | BPR evaluation ongoing |
| 5466-77-3 + 83834-59-7 | Ethylhexyl methoxycinnamate(EHMC)/octyl methoxycinnamate (OMC)/octinoxate | REACH evaluation ongoing | REACH evaluation ongoing |
| 51630-58-1 | Fenvalerate | Not registered under REACH | Not registered under REACH |
| 9036-19-5 | Glycols, polyethylene, mono((1,1,3,3-tet = Poly(oxy-1,2-ethanediyl), .alpha.-[(1,1,3,3-tetramethylbutyl) phenyl]-.omega.-hydroxy- | Not registered under REACH | SVHC for ED (ENV) |
| 520-33-2 | Hesperetin | Not registered under REACH + Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Not registered under REACH |
| 25637-99-4 | Hexabromocyclododecane | Strictly regulated as POP + Registered under REACH but currently not used in the EU | Strictly regulated as POP + Registered under REACH but currently not used in the EU |
| 70-30-4 | Hexachlorophene | Evaluated as suspected ED (HH) by CEHOS (2018) + Registered under REACH but currently not used in the EU | Registered under REACH but currently not used in the EU |
| 6259-76-3 | Hexyl salicylate | New data under development | New data under development |

## Annex 3 - 95 excluded substances

| CAS no | Name | Exclusion criteria (HH) | Exclusion criteria (ENV) |
|---|---|---|---|
| 35554-44-0 | Imazalil | Not registered under REACH (2023) | Not registered under REACH |
| 193-39-5 | Indeno[1 , 2 , 3-cd]pyrene | Strictly regulated as POP + Not registered under REACH | Not registered under REACH |
| 115-95-7 | Linalyl acetate | Lack of ED relevant data (CEHOS 2018 + DTU 2019). | Lack of ED relevant data (CEHOS 2018 + SDU 2021). |
| 2032-65-7 | Methiocarb | Not registered under REACH | Not registered under REACH |
| 99-76-3 | methylparaben | REACH evaluation ongoing | REACH evaluation ongoing |
| 119-36-8 | Methyl salicylate | REACH evaluation ongoing | REACH evaluation ongoing |
| 298-00-0 | Methylparathion | Not registered under REACH | Not registered under REACH |
| 142-59-6 | Nabam | Not registered under REACH | Not registered under REACH |
| 97-78-9 | N-lauroylsarcosine | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Lack of ED relevant data (CEHOS 2018 + SDU 2021). |
| 6197-30-4 | Octocrilene | REACH evaluation ongoing | REACH evaluation ongoing |
| 52645-53-1 | Permethrin | Not registered under REACH | Not registered under REACH |
| 85-01-8 | Phenanthrene | Not registered under REACH | Not registered under REACH |
| 143-74-8 | Phenol red | Not registered under REACH | Not registered under REACH |
| 121158-58-5 ?? EC 310-154-3 | Phenol, dodecyl-, branched | SVHC for ED (HH+ENV) | SVHC for ED (HH+ENV) |
| 68937-41-7 | Phenol, isopropylated, phosphate (3:1) | REACH evaluation ongoing | REACH evaluation ongoing |
| 2310-17-0 | Phosalone | Not registered under REACH | Not registered under REACH |
| 80-26-2 | P-menth-1-en-8-yl acetate | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Lack of ED relevant data (CEHOS 2018 + SDU 2021) |
| 9014-90-8 | Poly(oxy-1,2-ethanediyl) | Not registered under REACH | Not registered under REACH |
| 68987-90-6 | Poly(oxy-1,2-ethanediyl), alpha-(octylphenyl)-omega-hydroxy-branched | Not registered under REACH | Not registered under REACH |
| 67747-09-5 | Prochloraz | Not registered under REACH + Evaluated as ED (HH) by CEHOS (2018) | Not registered under REACH + Evaluated as ED (ENV) by CEHOS (2018) |
| 60207-90-1 | Propiconazole | ED HH (BPR) | ED ENV (BPR) |

## Annex 3 - 95 excluded substances

| CAS no | Name | Exclusion criteria (HH) | Exclusion criteria (ENV) |
|---|---|---|---|
| 94-13-3 | Propylparaben, propyl 4-hydroxybenzoate | REACH evaluation concluded in 2023 with no follow up on ED | REACH evaluation concluded in 2023 with follow up on ED |
| 121-29-9 | Pyrethrin | Not registered under REACH | Not registered under REACH |
| 117-39-5 | Quercetin | Not registered under REACH | Not registered under REACH |
| 9005-65-6 | Sorbitan monooleate, ethoxylated | Not registered under REACH | Not registered under REACH + Lack of ED relevant data (SDU 2021) |
| 85586-07-8 | Sulfuric acid, mono-C12-14-alkyl esters, sodium salts | Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Lack of ED relevant data (CEHOS 2018 + SDU 2021) |
| 26002-80-2 | Sumithrin | Not registered under REACH + Lack of ED relevant data (CEHOS 2018 + DTU 2019) | Not registered under REACH |
| 107534-96-3 | Tebuconazole | Registered under REACH but currently not used in the EU | Registered under REACH but currently not used in the EU |
| 25013-16-5 | Tert-butylhydroxyanisole/Butylated hydroxyanisole/tert-butyl-4-methoxyphenol/BHA | Not registered under REACH | Not registered under REACH |
| 57018-04-9 | Tolclofos-methyl | Not registered under REACH | Lack of ED relevant data (SDU 2021) |
| 731-27-1 | Tolylfluanid | Not registered under REACH | Not registered under REACH |
| 101-20-2 | Triclocarban | Evaluated as ED (HH) by CEHOS (2018)<br>+<br>Registered under REACH but currently not used in the EU | Registered under REACH but currently not used in the EU |
| 115-86-6 | Triphenyl phosphate | New data under development | REACH evaluation concluded in 2023 with follow up on ED |
| 13674-87-8 | Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP) | REACH evaluation ongoing | REACH evaluation ongoing |

## Annex 3 - 95 excluded substances

| CAS no | Name | Exclusion criteria (HH) | Exclusion criteria (ENV) |
|---|---|---|---|
| 52918-63-5 | α-cyano-3-phenoxybenzyl [1R-[1α(S*),3α]]-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate/deltamethrin | Not registered under REACH + Evaluated as suspected ED (HH) by CEHOS (2018) | Not registered under REACH |

# Annex 4 Preliminary literature screening of 10 selected focus substances

A subsample of 10 out of the 97 focus substances were selected for a preliminary literature screening. The aim of the literature screening was to investigate whether information was available on endocrine activity and ED-related adversity respectively, with focus on the EATS modalities.

The following methodology was used:

1. Information was retrieved from ECHA's publicly available database of registered substances by entering the CAS no. (ECHA 2023). Available synonyms were noted together with information on tonnage level.
2. ECHA registration dossiers were screened for effects relevant for endocrine disruption. The section on reproductive toxicity was always screened, and in some cases also the section on repeated dose toxicity.
3. Search strings were developed based on CAS numbers and synonyms.
4. The open literature was searched using "Web of Science (all database mode)"
5. Retrieved abstracts were screened for information relevant for endocrine disruption.
6. Relevant information was summarised and compiled in a tabular format for each substance.

In this annex, information about each of the 10 selected focus substances was compiled, including substance name, CAS number, synonyms, structure, descriptions of data searches, summaries of ED relevant information and summarised information in a tabular format. In the tables with summarised information, "EAS" refers to endpoints relevant for the sex hormone system, and "T" refers to endpoints relevant for the thyroid hormone system. "HH" refers to endpoints considered relevant for human health, and "ENV" refers to endpoints considered relevant for the environment. It should however be noted that cross-talk between "EAS" and "T" modalities may occur, and cross-species extrapolations may be relevant and should be considered when possible, including use of rodent data in evaluation of ED relevant for the environment.

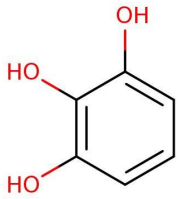
Since only a preliminary screening of the literature was conducted, simple search strings were developed, and it could not be excluded that some relevant studies were not identified. Further, the studies were not systematically evaluated for reliability or quality, and no weight of evidence analysis was applied. When effects in animal studies were identified, they were noted without a thorough evaluation of other signs of systemic or general toxicity, effects on body weights or other observations that might affect the findings.

# 1,2,3-trihydroxybenzene, CAS no. 87-66-1

**Synonyms**

Pyrogallol, 1,2,3-Benzenetriol, Benzene-1,2,3-triol, 1,2,3-Trihydroxybenzol, 1,2,3-trihydroxybenzen, Pyrogallic acid, Fourrine 85, Fouramine Brown AP, CI 76515, C.I. Oxidation Base 32, Benzene, 1,2,3-trihydroxy-

**Structure**




**Searches and dates**

1) ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/25683
CAS no. 87-66-1
Searched 7th of August 2023
Two active registrations: A) 10-100 t/a (25-11-2019), B) Intermediate use only (24-05-2021)

2) Web of Science -All databases - all fields
((“87-66-1” OR “1 , 2 , 3-trihydroxybenzene” OR “Pyrogallol” OR “1,2,3-Benzenetriol” OR “Benzene-1,2,3-triol” OR “1,2,3-Trihydroxybenzol” OR “1,2,3-trihydroxybenzen” OR “Pyrogallic acid” OR “Fourrine 85” OR “Fouramine Brown AP” OR “CI 76515” OR “C.I. Oxidation Base 32” OR “Benzene, 1,2,3-trihydroxy-”) AND endocrine)
Searched 28th of August 2023, 292 results

3) Web of Science - All databases
(87-66-1 OR “1,2,3-trihydroxybenzene” OR “pyrogallol” OR 1,2,3-benzenetriol OR benzene-1,2,3-triol OR 1,2,3-trihydroxybenzol OR 1,2,3-trihydroxybenzen OR “pyrogallic acid” OR “fourrine 85” OR “fouramine brown ap” OR “CI 76515” OR “C.I. oxidation base 32” OR “benzene, 1,2,3-trihydroxy-”) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 29th of August 2023, 13 results

**Overview**

**Summary - *in vitro***
One relevant study was found in the literature screening. The composition/identity of the test substances (extracts) was not clear and it was not clear exactly which effects were induced by

which extracts. It was reported that all extracts affected luteinizing hormone-stimulated testosterone secretion in mouse Leydig cells *in vitro* (Burczyk et al., 1996).

**Summary - human health related**

One relevant study was found in the literature screen. Rats were exposed to two different doses of pyrogallol, and the following effects were reported in the thyroid of the rats in the high dose group: Increased mean epithelial cell height of follicular epithelial cells and decreased follicular diameter. Both were reported to be precursors of goitre (Seffner et al., 1995).

| Summarised information, 1,2,3-trihydroxybenzene, CAS no. 87-66-1 | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro*:** Some information on LH stimulated testosterone secretion *in vitro*. | | | **EAS:** Some info on endocrine activity | |
| **T** | | Some information on effect on the thyroid system: Increased mean epithelial cell height of follicular epithelial cells and decreased follicular diameter in rodents. | | **T:** Some info on adversity | |

**References**

Burczyk et al., 1996: Burczyk, J; Stolarczyk, A; Wojtusiak, A; Bilinska, B, Title: Time- and dose-dependent antigonadotropic activity of oxidation products of gallic acid and pyrogallol on Leydig cells in vitro., Source: Cytobios, Volume: 86, Issue: 344, Pages: 7-16, Document Type: Journal Article; Research Support, Non-U.S. Gov't, Published: 1996

Seffner et al., 1995: Seffner, W; Schiller, F; Heinze, R; Breng, R. Experimental And Toxicologic Pathology, Volume: 47, Issue: 1, Pages: 63-70, DOI: 10.1016/S0940-2993(11)80288-5, Document Type: Article, Published: JAN 1995

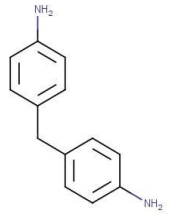
ECHA reg. dossier https://echa.europa.eu/da/registration-dossier/-/registered-dossier/25683

# 4,4'-methylenedianiline, CAS no. 101-77-9

**Synonyms**

MDA, 4,4´-MDA, 4,4'-diaminodiphenylmethane, 4-[(4-aminophenyl)methyl]aniline, Bis (4-aminophenyl)methane, DIAMINODIPHENYLMETHANE, 4,4'- Diaminodiphenylmethane (MDA), 4,4'-DIAMINODIPHENYL-METHANE, 4,4'-Diaminodiphenylmethan, DIPHENYLMETHANE-4, 4'-DIISOCYANATE, 4,4'-methylenedianiline

**Structure**



**Searches and dates**

1) ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15201
101-77-9
Searched 7th of August 2023
Latest update 25-04-2023: 1000-10.000t/a

2) Web of Science - All databases - All fields
(("101-77-9" OR "4,4'-methylenedianiline" OR "4,4´-MDA" OR "4,4'-diaminodiphenylmethane" OR "4-[(4-aminophenyl)methyl]aniline" OR "Bis(4-aminophenyl)methane" OR "DIAMINODIPHENYLMETHANE" OR "4,4'- Diaminodiphenylmethane" OR "4,4'-DIAMINODIPHENYL-METHANE" OR "4,4'-Diaminodiphenylmethane" OR "DIPHENYLMETHANE-4, 4'-DIISOCYANATE") AND endocrine)
Searched 7th of August 2023, 43 results

3) Web of Science - All databases
(101-77-9 OR 4,4'-methylenedianiline OR 4,4´-MDA OR 4,4'-diaminodiphenylmethane OR 4-[(4-aminophenyl)methyl]aniline OR bis(4-aminophenyl)methane OR diaminodiphenylmethane OR 4,4'- diaminodiphenylmethane OR 4,4'-diaminodiphenyl-methane OR 4,4'-diaminodiphenylmethane OR diphenylmethane-4,4'-diisocyanate) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 11th of August 2023, 3 results

# Annex 4 - Preliminary literature screening of 10 selected focus substances

**Overview**

**Summary - *in vitro***

*In vitro*, the substance has been shown to inhibit steroidogenesis in H295R cells (Bhuiyan et al., 2019) and markedly inhibit TPO (Friedman et al., 2016).

**Summary - human health related**

No guideline studies investigating reproductive toxicity were available, but in two repeated dose tox studies from 1982 and 1983, effects on the thyroid, including thyroid follicular cell hyperplasia and development of goiter were reported (REACH reg dossier). The substance is classified for carcinogenicity (1B) and mutagenicity (2), with the target organs being liver and thyroid.

Further, older publications (1960's´1970's) indicated that the substance inhibited steroidogenesis *in vivo* (Egashira et al., 1971, Yamashita et al., 1963, Yamashita et al., 1969).

**Summary - environment related**

4,4'-methylenedianiline was shown to decrease reproduction, disrupt sex hormone balance and alter the mRNA expression levels of key steroidogenic genes in adult zebrafish (Bhuiyan et al., 2019; 2021). Following 21 d exposure to 4,4'-methylenedianiline, significantly decreased reproduction was observed. Moreover, exposure to 4,4'-methylenedianiline significantly decreased plasma concentrations of testosterone (T) and 17β-estradiol (E2) in both male and female fish leading to a significantly increased E2/T ratio in males and a significantly decreased E2/T ratio in females. Significant down-regulations of mRNA expression levels of genes along the hypothalamic-pituitary-gonadal axis following exposure to 4,4'-methylenedianiline were observed, which could explain the decreases in sex hormone concentrations. Moreover, significantly down-regulated prostaglandin-endoperoxide synthase 2 (ptgs2) gene expression was observed, suggesting potential disruption of oocyte maturation and ovulation by the exposure.

## Annex 4 - Preliminary literature screening of 10 selected focus substances

| Summarised information, 4,4'-methylenedianiline, CAS no. 101-77-9 | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro*:** Some information on inhibition of steroidogenesis<br>***In vivo* mammalian:** Some information on inhibition of steroidogenesis in dogs and rabbits.<br>***In vivo* non-mammalian:** Some information on inhibition of steroidogenesis in fish. | | Some information on decreased reproduction in fish. | **EAS:** Some info on endocrine activity | **EAS:** Some info on endocrine activity and adversity |
| **T** | ***In vitro*:** Some information on inhibition of TPO.<br>***In vivo* mammalian:** Some information on adverse effects, e.g. thyroid follicular cell hyperplasia, development of goiter and thyroid tumours | **Some information** on effect on the thyroid hormone system: Thyroid follicular cell hyperplasia, development of goiter and thyroid tumours in rodents. | | **T:** Some info on endocrine activity and adversity | **T:** Some info on endocrine activity |

**References**

Bhuiyan et al. 2019:Bhuiyan, MNH; Kang, H; Kim, JH; Kim, S; Kho, Y; Choi, K. 2019. Endocrine disruption by several aniline derivatives and related mechanisms in a human adrenal H295R cell line and adult male zebrafish. Ecotoxicology And Environmental Safety, Volume: 180, Pages: 326-332, DOI: 10.1016/j.ecoenv.2019.05.003

Bhuiyan et al. 2021: Bhuiyan, MNH; Kang, H; Choi, J; Lim, S; Kho, Y; Choi, K. 2021. Effects of 3,4-dichloroaniline (3,4-DCA) and 4,4 '-methylenedianiline (4,4 '-MDA) on sex hormone regulation and reproduction of adult zebrafish (Danio rerio).CHEMOSPHERE, Volume: 269, Article Number: 128768, DOI: 10.1016/j.chemosphere.2020.128768

ECHA registration dossier: https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15201

Egashira et al. 1971: Egashira K. 1971. Some Aspects of Androgen Secretion by the Canine Adrenal. Tohoku J. exp. Med., 1971, 105 (1), 35-43

Friedman et al. 2016: Friedman KP, Watt ED, Hornung MW, Hedge JM, Judson RS, Crofton KM, Houck KA, Simmons SO. 2016. Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries. Toxicol Sci. 2016 May;151(1):160-80. doi: 10.1093/toxsci/kfw034. Epub 2016 Feb 15. PMID: 26884060; PMCID: PMC4914804.

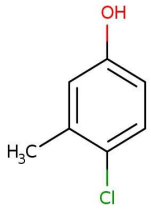
Yamashita et al. 1963: Yamashita K .1963.Adrenal-Dependent Effect Of 4,4-Methylenedianiline On Endometrial Carbonic Anhydrase, Source: AMERICAN JOURNAL OF PHYSIOLOGY, Volume: 205, Issue: 1, Pages: 195-& DOI: 10.1152/ajplegacy.1963.205.1.195, Document Type: Article, Published: 1963

Yamashita et al. 1969: Yamashita, K; Nakasone, K; Kawao, K. 1969. Inhibitory Effect Of Methylenedianiline Of Testicular 17-Oxosteroid Secretion Produced By Pregnant Mare Serum, Source: Tohoku Journal Of Experimental Medicine, Volume: 97, Issue: 2, Pages: 175-180, DOI: 10.1620/tjem.97.175

# Chlorocresol, CAS no. 59-50-7

**Synonyms**
4-Chloro-m-cresol, 4-chloro-3-methylphenol, 4-chlor-3-methylphenol

**Structure**




**Searches and dates**
1) ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/10359
59-50-7
Searched 3rd of August 2023
latest update 29th September 2021: 10-100 t/a

2) Web of Science -All databases - all fields
("59-50-7" or "chlorocresol" or "4-chloro-3-methylphenol" or "4-chloro-m-cresol" or "4-Chlor-3-methylphenol") and endocrine
Searched 3rd of August 2023, 34 results

3) Web of Science - All databases
(59-50-7 OR chlorocresol OR 4-chloro-3-methylphenol OR 4-chloro-m-cresol OR 4-chlor-3-methylphenol) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 4th of August 2023, 3 results

**Overview**

**Summary - *in vitro***
*In vitro*, chlorocresol was found to be able to bind to and activate the estrogen receptor in some studies (Han et al., 2004; Nakama et al., 2007; Onishi et al., 2020), but not in others (Fang et al., 2001; Ghisari et al., 2009). Differences in results may be due to differences in designs of the studies including cell lines. Two studies found that the substance interfered with the AR (Kruger et al., 2008; Onishi et al., 2020). One study found that the substance was positive in the T-screen assay (Ghisari et al., 2009), and one study found that the substance interfered with the AhR (Kruger et al., 2008).

## Annex 4 - Preliminary literature screening of 10 selected focus substances

**Summary - human health related**

No *in vivo* studies in rodents were identified in the literature screen. In the REACH Registration dossier, a 2-generation reproductive toxicity study was reported, in which some results pointed towards endocrine disruptive effects of the substance. Estrous cyclicity was affected in both P0 and P1 and the weight of a number of reproductive organs sensitive to endocrine disruptors were affected across generations. Further, some effects on sexual maturation in the offspring were observed and in the P1 generation, there was a decrease in the number of growing follicles and corpora lutea. There were also some sporadic effects observed on thyroid and pituitary weights in some generations and sexes.

**Summary - environment related**

Chlorocresol was used in the validation of the MEOGRT as a weak estrogenic substance (Flynn et al., 2017; US EPA, 2013). However, no effects indicative of endocrine disruption was consistently observed across generations and life stages after exposure to up to 345 µg/L chlorocresol, except for a small increase (~2x of controls) in vitellogenin gene expression in adult fish in F0 only starting at 88 µg/L of chlorocresol. Reproduction was not decreased in any of the generations and no indication of toxicity was observed via histopathology. Chlorocresol was also evaluated by the 21-day fish short-term reproduction assay, OECD TG 229, using Japanese medaka (Onishi et al., 2020). No significant effects on fecundity, fertility or secondary sex characteristics were observed in the fish exposed to up to 1060 µg/L chlorocresol. However, the hepatic vitellogenin concentrations were significantly increased by exposure to chlorocresol, with a LOEC for vitellogenin induction of 1060 µg/L. For male vitellogenin, the LOEC (in molarity) was 110,000 times higher than that of E2.

In a paper in Chinese with English abstract, chlorocresol was reported to mediate endocrine disrupting effects and affect the early growth and development of zebrafish through the ER, AhR and PXR (pregnane X receptor) (Song et al., 2020). q-RT-PCR analysis in zebrafish larvae at 120 hpf showed that *erl* was significantly down-regulated at 0.5 and 2.5 mg/L, *ahr2* was down-regulated, and *pxr* was up-regulated at 0.1 and 2.5 mg/L chlorocresol. Furthermore, the results indicated that chlorocresol concentrations of 5 mg/L and 10 mg/L resulted in adverse effects on spinal curvature and edema in zebrafish embryos and larvae. However, these developmental endpoints are not specifically indicative of endocrine mediated mechanisms of toxicity but may also be affected through other mechanisms.

| Summarised information, Chlorocresol, CAS no. 59-50-7 | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro*:** Some information on ER agonism, AR antagonism, AhR antagonism<br><br>***In vivo* mammalian:** Some information implicit in adverse effects, e.g. disturbance of estrous cyclicity.<br>***In vivo* non-mammalian:** Some information on down-regulation of *er1* and *ahr2* expression; and induction of vitellogenin in fish. | **Some information** on effects on estrous cyclicity, sexual maturation, reproductive organ weights, ovarian follicles in 2-gen study in rodents (OECD TG 416). | | **EAS:** Some info on endocrine activity and adversity | **EAS:** Some info on endocrine activity |
| **T** | ***In vitro*:** Some information on TH dependent cell proliferation (T-screen). | | | **T:** Some info on endocrine activity | **T:** Some info on endocrine activity |

**References**

Fang et al. 2001: Fang H, Tong W, Shi LM, Blair R, Perkins R, Branham W, Hass BS, Xie Q, Dial SL, Moland CL, Sheehan DM. 2001. Structure-Activity Relationships for a Large Diverse Set of Natural, Synthetic, and Environmental Estrogens, Chem. Res. Toxicol. 2001, 14, 280-294

Flynn et al. 2017: Flynn, K., Lothenbach, D., Whiteman, F., Hammermeister, D., Touart, L.W., Swintek, J., Tatarazako, N., Onishi, Y., Iguchi, T. and Johnson, R. (2017). Summary of the development of the US Environmental Protection Agency's Medaka Extended One Generation Reproduction Test (MEOGRT) using data from 9 multigenerational medaka tests. Environmental Toxicology and Chemistry 36(12), 3387-3403. doi:10.1002/etc.3923.

Ghisari et al. 2009: Ghisari, M; Bonefeld-Jorgensen, EC. 2009. Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions, Source: Toxicology Letters, Volume: 189, Issue: 1, Pages: 67-77, DOI: 10.1016/j.toxlet.2009.05.004

Han Sang-Kuk, 2004: Han, Sang-Kuk, 2004. Study on Estrogenic Activities of Pesticide Chemicals using E-screen Assay, Source: Journal Of Environmental Science International,

Source: The Korean Environmental Sciences Society, vol. 13, no. 6: 591-597, Document Type: research-article, Published: 2004 https://doi.org/10.5322/jes.2004.13.6.591

Kruger et al. 2008: Kruger, T; Long, M; Bonefeld-Jorgensen, EC, 2008. Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor, Source: TOXICOLOGY, Volume: 246, issue: 2-3, Pages: 112-123, DOI: 10.1016/j.tox.2007.12.028.

Nakama et al. 2007: Nakama, A; Funasaka, K; Shimizu, M . 2007.Evaluation of estrogenic activity of organic biocides using ER-binding and YES assay, Source: FOOD AND CHEMICAL TOXICOLOGY, Volume: 45, Issue: 9, Pages: 1558-1564, DOI: 10.1016/j.fct.2007.02.014

Onishi et al. 2021: Onishi, Y., Tatarazako, N., Koshio, M., Okamura, T., Watanabe, H., Sawai, A., Yamamoto, J., Ishikawa, H., Sato, T., Kawashima, Y., Yamazaki, K. and Iguchi, T. (2021). Summary of reference chemicals evaluated by the fish short-term reproduction assay, OECD TG229, using Japanese medaka, Oryzias latipes. Journal of Applied Toxicology 41(8), 1200-1221. doi:10.1002/jat.4104.

REACH registration dossier: Registered substances - ECHA (europa.eu) https://echa.europa.eu/da/registration-dossier/-/registered-dossier/10359

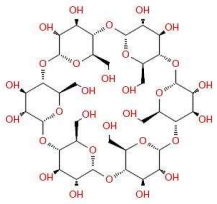
Song et al. 2020: Song, L.-w., Jin, Y.-r. and Liu, H.-l. (2020). Research on the endocrine disruption effect of typical phenolic pollutants: The embryonic development effects and molecule effects of gene regulation mediated by nuclear receptor on zebrafish. China Environmental Science 40(9), 4065-4076.

US EPA. 2013: US EPA. (2013). Validation of the Medaka multigeneration test: Integrated summary report. Washington, DC: Environmental Protection Agency.

# Cyclohexapentylose, CAS no. 10016-20-3

**Synonyms**
Cyclohexapentylose" or "α-cyclodextrin" or "α-Schardinger dextrin" or "cyclomaltohexaose" or "alpha-cyclodextrin" or "cyclohexaamylose" or "alpha-cyclodextrin hydrate"

**Structure**



**Searches and dates**
1) ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/10199
10016-20-3
Searched 3rd of August 2023
latest update 18th July 2017: 10-100 t/a

2) Web of Science
("10016-20-3" or "cyclohexapentylose" or "α-cyclodextrin" or "α-Schardinger dextrin" or "cyclomaltohexaose" or "alpha-cyclodextrin" or "cyclohexaamylose" or "alpha-cyclodextrin hydrate") and endocrine
Searched 3rd of August 2023, 124 results

3) Web of Science - All databases
(10016-20-3 OR cyclohexapentylose OR α-cyclodextrin OR "α-schardinger dextrin" OR cyclomaltohexaose OR alpha-cyclodextrin OR cyclohexaamylose OR "alpha-cyclodextrin hydrate") AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 8th of August 2023, 3 results

**Overview**

**Summary - *in vitro***
*In vitro* and in silico studies show that cyclodextrin forms a cavity in which molecules like steroids and cholesterol can bind (Araj et al., 2023, Marzona et al., 1992, Schwartz et al., 2017, Sadler et al., 2004).

**Summary - human health related**
*In vivo* studies investigate the ability of cyclodextrin to e.g. bind radioactive iodine for slower release to the thyroid and as co-administration with HcG (for medical purposes)(Nishi et al., 2023). Such applications and uses are further described in a review, which also lists the

following uses of cyclodextrins (CDs): "CDs have been used as oestrogen solubilizers and absorption boosters in pharmaceutical formulations, as well as in chromatographic and electrophoretic procedures for their separation and quantification. Other applications include the removal of the endocrine disruptors from environmental materials, the preparation of the samples for mass spectrometric analysis, or solid-phase extractions based on complex formation with CDs" (Araj et al., 2023). No adverse effects are reported in a standard embryotox/teratogenicity study (Waalkens-Berendsen, 2004) or in a 4-week range finding study and a 13-week oral toxicity study in rats (Lina et al., 2004).

No other repro/ED studies on alpha-cyclodextrin are available. In the REACH reg dossier, read across to beta-cyclodextrin is applied, for which two older TG416 are available (1982 and 1992). No alerts for ED are observed.

In conclusion, the substance has the ability to bind molecules, including steroid hormones, and this ability is utilised in medicine, environmental monitoring etc. How this ability affects molecules in the body, including endogenous hormones, is not thoroughly investigated.

**Summary - environment related**

No information

| **Summarised information, Cyclohexapentylose, CAS no. 10016-20-3** | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro*:** Some information on binding of molecules, including steroid hormones and cholesterol.<br>***In vivo* mammalian:** Some information on binding of molecules, including steroid hormones and cholesterol. | | | **EAS:** Some info on endocrine activity | **EAS:** Some info on endocrine activity |

**References**

Araj et al. 2023: Araj, SK; Szeleszczuk, L. 2023. A Review On Cyclodextrins/Estrogens Inclusion Complexes, Source: International Journal Of Molecular Sciences, Volume: 24, Issue: 10, Article Number: 8780, DOI: 10.3390/Ijms24108780

Lina & Bär 2004: Lina, BAR; Bar, A. 2004. Subchronic Oral Toxicity Studies With Alpha-Cyclodextrin In Rats, Source: Regulatory Toxicology And Pharmacology, Volume: 39, Pages: S14-S26, DOI: 10.1016/J.Yrtph.2004.05.006, Supplement: 1

Marzona et al. 1992: Marzona, M; Carpignano, R; Quagliotto, P. 1992. Quantitative Structure-Stability Relationships In The Inclusion Complexes Of Steroids With Cyclodextrins. Annali Di Chimica, Volume: 82, Issue: 9-10, Pages: 517-537

Nishi ET AL. 2023: Nishi, K; Hirota, M; Higaki, S; Shiraishi, S; Kudo, T; Matsuda, N; Ito, S. 2023. Reduction of thyroid radioactive iodine exposure by oral administration of cyclic oligosaccharides, Source: Scientific Reports, Volume: 13, Issue: 1, DOI: 10.1038/s41598-023-34254-0

REACH registration dossier: Registered substances - ECHA (europa.eu) https://echa.europa.eu/da/registration-dossier/-/registered-dossier/10199

Sadler et al. 2004: Sadler, SE; Jacobs, ND. 2004.Stimulation of Xenopus laevis oocyte maturation by methyl-beta-cyclodextrin, Source: BIOLOGY OF REPRODUCTION, Volume: 70, Issue: 6, Pages: 1685-1692, DOI: 10.1095/biolreprod.103.026161,

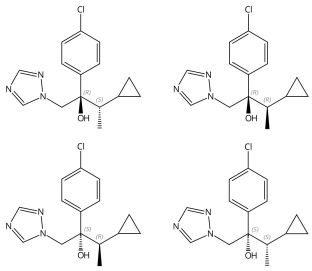
Schwarz et al. 2017: Schwarz, DH; Engelke, A; Wenz, G. 2017.Solubilizing steroidal drugs by beta-cyclodextrin derivatives, Source: International Journal Of Pharmaceutics, Volume: 531, Issue: 2, Special Issue: SI, Pages: 559-567, DOI: 10.1016/j.ijpharm.2017.07.046

Waalkens-Berendsen et al. 2004: Waalkens-Berendsen DH; Smits-van Prooije, AE; Bar, A.2004. Embryotoxicity and teratogenicity study with alpha-cyclodextrin in rabbits, Source: Regulatory Toxicology And Pharmacology, Volume: 39, Pages: S40-S46, DOI: 10.1016/j.yrtph.2004.05.003, Supplement: 1.

# Cyproconazole, CAS no. 94361-06-5

**Synonyms**
2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol,
(3R, 2S / 3S, 2R)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1,2,4-triazol-1-yl)-butan-2-ol,
(3R, 2R / 3S, 2S)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1,2,4-triazol-1-yl)-butan-2-ol

**Structure**



**Searches and dates**
1) ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/32337
94361-06-5
Searched 1st of August 2023
latest update 25th April 2023: 0-10 t/a

2) Web of Science - All databases - All fields
(("94361-06-5" OR "cyproconazole" OR "2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol" OR "4-Allylguaiacol" OR "(3R, 2S / 3S, 2R)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1,2,4-triazol-1-yl)-butan-2-ol" OR "(3R, 2R / 3S, 2S)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1,2,4-triazol-1-yl)-butan-2-ol") AND endocrine)
Searched 1st of August, 26 results

3) Web of Science - All databases
(94361-06-5 OR cyproconazole OR 2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol OR 4-allylguaiacol OR "(3R, 2S / 3S, 2R)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1,2,4-triazol-1-yl)-butan-2-ol" OR "(3R, 2R / 3S, 2S)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1,2,4-triazol-1-yl)-butan-2-ol") AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 3rd of August, 4 results.

**Overview**

**Summary - *in vitro***

The identified *in vitro* studies suggested that cyproconazole may interfere with sex hormone signalling. In one study, the substance was found to act as an agonist on estrogen receptor alpha and to bind to the estrogen receptor in a molecular docking model, as well as to induce mRNA expression of a number of enzymes in the steroidogenesis pathway (CYP11a1, 3BHSD, CYP17, CYP19, SCP2) in HepG2 cells (Wang et al., 2022). Another study did not find that cyproconazole affected expression of genes in the steroid synthesis pathway. In that study, it was however shown that cyproconazole reduced testosterone production in MA-10 cells (a murine Leydig tumour cell line) and inhibited testosterone-induced androgen receptor activation in T47D-ARE cells (Roelofs et al., 2014). Further, decreased progesterone production in Jeg-3 cells (a human choriocarcinoma cell line) was observed in one study (Rieke et al., 2014).

**Summary - human health related**

One newer study in the open literature investigated effects which could be related to endocrine disruption (Rieke et al., 2017). In this study, both cyproconazole, epoxiconazole, prochloraz and mixtures of those substances were investigated with focus on adrenal toxicity. Different effect profiles were observed between the different studies. No adverse effects were observed on the adrenals after cyproconazole exposure. However, the study used a group size of only 5 animals in the cyproconazole groups, which may have been too small to detect effects induced by the exposure, and the study can therefore not be viewed as reliable.

Cyproconazole is classified for reproductive toxicity (REP 1B) based on developmental toxicity. In the REACH registration dossier and the RAC CLH report, it was specified that gestation is prolonged. This is an effect, which is also observed for several other azole fungicides - often in combination with increased foetal and postnatal pup mortality. The prolonged gestation was statistically significant in the second generation, but not the first generation of the 2-generation reproductive toxicity study.

Increased incidences of thyroid follicular hypertrophy were observed after exposure to cyproconazole in two repeated dose toxicity studies: A 90-days study and a 28-days study in rats from 1999 and 2000, respectively. In contrast to these findings, no effects on the thyroid were observed in 9 older reproductive/developmental/repeated dose toxicity studies in rats/mice/dogs conducted between 1981-1992.

Repeated dose toxicity studies in rats and dogs also showed changes in weight and histopathology of ovaries, adrenals, and pituitaries, further indicating that adverse endocrine effects may occur after cyproconazole exposure.

**Summary - environment related**

One study examining endocrine disruption related effects of cyproconazole exposure in amphibians was identified. This study investigated the effects of cyproconazole (1 and 10 mg/L) on *Rana nigromaculata* during a chronic 90 days exposure period and showed that cyproconazole affected both the HPT and HPG axes (Zhang et al., 2019). Tadpoles exposed to 10 mg/L did not survive beyond 42 days. Although tadpoles exposed to 1 mg/L cyproconazole and controls had no significant difference in survival, snout-vent length, weight, developmental stage or behavioural endpoints at 90 days, thyroid tissue was significantly altered histologically showing follicular cell degeneration and vacuolated colloid in the exposed animals. Moreover, T3 and T4, and thyroid hormone system related gene transcript levels were significantly affected in a stage-dependent manner. At 14 days, following exposure to 1 mg/L cyproconazole, T3 was significantly reduced, while T4 was not significantly affected, when compared to controls. At 28 days, following exposure to 1 mg/L cyproconazole, the concentration of T3 was not significantly affected, while T4 was significantly increased, when compared to controls. Exposure to 1 mg/L cyproconazole significantly increased the concentration of T3 as compared to controls, while T4 was significantly reduced compared to controls at 42- and 90-days exposure. The relative expression of thyroid hormone system related genes in brain tissue was also affected by exposure to 1 mg/L cyproconazole. The expression of *trh* and *tshβ*, was upregulated following cyproconazole exposure at 14 days. However, no significant differences were observed at the later stages. Expression levels of *trα* were down-regulated in the treated animals at 28 days, followed by stabilisation with no significant difference to controls after 28 days. The expression levels of *dio2* and *dio3* following 1 mg/L cyproconazole exposure showed no significant difference to control levels.

The levels of estradiol were significantly increased in males exposed to 1 mg/L cyproconazole, while the estradiol levels were not significantly affected in females. The concentrations of testosterone in male and female tadpoles exposed to 1 mg/L cyproconazole showed no significant difference from controls. For both males and females, the relative expression of gonad hormone related genes in the gonads was affected by exposure to 1 mg/L cyproconazole. For *er* expression levels, both female and male genes were significantly upregulated with exposure to 1 mg/L cyproconazole, with upregulation occurring in relation to significantly increased expression of the aromatase gene in males. Furthermore, a significant down-regulation of *ar* expression was observed in females treated with 1 mg/L cyproconazole.

Overall, the authors concluded that the thyroid and gonad development of tadpoles, at both the gene and hormone levels, were more sensitive to cyproconazole exposure than phenotypic biological indicators such as body weight, snout-vent length, age at metamorphosis or abnormal behavioural endpoints.

As mentioned in the abstract of the recent review of endocrine disruption by azole fungicides in fish, data on endocrine disrupting effects of cyproconazole in fish are wanted for, and cyproconazole is not further mentioned in the review (Huang et al., 2022).

| Summarised information, Cyproconazole, CAS no. 94361-06-5 | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro*:** Some information on interference with sex hormone signalling<br>***In vivo* mammalian:** Some information implicit in adverse effects, e.g. histopathological alterations in hormone-regulated tissues like pituitary, adrenals, and ovaries<br>***In vivo* non-mammalian:** Some information on decreased *ar* expression levels and increased *er* expression and estradiol levels in amphibians. | Some information on prolonged gestation in second generation in a 2-gen. study (OECD TG 416) and histopathological changes in ovaries, adrenals, and pituitaries in repeated dose toxicity studies in rats and dogs. | | **EAS:** Some info on endocrine activity and adversity | **EAS:** Some info on endocrine activity |
| **T** | ***In vivo* mammalian:** Some information implicit in adverse effects, e.g. altered histopathology of the thyroid gland.<br>***In vivo* non-mammalian:** Some information on affected T3, T4 and thyroid hormone system related gene transcript levels in amphibians. | Some information on increased incidences of thyroid follicular hypertrophy in repeated dose toxicity studies in rodents. | Some information on altered thyroid histology in amphibians. | **T:** Some info on adversity | **T:** Some info on endocrine activity and adversity |

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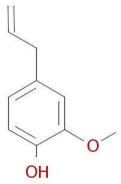
# Eugenol, CAS no. 97-53-0

**Synonyms:** 4-Allyl-2-methoxyphenol, 2-methoxy-4-(prop-2-en-1-yl)phenol, clove bud oil, clover leaf oil, rectified clover leaf oil

**Structure**




**Searches and dates**

1) ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13694
Searched 1st of August 2023
latest update ,13th October 2022: 1000-10.000 t/a

2) Web of Science - All databases - All fields
(("97-53-0" OR "eugenol" OR "2-Methoxy-4-phenol" OR "4-Allylguaiacol" OR "4-Allyl-2-methoxyphenol") AND endocrine)
Searched 31st of July 2023, 362 results

3) Web of Science - All databases - All fields
(("97-54-1" OR "isoeugenol" OR "4-Hydroxy-3-methoxy-1-propen-1-yl benzene") AND endocrine).
Searched 1st of August 2023, 45 results

4) Web of Science - All databases
(97-53-0 OR eugenol OR 2-methoxy-4-phenol OR 4-allylguaiacol OR 4-allyl-2-methoxyphenol) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 1st of August 2023, 21 results

5) Web Of Science - All databases
(97-54-1 OR isoeugenol OR 4-Hydroxy-3-methoxy-1-propen-1-yl benzene) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc*) AND endocrine
Searched 2nd of August 2023, 3 results

**Overview**

**Summary - *in vitro***

Some *in vitro* studies have shown that eugenol and its analogue isoeugenol may antagonize the androgen receptor *in vitro* (Ogawa et al., 2010, Park et al., 2021). One study did not find anti-androgenic effects of eugenol in a yeast screen (Howes et al., 2002). Several studies found that eugenol did not affect the estrogen receptor *in vitro* (Howes et al., 2002, Garrison et al., 2021, REACH registration dossier). No investigations of effects on steroidogenesis or the thyroid system *in vitro* were identified in the literature search. The REACH registration dossier reports that the Derek Nexus (Q)SAR system predicted no thyroid toxicity for eugenol, whereas the Danish (Q)SAR database predicts that both eugenol and isoeugenol are positive for TPO inhibition and part of the experimental data set where they test positive (DK (Q)SAR database). Regarding non-EATS-modalities, eugenol seems to act *in vitro* as an AhR antagonist (Bartonkova et al., 2018) and a prostaglandin inhibitor (PONGPRAYOON et al., 1991), but not as a PPAR gamma agonist (Fakhrudin et al., 2010).

**Summary - human health related**

*In vivo*, one study investigating effects on adult male reproduction was identified. Eugenol exposure for 60 days lowered testosterone levels and affected sperm motility and morphology in adult male rats (Carvalho et al., 2022). The study did however have some weaknesses, including low group size and unclear reporting of doses), which lowered its reliability. Other *in vivo* studies found compensatory effects of eugenol treatment, suppressing disrupting effects on reproduction induced by diabetes (Kokabiyan et al., 2023, Yilmaz-Oral, 2020), PCOS (Kokabiyan et al., 2022) and chlorpyrifos (Nikbin et al., 2020), respectively.
In the REACH registration dossier, read across to isoeugenol is conducted to fill the data gap for reproductive toxicity.
In a two-generation reproductive toxicity study (OECD TG 416) on isoeugenol from 2003, some sporadic effects indicative of EAS-mediated effects was observed: decrease in absolute female anogenital distance (F1 pups); increase in female anogenital distance relative to body weight, increase in relative weights of right epididymis and right testis in the high dose group; decrease in ovary weights in mid-dose group. From the study summary, sperm parameters did not seem to be affected.

**Summary - environment related**

Eugenol is used as an anaesthetic in fish. One study examining the effects of eugenol on thyroid hormone levels in fish was identified. In sexually immature rainbow trouts, eugenol exposure for approximately 2 min caused no significant effects on plasma T3 or T4 levels when compared to fish exposed to MS-222 or stunned fish (Holloway et al., 2004). In a second experiment in the same study, plasma T3 and T4 levels in the 10-min eugenol exposure group were significantly higher than the plasma T3 and T4 levels in the 2-min control group; whereas cortisol levels were significantly decreased in the 10-min eugenol exposure group when compared to the 2-min control group. However, a 10-min control group was lacking, and no differences in plasma T3, T4 and cortisol levels were observed in the 2-min eugenol exposure group when compared to the 2-min control group. Thus, it is not known whether the observed

significant effects were exposure or time related. Five other studies investigating effects of eugenol or isoeugenol on cortisol levels in fish were identified (Iversen et al., 2003; Zahl et al., 2010; Renault et al., 2011; Fraser et al., 2014; Zahran et al., 2021). These studies showed varied responses in cortisol levels in fish following exposure to eugenol or isoeugenol. No significant effects were observed on growth hormone in eugenol treated rainbow trouts (Holloway et al., 2004).

The acaricidal effect of eugenol was measured and its mechanism of action investigated in the mite *Psoroptes cuniculi* (Ma et al., 2019). Transcriptome analysis showed that the main up-regulated pathway in the eugenol treated mites was the PPAR signalling pathway, while the main down-regulated pathways were associated with NF-kappa B, TNF, Rap 1, and Ras signalling.

| **Summarised information, Eugenol, CAS no. 97-53-0** | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro*:** Some information on anti-androgenicity, AhR agonistic activity and prostaglandin inhibition. ***In vivo* mammalian:** Some information on decreased testosterone in adult rats *in vivo*. ***In vivo* non-mammalian:** Some information on up-regulated PPAR signalling pathway in mites. | Some information on decreased sperm motility and changed sperm morphology in rodents. Some information from read-across to isoeugenol; affected female anogenital distance, increases in relative weights of epididymis testis and decreases in ovary weights in a 2-gen study in rodents. | | **EAS:** Some info on endocrine activity and adversity | |

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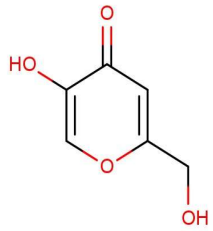
# Kojic acid, CAS no. 501-30-4

**Synonyms**

5-hydroxy-2-hydroxymethyl-4-pyrone, 5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one

**Structure**




**Search string and date**

1) ECHA registration dossier
Registered substances - ECHA (europa.eu)
501-30-4
Searched 28th of August 2023: Not registered.

2) Web of Science - All databases - All fields
(("501-30-4" OR "kojic acid" OR "5-hydroxy-2-hydroxymethyl-4-pyrone" OR "5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one") AND endocrine)
Searched 28th of August 2023, 69 results

3) Web of Science - All databases
(501-30-4 OR "kojic acid" OR 5-hydroxy-2-hydroxymethyl-4-pyrone OR "5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one") AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 29th of August 2023, 1 result

**Overview**

**Summary - *in vitro***

No information

**Summary - human health related**

Three relevant *in vivo* studies in rodents were identified in the open literature. In a 28- day study in rats exposure to 5 different doses of kojic acid showed very clear and dose-dependent decreases in serum T3 and T4, increases in TSH concentrations and adverse effects on the thyroid gland weight and histopathology. The mechanism behind these effects was investigated and the study showed that uptake of radiolabelled iodine by the gland was affected (Tamura et al. 1999). Another 28-day study in male rats found similar effects (low TH, increased thyroid gland weight and hypertrophy of the epithelial cells of the thyroid gland follicles) but no

significant effects on TSH (Higa et al., 1999). In contrast to the Taura study, effects were only seen in the highest dose group. The third study (Mitsumori et al., 1999) investigated the effects of a 13-week exposure period in rats. This study also showed decreased T3 and T4 concentration, increased TSH, increased thyroid gland weights and adverse thyroid histopathology. No effect on liver enzyme induction were seen in this study, indicating that other mechanisms were responsible for the observed thyroid-related effects (Mitsumori et al., 1999).

Overall, kojic acid is consistently seen to cause adverse effects on thyroid function in rats. The underlying mechanism is either altered iodine organification or iodine uptake by the thyroid (Tamura et al., 1999; Higa et al., 2002) and not liver enzyme induction (Mitsumori et al., 1999). A similar conclusion was reached by the SCCS, in their most recent evaluation of kojic acid (SCCS 2022)

**Summary - environment related**

No information

| Summarised information, Kojic acid, CAS no. 501-30-4 | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **T** | ***In vivo* mammalian:** Some information on decreased uptake of iodine in thyroid, decreased organic formation of iodine in the thyroid, decreased serum T3 and T4 and increased TSH in rodents. | Some information on increased thyroid weights and follicular cell hypertrophy and decreased colloid in thyroid follicles in rodents. | | **T:** Some info on endocrine activity and adversity | |

**References**

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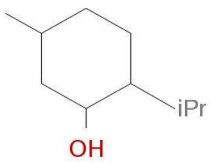
# Menthol, CAS no. 89-78-1 (and 15356-70-4)
# AND
# (+/-) Menthol, CAS no. 15356-60-2

**Synonyms**

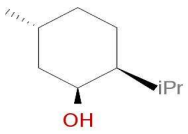
Menthol:
(1R,2S,5R)-2-isopropyl-5-methylcyclohexanol, (1R,2S,5R)-5-methyl-2-(methylethyl) cyclohexanol, (1R,2S,5R)-5-methyl-2-propan-2-ylcyclohexan-1-ol, 2-isopropyl-5-methylcyclohexanol, 5-methyl-2-(propan-2-yl)cyclohexan-1-ol, 5-methyl-2-propan-2-ylcyclohexan-1-ol, Cyclohexanol, 5-methyl-2-(1-methylethyl)-, L-menthol, methyl-2-(propan-2-yl) cyclohexan-1-ol, Menthol racemic, Racementhol, 15356-70-4

(+/-) Menthol:
(+)-Menthol, (1S,2R,5S)-5-methyl-2-propan-2-ylcyclohexan-1-ol, 2-isopropyl-5-methylcyclohexanol, 5-methyl-2-propan-2-ylcyclohexan-1-ol, Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1S,2R,5S)-, Menthol D dist.

**Structure**




**Menthol**




**(+/-) Menthol**

**Searches and dates**

1) Menthol: ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13758
89-78-1
Searched 23rd of August
latest update 23-09-2022
10.000-100.000 t/a

2) (+/-) Menthol: ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/12128
15356-60-2
Searched 23rd of August 2023
1-10 t/a
Updated 13-10-2010

3) Menthol:Web of Science- All databases - All fields
(("89-78-1" OR "15356-70-4" OR "menthol" OR "(1R,2S,5R)-2-isopropyl-5-methylcyclohexanol" OR "(1R,2S,5R)-5-methyl-2-(methylethyl) cyclohexanol" OR "(1R,2S,5R)-5-methyl-2-propan-2-ylcyclohexan-1-ol" OR "2-isopropyl-5-methylcyclohexanol" OR "5-methyl-2-(propan-2-yl)cyclohexan-1-ol" OR "5-methyl-2-propan-2-ylcyclohexan-1-ol" OR "Cyclohexanol, 5-methyl-2-(1-methylethyl)-" OR "L-menthol" OR "methyl-2-(propan-2-yl) cyclohexan-1-ol" OR "Menthol racemic" OR "Racementhol") AND endocrine)
https://www.webofscience.com/wos/alldb/summary/6afcc43f-0bba-4227-b52c-81027c48bacc-9f4ef8a5/date-descending/1
Searched 23rd of August 2023, 233 results

4) (+/-) Menthol:Web of Science- All databases - All fields
(("15356-60-2" OR "(+)-Menthol" OR "(1S,2R,5S)-5-methyl-2-propan-2-ylcyclohexan-1-ol" OR "2-isopropyl-5-methylcyclohexanol" OR "5-methyl-2-propan-2-ylcyclohexan-1-ol" OR "Cyclohexanol, 5-methyl-2-(1-methylethyl)-" OR "(1S,2R,5S)-" OR "Menthol D dist.") AND endocrine)
Searched 24th of August 2023, 234 results (overlapping with search on menthol)

5) Web of Science - All databases
(89-78-1 OR 15356-70-4 OR menthol OR (1R,2S,5R)-2-isopropyl-5-methylcyclohexanol OR "(1R,2S,5R)-5-methyl-2-(methylethyl) cyclohexanol" OR (1R,2S,5R)-5-methyl-2-propan-2-ylcyclohexan-1-ol OR 2-isopropyl-5-methylcyclohexanol OR 5-methyl-2-(propan-2-yl)cyclohexan-1-ol OR 5-methyl-2-propan-2-ylcyclohexan-1-ol OR "cyclohexanol, 5-methyl-2-(1-methylethyl)-" OR L-menthol OR "methyl-2-(propan-2-yl) cyclohexan-1-ol" OR "Menthol racemic" OR Racementhol) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc*) AND endocrine
Searched 23rd of August 2023, 7 results

6) Web of Science - All databases
(15356-60-2 OR "(+)-menthol" OR (1S,2R,5S)-5-methyl-2-propan-2-ylcyclohexan-1-ol OR 2-isopropyl-5-methylcyclohexanol OR 5-methyl-2-propan-2-ylcyclohexan-1-ol OR "cyclohexanol, 5-methyl-2-(1-methylethyl)-" OR (1S,2R,5S)- OR "menthol D dist.") AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc*) AND endocrine
Searched 24th of August 2023 *(corrected 21/9 2023),* 7 results

# Annex 4 - Preliminary literature screening of 10 selected focus substances

## Overview

### Summary - *in vitro*

One study showed that menthol can antagonise human ER and AR (Michalikova et al., 2019). Other studies have indicated that menthol may be able to interact with estrogens, though the mechanisms are unknown and not "classic EAS"-types (and therefore more difficult to interpret and use in a regulatory setting): Menthol was shown to be an agonist of *transient receptor potential melastatin type* 8 (TRPM8), which is a peripheral receptor expressed in multiple genito-urinary organs in the rat and human (Stein et al., 2004). Endogenous estrogen, E-2 suppresses menthol-induced elevation of body temperature, but the mechanism is unknown (Uchida et al., 2021).
Menthol is used in hydrophobic solvents to extract natural and synthetic estrogens from e.g. wastewater (Hlozek et al., 2022). There was also one study pointing at increased prostaglandin synthesis after administration of menthol orally to rats (Rozza et al., 2013).

### Summary - human health related

No relevant studies were found in the open literature, apart from those with mechanistic information summarised above. In the REACH registration dossier, an EOGRTS from 2020 was summarised. Several effects were observed which could be linked to endocrine disruptive properties. While some of the effects were relatively weak, the effects on nipple retention were very marked, dose related and seen in both F1 and F2 pups. The other observed effects included: estrous cycle irregularity and decreased testicular sperm count in F0, delayed preputial separation (and vaginal opening) in F1, decreased AGD in F1 (both male and female offspring), but not F2, and increased ovarian follicle counts and corpora lutea counts in F1.

### Summary - environment related

No information

| **Summarised information, Menthol, CAS no. 89-78-1 (and 15356-70-4) AND (+/-) Menthol, CAS no. 15356-60-2** | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro***: Some information on antagonism of ER and AR.<br>***In vivo* mammalian:** Some information on increased prostaglandin synthesis | Some information on increase in retained nipples and estrous cycle irregularity, delayed sexual maturation, and deceased AGD (both males and females) in rodents. | | **EAS:** Some info on endocrine activity and adversity | |

**References**

ECHA REACH reg. dossier: Menthol
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13758
(+/-) Menthol: ECHA registration dossier https://echa.europa.eu/da/registration-dossier/-/registered-dossier/12128

Hlozek et al. 2022: Hlozek, T; Bosakova, T; Bosakova, Z; Tuma, P. 2022. Hydrophobic eutectic solvents for endocrine disruptors purification from water: Natural and synthetic estrogens study. Separation And Purification Technology, Volume: 303, Article Number: 122310, DOI: 10.1016/j.seppur.2022.122310

Michalikova et al. 2019: Michalikova, K; Linhartova, L; Ezechias, M; Cajthaml, T. 2019. Assessment of agonistic and antagonistic properties of widely used oral care antimicrobial substances toward steroid estrogenic and androgenic receptors. Chemosphere, Volume: 217, Pages: 534-541, DOI: 10.1016/j.chemosphere.2018.11.006

Rozza et al. 2013: Rozza, AL; Hiruma-Lima, CA; Takahira, RK; Padovani, CR; Pellizzon, CH. 2013. Effect of menthol in experimentally induced ulcers: Pathways of gastroprotection. Chemico-Biological Interactions, Volume: 206, Issue: 2, Pages: 272-278, DOI: 10.1016/j.cbi.2013.10.003

Stein et al. 2004: Stein, RJ; Santos, S; Nagatomi, J; Hayashi, Y; Minnery, BS; Xavier, M; Patel, AS; Nelson, JB; Futrell, WJ; Yoshimura, N; Chancellor, MB; De Miguel, F. 2004. Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. JOURNAL OF UROLOGY, Volume: 172, Issue: 3, Pages: 1175-1178, DOI: 10.1097/01.ju.0000134880.55119.cf,

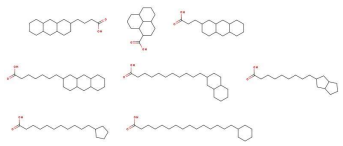
Uchida et al. 2021: Uchida, Y; Sato, I; Atsumi, K; Tsunekawa, C. 2021.UCP1 and TRPM8 Expression in the Brown Fat Did Not Affect the Restriction of Menthol-Induced Hyperthermia by Estradiol in Ovariectomized Rats. Journal Of Nutritional Science And Vitaminology, Volume: 67, Issue: 2, Pages: 130-134, DOI: 10.3177/jnsv.67.130.

# Naphthenic acids, CAS no. 1338-24-5

**Synonyms**

11-(decahydronaphthalen-2-yl)undecanoic acid; 11-cyclopentylundecanoic acid; 15-cyclohexylpentadecanoic acid; 3-(tetradecahydroanthracen-2-yl)propanoic acid; 4-(tetradecahydroanthracen-2-yl)butanoic acid; 7-(tetradecahydroanthracen-2-yl)heptanoic acid; 9-(octahydropentalen-2-yl)nonanoic acid; dodecahydro-1H-phenalene-1-carboxylic acid; 3-(3-ethylcyclopentyl)propanoic acid

**Structure**



**Searches and dates**

1) ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13528
1338-24-5
Searched 8th of August 2023
latest update 19th May 2021: 100-1000 t/a

2) Web Of Science - All databases - All fields
(("1338-24-5" OR "Naphthenic acids" OR "11-(decahydronaphthalen-2-yl)undecanoic acid" OR "11-cyclopentylundecanoic acid" OR "15-cyclohexylpentadecanoic acid" OR "3-(tetradecahydroanthracen-2-yl)propanoic acid" OR "4-(tetradecahydroanthracen-2-yl)butanoic acid" OR "7-(tetradecahydroanthracen-2-yl)heptanoic acid" OR "9-(octahydropentalen-2-yl)nonanoic acid" OR "dodecahydro-1H-phenalene-1-carboxylic acid" OR "3-(3-ethylcyclopentyl)propanoic acid") AND endocrine)
Searched 8th of August 2023, 53 results

3) Web of Science - All databases
(1338-24-5 OR "naphthenic acid*" OR "11-(decahydronaphthalen-2-yl)undecanoic acid" OR "11-cyclopentylundecanoic acid" OR "15-cyclohexylpentadecanoic acid" OR "3-(tetradecahydroanthracen-2-yl)propanoic acid" OR "4-(tetradecahydroanthracen-2-yl)butanoic acid" OR "7-(tetradecahydroanthracen-2-yl)heptanoic acid" OR "9-(octahydropentalen-2-yl)nonanoic acid" OR "dodecahydro-1H-phenalene-1-carboxylic acid" OR "3-(3-ethylcyclopentyl)propanoic acid") AND (*fish OR amphibian* OR bird* OR reptile* OR

mollusc* OR "aquatic toxicology") AND endocrine
Searched 21st of August 2023, 35 results

**Overview**

**Summary - *in vitro***

Naphthenic acids are UVCBs and components in polluted water from oil production. Toxicity studies are typically conducted on extracted fractions of polluted water or commercial mixtures. Some variations in the composition are therefore expected.

Naphthenic acids have been shown to interfere with steroid synthesis *in vitro* in the H295R assay. Two studies observed increased estradiol production (Wang et al., 2015a, Knag et al., 2013a), increased progesterone production (Wang et al., 2015a, Knag et al., 2013a) and decreased testosterone production (Wang et al., 2015a, Knag et al., 2013a) and one study also observe changes in expression of genes involved in steroid synthesis (Wang et al., 2015a). Another study does not find effects on steroidogenesis in the H2956R assay after exposure to NAs extracted from aged oil-sands influenced waters (Leclair et al., 2015).

In a study using Htr-8/Vneo cells (placental trophoblast cells), interference of steroid synthesis was also observed but with another profile. Progesterone was decreased and testosterone was increased while no effect was observed on estradiol (Raez-Villanuevala et al., 2019).

Naphthenic acids have been shown to act as weak ER agonists in one study (Thomas et al., 2009) and ER antagonists in another (Leclair et al., 2015) using yeast ER assays.

Naphthenic acids have been shown to act as AR antagonists (Thomas et al., 2009, Leclair et al., 2015) using yeast AR assays.

No studies on thyroid effects *in vitro.*

**Summary - human health related**
There is very little information on ED-related effects in mammalians. In the open literature, no studies investigating effects of extracted or commercial naphthenic acids in rodents were identified. Only studies on mixtures which included naphthenic acids were found, but they were not included or discussed in the current document.
The publicly available REACH registration dossier showed results from an TG408 and an TG 422 study in rats. In the TG422 developmental toxicity screening study, increased uterine weights were observed in the high dose females after parturition. This effect was dismissed as being an effect of “lactational anestrus”. However, since the control-, low- and mid- dose females were also in lactational anestrus, this explanation does not seem very plausible. In the males, increased epididymis weights were seen. The reporting differed between the repeated dose toxicity section and the reproductive/ developmental toxicity section, making it unclear from the study summaries whether absolute or the relative epididymis weights were significantly affected. The study summary also included information indicating that high dose exposure also caused fewer implantation sites, fewer live pups, and an increased percentage of

post implantation loss.
T: There were signs of thyroid toxicity in two of the conducted guideline studies: Increased incidences of minimal follicular cell hypertrophy were observed in the thyroid in high dose males in the 90-days study (OECD TG 408). Higher mean thyroid/parathyroid weights with corresponding epithelial hypertrophy and cytoplasmic vacuolation were observed in TG422.

**Summary - environment related**
The expressions of genes coding for CYP19b, ERα, and vitellogenin were significantly up-regulated in zebrafish larvae exposed to a commercial mixture of naphthenic acids, which suggested estrogenic properties of the mixture. (Wang et al., 2015a; 2015b). Estrogenic and antiandrogenic properties of two commercial mixtures of naphthenic acids were also evaluated in sticklebacks using a variant of the 21-day fish screen (TG 230) and the androgenized female stickleback screen, respectively (Knag et al., 2013b). Exposure to naphthenic acids did not have a statistically significant effect on vitellogenin production in male sticklebacks. Naphthenic acids enhanced the androgenic potency of DHT (when co-administered) without inducing spiggin when tested in the absence of DHT. Thus, naphthenic acids did not exhibit estrogenic or antiandrogenic properties in sticklebacks.

Androgen-dependent courtship behaviours were significantly affected in male Western clawed frogs, *Silurana tropicalis*, exposed to a commercial mixture of naphthenic acids (Zhang et al., 2022). The duration of calling activity and the ability to amplex females were significantly reduced by exposure to naphthenic acids but were restored after 2 weeks of recovery in clean water. To determine possible disruption at the level of the testes, the effects of exposure to naphthenic acids on gene expression of key players in steroidogenesis were determined. Exposure to naphthenic acids decreased expression of *srd5a*, which encodes the enzyme 5α-reductase, which converts testosterone to its more bioactive form DHT. On the contrary, *lhr*, *star* and *cyp17a1* expressions were upregulated. There were no significant effects of treatment with a commercial mixture of naphthenic acids for 75 days on sex ratios or the relative size of male or female gonads in Northern leopard frogs, *Lithobates pipiens* (Melvin et al., 2013). On the other hand, exposure to naphthenic acids significantly delayed development by two Gosner stages, which could be suggestive of thyroid disruption. However, exposure to a commercial mixture of naphthenic acids for 28 days did not significantly affect thyroid hormone levels in Northern leopard frogs (Smits et al., 2012).

It should be noted that the studies discussed above utilised commercial mixtures of naphthenic acids which could contain unidentified impurities which may contribute to the observed effects of the mixtures. Since it is suggested that oil sands process water acid-extracted naphthenic acids mixtures contain an even wider range of other compounds than commercial mixtures of naphthenic acids (West et al., 2011) the studies utilising oil sands process water acid-extracted naphthenic acids mixtures have not been included in this report.

## Annex 4 - Preliminary literature screening of 10 selected focus substances

| Summarised information, Naphthenic acids, CAS no. 1338-24-5 | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro*:** Some information on steroid synthesis inhibition/ interference, ER agonism/antagonism and AR antagonism. ***In vivo* mammalian:** Some information on increased uterus weight in rat dams after birth in TG 422. ***In vivo* non-mammalian:** Some information on estrogenicity in fish and interference with steroidogenesis in amphibians. | Some information on fewer implantation sites, fewer number of pups in the high dose group, increased percentage of post implantation loss in rodents (TG422). | | **EAS:** Some info on endocrine activity and adversity | **EAS:** Some info on endocrine activity |
| **T** | | Some information on histopathological changes in the thyroid (TG408+TG422) and increased thyroid weights (TG422) in rats. | Some information on delayed development in amphibians. | **T:** Some info on adversity | **T:** Some info on adversity |

### References


ECHA REACH registration dossier: https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13528

Knag et al. 2013a: Knag, AC; Verhaegen, S; Ropstad, E; Mayer, I; Meier, S. 2013a. Effects of polar oil related hydrocarbons on steroidogenesis in vitro in H295R cells. Chemosphere, Volume: 92, Issue: 1, Pages: 106-115, DOI: 10.1016/j.chemosphere.2013.02.046

Knag et al. 2013b: Knag, A.C; Sebire, M; Mayer, I; Meier, S; Renner, P; Katsiadaki, I. 2013b. In vivo endocrine effects of naphthenic acids in fish. Chemosphere 93(10), 2356-2364. doi:10.1016/j.chemosphere.2013.08.033.

Leclair et al. 2015: Leclair, LA; Pohler, L; Wiseman, SB; He, YH; Arens, CJ; Giesy, JP; Scully, S; Wagner, BD; van den Heuvel, MR; Hogan, NS. 2015. In Vitro Assessment of Endocrine

Disrupting Potential of Naphthenic Acid Fractions Derived from Oil Sands-Influenced Water, Source: Environmental Science & Technology, Volume: 49, Issue: 9, Pages: 5743-5752, DOI: 10.1021/acs.est.5b00077

Melvin et al. 2013: Melvin, S.D., Lanctot, C.M., Craig, P.M., Moon, T.W., Peru, K.M., Headley, J.V. and Trudeau, V.L. 2013. Effects of naphthenic acid exposure on development and liver metabolic processes in anuran tadpoles. Environmental Pollution 177, 22-27. doi:10.1016/j.envpol.2013.02.003.

Raez-Villanuevala et al. 2019: Raez-Villanuevala, S; Jamshed, L; Ratnayake, G; Cheng, L; Thomas, PJ; Holloway, AC. 2019. Adverse effects of naphthenic acids on reproductive health: A focus on placental trophoblast cells. Reproductive Toxicology, Volume: 90, Pages: 126-133, DOI: 10.1016/j.reprotox.2019.09.002

Smits et al. 2012: Smits, J.E.G., Hersikorn, B.D., Young, R.F. and Fedorak, P.M. 2012. Physiological effects and tissue residues from exposure of leopard frogs to commercial naphthenic acids. Science of the Total Environment 437, 36-41. doi:10.1016/j.scitotenv.2012.07.043.

Thomas et al. 2009: Thomas, KV; Langford, K; Petersen, K; Smith, AJ; Tollefsen, KE. 2009. Effect-Directed Identification of Naphthenic Acids As Important in Vitro Xeno-Estrogens and Anti-Androgens in North Sea Offshore Produced Water Discharges. Environmental Science & Technology, Volume: 43, Issue: 21, Pages: 8066-8071, DOI: 10.1021/es9014212.

Wang et al. 2015a: Wang, J; Cao, XF; Sun, JH; Huang, Y; Tang, XY. 2015a. Disruption of endocrine function in H295R cell in vitro and in zebrafish in vivo by naphthenic acids. Journal Of Hazardous Materials, Volume: 299, Pages: 1-9, DOI: 10.1016/j.jhazmat.2015.06.004,

Wang et al. 2015b: Wang, J., Cao, X.F., Huang, Y. and Tang, X.Y. 2015b. Developmental toxicity and endocrine disruption of naphthenic acids on the early life stage of zebrafish (Danio rerio). Journal of Applied Toxicology 35(12), 1493-1501. doi:10.1002/jat.3166.

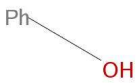
West et al. 2011: West, C.E., Jones, D., Scarlett, A.G. and Rowland, S.J. 2011. Compositional heterogeneity may limit the usefulness of some commercial naphthenic acids for toxicity assays. Science of the Total Environment 409(19), 4125-4131. doi:10.1016/j.scitotenv.2011.05.061.

Zhang et al. 2022: Zhang, W.S., Farmer, E.J., Muhanzi, D. and Trudeau, V.L. 2022. Petroleum-derived naphthenic acids disrupt hormone-dependent sexual behaviours in male Western clawed frogs. Conservation Physiology 10(1). doi:10.1093/conphys/coac030.

# Phenol, CAS no. 108-95-2

**Synonyms**
(Mono)hydroxybenzene, Idrossibenzene, Benzenol, Benzyl Alcohol, Carbolic acid, Monohydroxybenzol, Phenylalkohol, Benzophenol, Hydroxybenzene, oxybenzene, phenic acid, phenyl, phenyl hydrate, phenylic acid, Hydroxybenzol, Benzophenol, Monohydroxybenzene, Monoidrossibenzene, PHENYL HYDROXIDE, PhOH, Phenylalcohol, Phenylhydroxide, fenol, phenic acid, phenyl hydroxide, phenylic alcohol

**Structure**




**Searches and dates**

1) ECHA registration dossier
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15508
108-95-2
Searched 29th of August 2023
Intermediate use only (2018)
Latest update 4th May 2023: 1.000.000 -10.000.000 t/a

1)Web of Science- All databases - All fields
(("108-95-2" OR “Phenol” OR "(Mono)hydroxybenzene” OR “Idrossibenzene” OR “Benzenol” OR “Benzyl Alcohol” OR “Carbolic acid” OR “Monohydroxybenzol” OR “Phenylalkohol” OR “Benzophenol” OR “Hydroxybenzene” OR “oxybenzene” OR “phenic acid” OR “phenyl” OR “phenyl hydrate” OR “phenylic acid” OR “Hydroxybenzol” OR “Benzophenol” OR “Monohydroxybenzene” OR “Monoidrossibenzene” OR “PHENYL HYDROXIDE” OR “PhOH” OR “Phenylalcohol” OR “Phenylhydroxide” “fenol” OR “phenic acid” OR “phenyl hydroxide” OR “phenylic alcohol”) AND endocrine)
Searched 28th of July 2023, 24.473 results, not screened

2)Web of Science- All databases - All fields
(("108-95-2" OR “Phenol”) AND endocrine)
Searched 28th of July 2023, 5140 results, not screened

3) Web of Science - all databases
(("108-95-2" OR “Phenol”) AND "endocrine disruption")
Searched 20th of August, 89 results, not screened

4) Web of Science - All databases
(108-95-2 OR “phenol” OR (mono)hydroxybenzene OR idrossibenzene OR benzenol OR ”benzyl alcohol” OR “carbolic acid” OR monohydroxybenzol OR phenylalkohol OR benzophenol OR hydroxybenzene OR oxybenzene OR “phenic acid” OR phenyl OR “phenyl hydrate” OR “phenylic acid” OR hydroxybenzol OR benzophenol OR monohydroxybenzene OR monoidrossibenzene OR ”phenyl hydroxide” OR PhOH OR phenylalcohol OR phenylhydroxide OR “fenol” OR “phenic acid” OR “phenyl hydroxide” OR “phenylic alcohol”) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 30th of August 2023, 1168 results, not screened

5) Web of Science - All databases
(108-95-2 OR “phenol”) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 30th of August 2023, 404 results, not screened

6) Web of Science - All databases
(108-95-2 OR “phenol”) AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND “endocrine disruption”
Searched 30th of August 2023, 33 results, Screened: 1 relevant result - search string too restrictive

7) Web of Science - All databases
108-95-2 AND (*fish OR amphibian* OR bird* OR reptile* OR mollusc* OR "aquatic toxicology") AND endocrine
Searched 30th of August 2023, 64 results, screened

8) SciFinder CAS
Search: 108-95-2
Filter:
Freetext: Endocrine
30th August, 2023619 results
Filter:
Substance role: Adverse effect
120 results, screened

9) SciFinder CAS
Search: 108-95-2
Filter:
Freetext: Endocrine disruption
Filter:
Substance role: Adverse effect
30th August 2023, 135 results, partly screened

It should be noted that the literature search for phenol has been challenging since if "phenol" is included in the search string, the results include a broad variety of "environmental phenols", including e.g. bisphenols, nonyl- and octylphenol, alkylphenols etc.
Some studies investigating effects of phenol have been identified and are included below, but further literature search may lead to identification of additional relevant studies.

**Overview**

**Summary - *in vitro***

Phenol significantly inhibited LH-induced secretion of 17β-estradiol, LH-stimulated aromatase activity, P450arom gene expression, LH-induced activation of ovarian steroidogenic factor-1 (SF-1), and gonadotropin-induced stimulation of the activity of the steroidogenic enzymes, delta 5-3 beta-HSD and 17 beta-HSD, in carp ovarian follicles *in vitro* (Mukherjee et al., 1992; Das et al., 2013). Danish QSAR database: Part of experimental data sets and negative for: ER binding and activation, AR binding and activation, TPO inhibition, NIS, CAR inhibition and activation. It is predicted Negative and in domain for PXR binding and activation and AhR activation.

**Summary - human health related**

In one study from the open literature showed reduced levels of estrogen and FSH and reductions in the number of ovarian follicles, increased number of atretic follicles, increased thickness of the tunica albuginea and decreased absolute and relative ovarian weights after exposure of adult mice to phenol (Monfared et al., 2013).

In the REACH registration dossier, summary of a two-generation study from 1999 was available. In this study, reduced litter survival, changed reproductive organ to body weight ratios (e.g. increased relative epididymis weight in F1) and delayed sexual maturation was observed. Some of these effects may be explained by decreased food/water consumption and decreased body weight gains. Other changes in organ weights were evaluated to be independent of the affected body weight gain: Reduced relative prostate weights were observed in F1 and reduced absolute and relative uterus weights were observed in F1 but not in P1. Further, in F1, the estrous cyclicity of the rats in the high dose group seemed to be affected with fewer animals in proestrus/estrous in the high dose group compared to the controls based on vaginal smears and supported by histology showing fewer dilated uterus (indicating that the estrous cycle was either estrous or proestrus) in the high dose group compared to controls. Further, reduced average foetal body weights and cleft palate was observed in developmental tox studies (OECD TG 414 and TG414-like study).

**Summary - environment related**

Exposure of female common carp to phenol decreased serum and ovarian 17β-estradiol levels (Das et al., 2013), and ovarian cyp19a1a gene expression and P450 aromatase activity (Das et al., 2016). Furthermore, exposure to phenol decreased blood T4 levels in catfish (Zaki et al., 2012) and *C. punctatus* (Bhattacharya et al., 1989). Additionally, phenol caused retardation in

development and reduced pigmentation in a zebrafish embryo test (Makarova et al., 2016). Moreover, activity levels were severely depressed and shoaling behaviour was also affected in minnows exposed to phenol (Stott and Buckley, 1979).

| **Summarised information, Phenol, CAS no. 108-95-2** | | | | | |
|---|---|---|---|---|---|
| | **Endocrine activity** | **Adversity** | | **Summary** | |
| | | **Mammalian** | **Non-mammalian** | **HH** | **ENV** |
| **EAS** | ***In vitro*: Some information on inhibited steroidogenesis in carp ovarian follicles *in vitro*. ***In vivo* mammalian**: Some information on reduced FSH and estrogen levels in mice. ***In vivo* non-mammalian**: Some information on reduced estrogen levels and inhibited steroidogenesis in fish. | **Some information on** adverse effects on ovaries in adult mice. Some information on reduced weight of prostate and uterus and changes in weight of other reproductive organs, changes in age of sexual maturation, changes in estrous cyclicity (TG416) in rodents. Some information on developmental effects in guideline studies in rodents. | | **EAS:** Some info on endocrine activity and adversity | **EAS:** Some info on endocrine activity |
| **T** | ***In vivo* non-mammalian:** Some information on decreased T4 levels in fish. | | | | **T:** Some info on endocrine activity |

**References**

Bhattacharya et al. 1989: Bhattacharya, T., Bhattacharya, S., Ray, A.K., Dey, S. 1989. Influence of industrial pollutants on thyroid-function in Channa-punctatus (Bloch). Indian Journal of Experimental Biology 27, 65-68.

Das et al. 2016: Das, S., Majumder, S., Gupta, S., Dutta, S., Mukherjee, D. 2016. Effects of phenol on ovarian P450arom gene expression and aromatase activity in vivo and antioxidant metabolism in common carp Cyprinus carpio. Fish Physiology and Biochemistry 42, 275-286. doi:10.1007/s10695-015-0135-9.

Das et al. 2013: Das, S., Majumder, S., Mukherjee, D. 2013. Effect of phenol on ovarian secretion of 17β-estradiol in common carp Cyprinus carpio. Archives of Environmental Contamination and Toxicology 65, 132-141. doi:10.1007/s00244-013-9875-7.

ECHA REACH registration dossier: https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15508

Makarova et al. 2016: Makarova, K., Siudem, P., Zawada, K., Kurkowiak, J. 2016. screening of toxic effects of bisphenol A and products of its degradation: zebrafish (Danio rerio) embryo test and molecular docking. Zebrafish 13, 466-474. doi:10.1089/zeb.2016.1261.

Monfared et al.,2013: Histo-morphological and functional alterations in the ovary of adult mice following phenol administration, By: Monfared, Ali Louei; Havasi, Leili; Soltani, Salman; Naward, Sahar Hamoon, Journal of Reproduction and Infertility (Dubai, United Arab Emirates) (2013), 4(1), 09-12

Mukherjee et al. 1992: Mukherjee, D., Guha, D., Kumar, V. 1992. Effect of certain toxicants on gonadotropin-induced ovarian non-esterified cholesterol depletion and steroidogenic enzyme stimulation of the common carp Cyprinus carpio in vitro. Biomedical and environmental sciences. Biomedical and Environmental Sciences 5, 92-98.

Stott et al. 1979: Stott, B., Buckley, B.R. 1979. Avoidance experiments with homing shoals of minnows, Phoxinus-phoxinus in a laboratory stream channel. Journal of Fish Biology 14, 135-146. doi:10.1111/j.1095-8649.1979.tb03503.x.

Zaki et al. 2012: Zaki, M.S., El-Batrawy, N., Taha, N.M. 2012. Dose phenol toxicity affected endocrine status in African catfish (Clarias gariepinus). Life Science Journal 9, 636-639.

# Annex 5 Preliminary literature screening of a group of 12 benzophenones

12 benzophenones were preliminarily screened for literature related to endocrine disruption. The aim of the preliminary literature screening was to investigate whether information was available on endocrine activity and ED-related adversity respectively, with focus on the EATS modalities. Information about metabolism that could be useful in a grouping context and information about other ED-related (non-EATS) effects was noted if it was identified in the preliminary screening.

The following methodology was used:

1. Information was retrieved from ECHA's publicly available database of registered substances by entering the CAS no. (ECHA 2023). Tonnage level was noted.
2. ECHA registration dossiers were screened for effects relevant for endocrine disruption in the sections on reproductive toxicity and repeated dose toxicity.
3. For most substances, the open literature was searched using "SciFinder" (CAS), using the CAS number and a filter for "Endocrine*". This was a change in methodology from the use of individual search strings based on synonyms used to search the "Web of Science" as applied when searching for literature for the 10 individual focus substances. The use of SciFinder led to a much more targeted search, leaving out publications investigating substances with e.g. part of the substance name in the nomenclature.
    - For Benzophenone-3 (BP-3), a comprehensive review published in 2023 was available (Mustieles et al., 2023). This review was used as the main source of information for BP-3. For benzophenone-1 (BP-1), which is a main metabolite of BP-3, Mustieles et al., 2023 was also used for data extraction, in addition to the search for publications in SciFinder (CAS), as described above.
4. Retrieved abstracts were screened for information relevant for endocrine disruption.
5. Relevant information was extracted to a tabular format, using the effect categories laid out in the ECHA/EFSA guidance on identification of endocrine disruptors (ECHA/EFSA, 2018) as a starting point.

In this annex, information about each of the benzophenones was compiled, including substance name, CAS number, synonyms, structure, descriptions of data searches and extracted information. The extracted information in the tables was targeted and showed only available information for each substance. This means that blank fields, as observed in table 3 and 4 in the main report were deleted here to provide a better overview for each individual substance. A full table with all the information fields was provided in the end of the annex for information.

## Annex 5 - Preliminary literature screening of a group of 12 benzophenones

In the tables with summarised information, “EAS-relevant” refers to endpoints usually considered relevant for the sex hormone system, and “Thyroid-relevant” refers to endpoints usually considered relevant for the thyroid hormone system. It should however be noted that cross-talk between “EAS” and “Thyroid” modalities may occur.

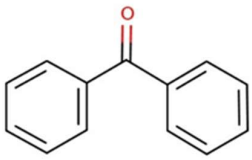
Since only a preliminary screening of the literature was conducted based on CAS number search in SciFinder, it cannot be excluded that some relevant studies were not identified. Further, the studies were not evaluated for reliability or quality, and no weight of evidence analysis was applied. When effects in animal studies were identified, they were noted without a thorough evaluation of other signs of systemic or general toxicity, effects on body weights or other observations that might affect the findings.

All references to annex 5 were collated and combined in one joint reference list.

# Benzophenone, CAS no. 119-61-9

**Synonyms:** BP

**Structure:**



**Searches and dates:**

**1) ECHA Substance Evaluation (SEV) report 2018**
**https://echa.europa.eu/documents/10162/5a195cc0-82f3-cd0a-8fa3-65c400911515**

**2) ECHA registration dossier**
**Registered substances - ECHA (europa.eu)** https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13823
Searched 21st of Sep 2023: CAS no 119-61-9
Latest update 3rd of June 2022: 1000-10.000 t/a

**3) SciFinder CAS**
Only for publications published after literature search conducted for ECHA SEV report 2018.
21st September 2023
Search: 119-61-9
42,303 Results
Filter: Freetext: Endocrine*
Filter: Language: English
Filter: Published after 2017
**103 results**

| **Benzophenone - CAS 119-61-9** | |
|---|---|
| **REACH registration status** | **1000-10.000 t/a** |
| **OECD TG studies in REACH dossier** | Rat TG408 (1993), Mice TG408 (1993), Rat TG408 (1982), Rat TG416 (2005), Rabbit TG414 (2004), Rat TG414 (2002) |
| **Metabolism** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone - CAS 119-61-9** | |
|---|---|
| Rodents | 4-OHBP, its sulfate conjugate and benzhydrol (Nagakawa et al., 2000 (SEV)) |
| **EAS-relevant** | |
| **In vitro** | |
| Estrogenicity (E) | 4Y, 4N (Y: Suzuki et al., 2005, Takatori et al., 2003, Zhang et al., 2017, Kawamura et al., 2005) (N: Kawamura et al., 2003, Nagakawa et al., 2000 (SEV), Yamasaki et al., 2002 (SEV), Hayashi et al., 2006 (SEV)) |
| Anti-A | 1Y,1N (N: Suzuki et al., 2005) (Y: Kawamura et al., 2005) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Estradiol level (EAS) | 1N (N: No effect on levels of estradiol in F1 and F2, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Follicle stimulating hormone level (EAS) | 1N (N: No effect on levels of FSH in F1 and F2, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Luteinising hormone (LH) level (EAS) | 1N (N: No effect on levels of LH in F1 and F2, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Testosterone level (EAS) | 1N (N: No effect on levels of testosterone in F1 and F2, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Uterotrophic (E) | 3Y, 3N (Y: ORAL: Suzuki et al., 2005, Nakagawa et al., 2002 (SEV)) (N: DERMAL: Nakagawa et al., 2001 (SEV), Yamasaki et al., 2002 (SEV), Hayashi et al., 2006 (SEV)) (Y: Sugihara et al., 2007) |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Age at puberty (EAS) | 1N (N: No effect on timing of sexual maturation in F1, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Anogenital distance | 1N (N: No effects on AGD in F1 or F2, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Epididymis weight/histopathology (EAS) | 2Y,1N (2Y: Decreased epididymis weights in high dose mice in NTP 14-week study, ECHA SEV 2018 + Decreased absolute and relative epididymis weights in range-finding for TG416, Hoshino et al., 2005 in ECHA SEV)) + (N: No effect |

| **Benzophenone - CAS 119-61-9** | |
|---|---|
| | on weights and histopathology of epididymis in F1 parental animals, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Estrous cyclicity (EAS) | 2N (N: No effect on estrous cyclicity in F1 and F2, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) (N: No effect on estrous cycle in NTP 14-week study in mice, ECHA SEV 2018) |
| Ovary weight/histopathology (EAS) | 2N (N: No effects on weights of other reproductive tissue than testes and epididymis + No effect on weights and histopathology of ovaries in F1 parental animals, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Prostate weight/histopathology (EAS) | 2N (N: No effects on weights of other reproductive tissue than testes and epididymis in NTP 14-week study in rats or mice in ECHA SEV 2018 + No effect on weights and histopathology of prostates in F1 parental animals, OECD TG 416, Hoshino et al., 2005 in ECHA SEV)) |
| Seminal vesicle weight/histopathology(EAS) | 2N (N: No effects on weights of other reproductive tissue than testes and epididymis in NTP 14-week study in rats or mice, ECHA SEV 2018 + No effect on weights and histopathology of seminal vesicles in F1 parental animals, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Testis weight/histopathology (EAS) | 2Y,1N (2Y: Decreased testis weights in high dose mice in NTP 14-week study, ECHA SEV 2018 + Increased absolute and relative testes weights in range-finding for TG416, Hoshino et al., 2005 in ECHA SEV) (N: No effects on weights and histopathology of testes in F1 parental animals in F1 and F2, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| Sperm parameters (EAS) | 2N (N: No effect on semen parameters in NTP 14-week study in rats or mice, ECHA SEV 2018 + No effect on semen quality in F1 and F2, OECD TG 416, Hoshino et al., 2005 in ECHA SEV)) |
| Uterus weight/histopathology (EAS) | 2N (N: No effects on weights of other reproductive tissue than testes and epididymis in NTP 14-week study in rats or mice in ECHA SEV 2018 + No effect on weights and histopathology of uterus in F1 parental animals, OECD TG 416, Hoshino et al., 2005 in ECHA SEV) |
| **Fish** | |
| **Other in vivo mechanistic** | |

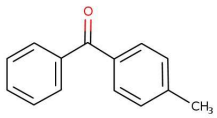
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone - CAS 119-61-9** | |
|---|---|
| Estrogenicity (E) | 1N (No expression of GFP in zebrafish, Brion et al., 2012 (ECHA SEV)) |
| **Thyroid-relevant** | |
| **In vitro** | |
| TPO inhibition | 1Y (Decreased TPO activity, Song et al., 2012) |
| Altered gene expression | 1Y (Lee et al., 2018) |
| **Rodents** | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Thyroid weight/histopathology | 1Y,2N (Y: Increases absolute and relative weight of left thyroid in males after 90 days of exposure (Burdock et al., 1991 (ECHA SEV)) (N: No effect on right thyroid weight or thyroid weight in female animals, also no effect in higher doses, though terminated after 28 days (Burdock et al., 1991 in ECHA SEV) + No effect on thyroid weights in other repeated dose studies after exposure for up to 2 years (ECHA SEV) |

# 4-methylbenzophenone, CAS no. 134-84-9

**Synonyms:** (4-methylphenyl)(phenyl)methanone, 4-MBP.

**Structure:**




**Searches and dates:**

**1) ECHA registration dossier**
**Registered substances - ECHA (europa.eu), https://echa.europa.eu/da/registration-dossier/-/registered-dossier/21484**
Searched 27th of Sep 2023: CAS no 134-84-9
Latest update December 2022: 100-1000 t/a

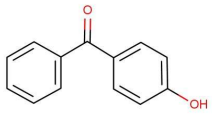
**3) SciFinder CAS**
27 September 2023
Search: 134-84-9
2830 Results
Filter: Freetext: Endocrine*
**16 results**

| **4-methylbenzophenone - CAS 134-84-9** | |
|---|---|
| **REACH registration status** | **100-1000 t/a** |
| **OECD TG studies in REACH dossier** | Read-across to benzophenone on repeated dose and reproductive toxicity |
| **EAS-relevant** | |
| **In vitro** | |
| Estrogenicity (E) | 1Y (Yamasaki et al., 2003) |

# 4-hydroxybenzophenone, CAS no. 1137-42-4

**Synonyms:** 4-OHBP, HBP

**Structure:**




**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu)
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/28385
Searched 27th of Sep 2023: CAS no 1137-42-4
Latest update May 2023: 10-100 t/a

**3) SciFinder CAS**
9 October 2023
Search: 1137-42-4
2902 Results
Filter: Freetext: Endocrine*
**122 results**

| **4-hydroxybenzophenone - CAS 1137-42-4** | |
|---|---|
| **REACH registration status** | **10-100 t/a** |
| **OECD TG studies in REACH dossier** | Rat TG421 (2019) |
| **EAS-relevant** | |
| **In vitro** | |
| Estrogenicity (E) | 9Y (Suzuki et al., 2005, Kawamura et al., 2003, Kawamura et al., 2005, Akahori et al., 2008, Kunz and Fent 2006, Nagakawi et al., 2002 (SEV), Yamasaki et al., 2002 (SEV), Hayashi et al.,2006 (SEV)) + (Y: recombinant rainbow trout tERa assay (Kunz et al., 2006) |

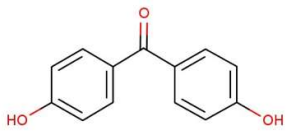
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **4-hydroxybenzophenone - CAS 1137-42-4** | |
|---|---|
| Anti-A | 3Y (Suzuki et al., 2005, Kunz and Fent 2006, Kawamura et al., 2005) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Uterotrophic (E) | 3Y (Yamasaki et al., 2003, Akahori et al., 2008, Nakagawa et al., 2001 (SEV), ) |
| Uterotrophic (anti-E) | 1Y (Yamasaki et al., 2003) |
| **Fish** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Vitellogenin | 1N (Kunz et al., 2006) |
| **Thyroid-relevant** | |
| **In vitro** | |
| TPO activation | 1N (Song et al., 2012) |
| TPO inhibition | 1N (Song et al., 2012) |
| TTR binding | 1Y (Cotrina et al., 2023) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| T3 and/or T4 level | 1Y (OECD TG 422 (2019): Decreased T4 levels in males (and increased cholesterol in females), ECHA 2023) |
| Thyroid stimulating hormone level (TSH) | 1Y (OECD TG 422 (2019): Increased TSH levels in females (and increased cholesterol in females), ECHA 2023) |
| **Other ED-related effects** | |
| ERR-gamma | 1Y (Zheng et al., 2020) |

# 4,4'-dihydroxybenzophenone, CAS no. 611-99-4

**Synonyms:** 4,4'-OHBP, 4-(4-hydroxybenzoyl)phenol, p,p'-Dihydroxybenzophenone

**Structure:**




**Searches and dates:**

**1) ECHA registration dossier**
**Registered substances - ECHA (europa.eu)**
**https://echa.europa.eu/da/registration-dossier/-/registered-dossier/23810**
Searched 27th of Sep 2023: CAS no 611-99-4
Latest update April 2018: 10-100 t/a

**3) SciFinder CAS**
27 September 2023
Search: 611-99-4
2376 Results
Filter: Freetext: Endocrine*
**96 results**

| **4,4'-dihydroxybenzophenone – CAS 611-99-4** | |
|---|---|
| **REACH registration status** | **10-100 t/a** |
| **OECD TG studies in REACH dossier** | Read-across to benzophenone (and benzophenone-1): Rat TG408-like (1983) on benzophenone-1 and Rat TG408-like (1991) on benzophenone, TG416 (1983) on benzophenone |
| **EAS-relevant** | |
| **In vitro** | |
| Estrogenicity (E) | 6Y (Suzuki et al., 2005, Kawamura et al., 2003, Kawamura et al., 2005, Yamasaki et al., 2003, Akahori et al., 2008, Kunz and Fent 2006) |
| Anti-A | 2Y (Kunz and Fent, 2006, Kawamura et al., 2005) |

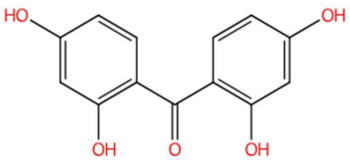
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **4,4'-dihydroxybenzophenone – CAS 611-99-4** | |
|---|---|
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Uterotrophic (E) | 4Y (Yamasaki et al., 2002 (SEV), Akahori et al., 2008, Hayashi et al., 2006 (SEV), Yamasaki et al., 2003) |
| Uterotrophic (anti-E) | 1Y (Yamasaki et al., 2003) |
| **Thyroid-relevant** | |
| **In vitro** | |
| TTR binding | 1Y (Cotrina et al., 2023) |
| **Other ED-related effects** | |
| ERR-gamma | 1Y (Zheng et al., 2020) |

# Benzophenone-2, CAS no. 131-55-5

**Synonyms:** BP-2, 2,2′,4,4′-Tetrahydroxybenzophenone

**Structure:**




**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu)
Searched 9th of Oct 2023: 131-55-5
No results found

**3) SciFinder CAS**
9 October 2023
Search: 131-55-5
1774 Results
Filter: Freetext: Endocrine*
**165 results**

| **Benzophenone-2 - CAS 131-55-5** | |
|---|---|
| **REACH registration status** | **Not registered** |
| **OECD TG studies in REACH dossier** | Not registered |
| **Metabolism** | |
| Rodents | Glucuronide - and sulfate-conjugates in rats (Schlecht et al., 2008) |
| Fish | Glucuronide - and sulfate-conjugates in zebrafish adults and larvae (Le Fol et al., 2015; Le Fol et al., 2017) |
| | |
| **EAS-relevant** | |
| **In vitro** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-2 - CAS 131-55-5** | |
|---|---|
| Estrogenicity (E) | 11Y (Watanabe et al., 2015, Suzuki et al., 2005, Kawamura et al., 2003, Kawamura et al., 2005, Seidlova-Wuttke et al., 2004, Schlumpf et al., 2004, Yamasaki et al., 2003, Akahori et al., 2008, Kunz and Fent 2006, Molina-Molina et al., 2008, Sugihara et al., 2007)) |
| Estrogen-like changes in gene expression, motility etc. | 1Y (Y: Increased motility of estrogen-responsive MCF-7 human breast cancer cells and estrogen-non-responsive human breast cancer cells, Alamer et al., 2018) |
| Androgenicity | 1Y (Kunz and Fent 2006) |
| Anti-A | 5Y (Watanabe et al., 2015, Kunz and Fent 2006, Kawamura et al., 2005, Molina-Molina et al., 2008, Sugihara et al., 2007) |
| Steroid synthesis changes | 2Y (Y: changed expression of steroidogenic enzyme genes in MA-10 Leydig cell line, Kim et al., 2011) (Y: Increased aromatase mRNA, increased aromatase activity, increased synthesis of 17-beta-estradiol, Williams et al., 2019) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Estradiol level (EAS) | 1Y (Increased serum 17-b-estradiol levels in rats, Broniowska et al., 2023) |
| Testosterone level (EAS) | 2Y (Y: Decreased testosterone production in vivo by changes in expression of steroid synthesis genes, Kim et al., 2011)(Y: Decreased serum and testes testosterone levels in rats, Broniowska et al., 2023) |
| Uterotrophic (E) | 6Y (Seidlowa-Wutke et al., 2004, Schlumpf et al., 2004, Jarry et al., 2004, Yamasaki et al., 2003, Akahori et al., 2008, Ohta et al., 2012) |
| Uterotrophic (anti-E) | 1Y (Yamasaki et al., 2003) |
| **Other in vivo mechanistic** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-2 - CAS 131-55-5** | |
|---|---|
| Other mechanistic (E) | 5Y (Ovariectomized rats: Estrogen-like effects on LH, cholesterol, LDL, HDL and fat depot size, Seidlova-Wuttke et al., 2005)(Ovariectomized rats: Estrogen-like changes in gene-expression, Schlecht et al., 2006) (Ovariectomized rats: Estrogen-like changes in gene-expression in uterus, liver and vagina, Jarry et al., 2004) (Estrogen-like gene expression of steroid receptors in uterus, pituitary and thyroid of rats, Schlecht et al., 2004) (In ovariectomized rats: Estrogen-like effects in vagina, estrogen-like anti-osteoporotic effects in metaphysis of the tibia and estrogen-like effects on serum surrogate parameters of bone metabolism (Seidlova-Wuttke et al., 2004) |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Anogenital distance | 1N (N: No reduced AGD in male mice offspring, Hsieh et al., 2007) |
| Genital anomalies (EAS) | 1Y (Y: Hypospadias in male mice offspring, possibly through estrogenic MoA, Hsieh et al., 2007) |
| Sperm parameters (EAS) | 1Y (Y: Reduced number and motility of sperm, decreased changes in sperm morphology in rats, Broniowska et al., 2023) |
| **Fish** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Vitellogenin | 3Y (Weisbrod et al., 2007; Fent et al., 2008 |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| secondary sex characteristics | 2Y (Weisbrod et al., 2007; Fent et al., 2008 |
| **"Sensitive to, but not diagnostic of, EATS" (ECHA/EFSA guidance)** | |
| other adverse | 1Y (inhibited spermatocyte and oocyte development (Weisbrod et al., 2007) |
| **Amphibians** | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| vitellogenin | 1Y (Haselman et al., 2016) |

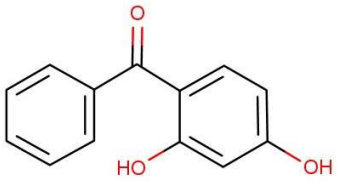
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-2 - CAS 131-55-5** | |
|---|---|
| sex ratio | 1Y (Haselman et al., 2016) |
| **Thyroid-relevant** | |
| **In vitro** | |
| TPO inhibition | 2Y (Y: Schmutzler et al., 2007 + Decreased TPO activity, Song et al., 2012) |
| TTR binding | 2Y (Y: 500 times higher binding potency to human TTR than to fish (Gilthead seabream) TTR, Zhang et al., 2018) (Y: Cotrina et al., 2023) |
| TR agonist | 2Y (Y:Hofman et al., 2009) (Y:Schmutzler et al., 2007) |
| Altered gene expression | 1Y (Lee et al., 2018) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| T3 and/or T4 level | 4Y (Reduced levels of T3 and T4 in ovariectomized rats, Jarry et al., 2004) (Decreased T4 but not T3 and TSH in ovariectomized rats, Seidlova-Wuttke et al., 2005) (Reduced levels of serum T4, increased level of serum TSH in rats, Schmutzler et al., 2007) (Reduced TSH, increased free fraction of T3 and T4 in serum in rats, Broniowska et al., 2018) |
| Thyroid stimulating hormone level (TSH) | 2Y,1N (N: Decreased T4 but not T3 and TSH in ovariectomized rats, Seidlova-Wuttke et al., 2005) (Y: Reduced levels of serum T4, increased level of serum TSH in rats, Schmutzler et al., 2007) (Y: Reduced TSH, increased free fraction of T3 and T4 in serum in rats, Broniowska et al., 2018) |

# Benzophenone-1, CAS no. 131-56-6

**Synonyms:** 2,4-OHBP, DHB, 2,4'-dihydroxybenzophenone, BP-1

**Structure:**




**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu) **https://echa.europa.eu/da/registration-dossier/-/registered-dossier/12687**
Searched 27th of Sep 2023: CAS no 131-56-6
Latest update May 2022: 100-1000 t/a

**2) Mustieles et al., 2023**
Comprehensive review based on literature search from January 2021

**2) SciFinder CAS**
4 December 2023
Search: 131-56-6
4955 Results
Filter: Freetext: Endocrine*
Filter: Language: English
**212 results**

| **Benzophenone-1 - CAS 131-56-6** | |
|---|---|
| **REACH registration status** | **100-1000 t/a** |
| **OECD TG studies in REACH dossier** | OECD Toolbox grouping for repeated dose tox, Reference to CeHoS SIN List report for reproductive toxicity |
| **Metabolism** | |
| Rodents | THBP, 2,4,5-triOH BP (Watanabe et al., 2015) |
| **EAS-relevant** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-1 - CAS 131-56-6** | |
|---|---|
| **In vitro** | |
| Estrogenicity (E) | 8Y (Watanabe et al., 2015, Suzuki et al., 2005, Kawamura et al., 2003, Kawamura et al., 2005, Schlumpf et al., 2004, Akahori et al., 2008, Molina-Molina et al., 2008, Habauzit et al., 2017, Kunz and Fent 2006) + More in Mustieles et al., 2023 |
| Estrogen-like changes in gene expression, motility etc. | 4Y (Y: Estrogen-like stimulation of proliferation of ovarian cancer via ER signalling, Park et al., 2013) (Y: Enhanced the migration capability of BG-1 cells, similar to E2, Shin et al., 2016) (Y: Promoted proliferation of MCF-7 cells similar to E2 and induced migration of MCF-7 cells similar to E2, In et al., 2015) (Y: Increased motility of estrogen-responsive MCF-7 human breast cancer cells and estrogen-non-responsive human breast cancer cells, Alamer et al., 2018) |
| Anti-A | 4Y (Watanabe et al., 2015, Molina-Molina et al., 2018, Kunz and Fent 2006, Kawamura et al., 2005) + More in Mustieles et al., 2023 |
| Steroid synthesis changes | 1Y (inhibition of 17-b-hydrosteroid dehydrogenase 3 activity in Leydig cells, Nashev et al., 2010) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Uterotrophic (E) | 7Y (Y: Suzuki et al., 2005, Schlumpf et al., 2004, Akahori et al., 2008, Subcutaneous and oral: Ohta et al., 2012. Subcutaneous: Koda et al., 2005. Yamasaki et al., 2004) (Y: Sugihara et al., 2007) |
| Uterotrophic (anti-E) | 1Y,1N (Y: Subcutaneous: Ohta et al., 2012) (N: Oral: Ohta et al., 2012) |
| **Other in vivo mechanistic** | |
| Other mechanistic (A/anti-A) | 1Y (Y: Increased LNCaP cell proliferative activity and migration as DHT, Kim et al., 2015) |
| **Fish** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Vitellogenin | 2Y (Kunz et al., 2006, Kunz and Fent., 2009 |
| **Thyroid-relevant** | |

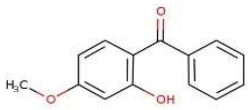
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-1 - CAS 131-56-6** | |
|---|---|
| **In vitro** | |
| TPO activation | 1Y (Increased TPO activity, Song et al., 2012) |
| TTR binding | 1Y (Cotrina et al., 2023) |
| Altered gene expression | 1Y (Lee et al., 2018 ) |
| **Fish** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Thyroid hormones | 1Y (Decreased T3,T4 (Lee et al. (2018)) |
| **Other ED-related effects** | |
| ERR-gamma | 1Y (Zheng et al., 2020) |

# Benzophenone-3, CAS no. 131-57-7

**Synonyms:** Oxybenzone, 2-hydroxy-4-methoxybenzophenone, HMB, 2-OH-4MeO BP

**Structure:**




**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu) **https://echa.europa.eu/da/registration-dossier/-/registered-dossier/5515**
Searched 27th of Sep 2023: CAS no 131-57-7
Latest update Feb 2021: 100-1000 t/a

**2) Mustieles et al., 2023**
Comprehensive review based on literature search from January 2021

| **Benzophenone-3 - CAS 131-57-7** | |
|---|---|
| **REACH registration status** | **100-1000 t/a** |
| **OECD TG studies in REACH dossier** | Rat TG408 (1992), Mice TG408 (1992), Rat TG 407 (1953), Rat TG411 (1992), Mice TG411 (1992), Rat TG414 (2005), Rat Hershberger Assay (2003), Rat TG440 (1998) |
| **Metabolism** | |
| Rodents | BP-1, BP-8, THBP, 3-OH BP-3 (Watanabe et al., 2015), 5-OH BP-3 (Watanabe et al., 2015) |
| **EAS-relevant** | |
| **In vitro** | |
| Estrogenicity (E) | 8Y (Watanabe et al, 2015, Suzuki et al., 2005, Takatori et a., 2003, Kawamura et al., 2003, Kawamura et al., 2005, Schlumpf et al., 2004, Zhang et al., 2017, Kunz and Fent 2006) + More in Mustieles et al., 2023 |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-3 - CAS 131-57-7** | |
|---|---|
| Estrogen-like changes in gene expression, motility etc. | 2Y (Y: Ex vivo study in mice showed effects on neuronal gene and protein expression and epigenetics - BP3 induced apoptosis, caused neurotoxicity, and decreased ER-alpha and ER-beta expression levels in neocortical cells. Also, hypermethylation of estrogenic genes, Wnuk et al., 2018 in Mustieles et al., 2023) (Y: Increased motility of estrogen-responsive MCF-7 human breast cancer cells and estrogen-non-responsive human breast cancer cells, Alamer et al., 2018) |
| Anti-E | 1Y (Kunz and Fent 2006) |
| Anti-A | 5Y,1N (Y: Watanabe et al., 2015, Schlumpf et al., 2004, Kunz and Fent 2006, Kawamura et al., 2005, Molina-Molina et al., 2008) (N: Suzuki et al., 2005) + More in Mustieles et al., 2023 |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Testosterone level (EAS) | 2Y (Decreased testosterone levels in male rat offspring in MOG study, Nakamura et al., 2015 in Mustieles et al., 2023) (Y: Decreased serum testosterone levels, Krzyzanowska et al., 2018 in Mustieles et al., 2023) |
| Uterotrophic (E) | 1Y, 6N (Y: At higher dose: 1525 mg/kg/d, Schlumpf et al., 2004) (N: At lower doses - Suzuki et al., 2005, Subcutaneous and oral: Ohta et al., 2012. Oral: LaPlante et al., 2018 - and more in Mustieles et al., 2023) |
| Uterotrophic (anti-E) | 2N (Subcutaneous and orally: Ohta et al., 2012) |
| Hershberger (A and anti-A) | 1N (No androgenic or antiandrogenic effect, NTP 2019 in Mustieles et al., 2023) |
| **Other in vivo mechanistic** | |
| Other mechanistic (E) | 5Y (3Y: Estrogen-like gene expression of steroid receptors in uterus, pituitary, and thyroid of rats, Schlecht et al., 2004, LaPlante et al., 2018, Majhi et al., 2020 - all in Mustieles et al., 2023) (Y: Changes in gene-expression patterns in testes, Nakamura et al., 2018 in Mustieles et al., 2023) (Y: Decreased expression of ERbeta in brain, Krzyzanowska et al., 2018 in Mustieles et al., 2023) |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-3 - CAS 131-57-7** | |
|---|---|
| Age at puberty (EAS) | 1N (No effect on sexual maturation in MOG study, NTP 2021 in Mustieles et al., 2023) |
| Anogenital distance | 1N (No effect on AGD in MOG study, NTP 2021 in Mustieles et al., 2023) |
| Estrous cyclicity (EAS) | 4Y,1N (4Y: Increased estrous cycle length in rats and mice, French et al., 1992 + effects on estrous cyclicity in adult F1 offspring, NTP 2021 + change in proportion of mice in different estrous stages, Kariagina et al., 2020 - all in Mustieles et al., 2023) (1N: No changes in estrous cycle length in F1 female mice, Chapin et al., 1997 - in Mustieles et al., 2023) |
| Mammary gland histopathology (EAS) | 2Y (2Y: Changes in mammary gland development (LaPlante et al., 2018 + Matouskova et al., 2020 in Mustieles et al., 2023)) |
| Nipple development (EAS) | 1N (No nipple retention in MOG study, NTP 2021 in Mustieles et al., 2023) |
| Sperm parameters (EAS) | 5Y,2N (Y: Oral 90-days study in rats: Decreased epididymal sperm concentration in highest dose group which also had marked decreased body weight, also 14% sperm concentration in lower dose, but non-significant (French et al., 1992 in Mustieles et al., 2023)) (Y: Oral 90-days study in mice: Decreased sperm concentration in highest dose group and increase in sperm abnormalities in all dose groups (French et al., 1992 in Mustieles et al., 2023) (Y: Dermal 90-days study in mice: Decreased sperm concentration in all dose groups, French et al., 1992 in Mustieles et al., 2023) (N: Dermal 90-days study in rats: No effects on sperm parameters, French et al., 1992 in Mustieles et al., 2023) (Y: Dermal 90-days study in mice: Non-significant 15-20% reductions in sperm concentration but conclusion that BP-3 did not affect male reproduction (Daston et al., 1993 in Mustieles et al., 2023) (N: RACB study in maci: No effect on sperm motility, morphology or number in F1, Chapin et al., 1997 in Mustieles et al., 2023) (Y: MOG study in rats: number of spermatocytes per seminiferous tubule reduced at increase in apoptotic germ cells, Nakamura et al., 2015 in Mustieles et al., 2023) |
| **"Sensitive to, but not diagnostic of, EATS" (ECHA/EFSA guidance)** | |
| Fetal development | 3N (3N: Only adverse developmental effects at doses causing maternal toxicity (Chapin et al., 1997, Nakamura et al., 2015, NTP, 2021 - all in Mustieles et al., 2023)) |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-3 - CAS 131-57-7** | |
|---|---|
| Implantation loss | 1Y (Lower percentage of mouse embryos that reached the blastocyst stage and increased early apoptosis in the oocytes (Jin et al., 2020 in Mustieles et al., 2023)) |
| Learning and memory in offspring | 1Y (Weakened offspring spatial memory (but no effect on short-term memory), Pomierny et al., 2019 in Mustieles et al., 2023) |
| Tumour types | 2Y (Y: Combination of high fat diet and BP-3 increased tumour cell proliferation and decreased tumour apoptosis, Kariagina et al., 2020 in Mustieles et al., 2023) (Y: Increased incidence of C-cell adenoma in thyroid glands in mid-dose females in 2-year carcinogenicity study, NTP, 2019 in Mustieles et al., 2023) |
| **Fish** | |
| **Other in vivo mechanistic** | |
| Effects on gene transcription | 5Y (3Y: Er-α and β upregulated (Meng et al., 2020; Thia et al., 2020; Xu et al., 2021)) (2Y: Steroid synthesis related genes downregulated (Blüthgen et al., 2012; Kim et al., 2014)) |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Vitellogenin | 4Y (Coronado et al., 2008, Kim et al., 2014, Kinnberg et al., 2015 (adult male zebrafish); Zhang et al., 2020). 3Y (upregulated Vtg gene expression (Rodriguez-Fuentes et al., 2015; Thia et al., 2020; Xu et al., 2021). 3N for Vtg protein (Blüthgen et al., 2012, Kunz et al., 2006, Kinnberg et al., 2015 (juvenile zebrafish), |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| secondary sex characteristics | 2Y (Kinnberg et al. (2015), Xu et al. (2021)) |
| sex ratio | 2Y (Kinnberg et al. (2015), Xu et al. (2021)) |
| **"Sensitive to, but not diagnostic of, EATS" (ECHA/EFSA guidance)** | |
| other activities | 7Y Behavioural effects and DNT (Chen et al., 2016; Portrais et al., 2019; Tao et al., 2020; Moreira & Luchiari. 2022; Bai et al., 2023; Tao et al., 2023; Wang et al., 2023)) |
| other adverse | 1Y (Decreased oocyte maturation and numbers) |
| **Amphibians** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-3 - CAS 131-57-7** | |
|---|---|
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Effects on testis | 1Y Decreased testis area, Decreased cell proliferation in testes, Down regulation testis related gene expression and upregulation of ovary related gene expression (Li et al., 2023) |
| **Thyroid-relevant** | |
| **In vitro** | |
| TPO activation | 1N (Song et al., 2012) |
| TPO inhibition | 2N (Song et al., 2012 + Schmutzler et al., 2007 in Mustieles et al., 2023) |
| TTR binding | 1Y (Weak interaction with TTR, Cotrina et al., 2023) |
| TR agonist | 2Y (Schmutzler et al., 2007 + Hofmann et al., 2009 in Mustieles et al., 2023) |
| Altered gene expression | 1Y (Lee et al., 2018) |
| Inhibition of Th synthesis | 1N (Paul et al., 2014 in Mustieles et al., 2023) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| T3 and/or T4 level | 2Y,1N (Y: Non-significant reduction in serum T3 levels in high dose males, Nakamura et al., 2015 in Mustieles et al., 2023 Y: Increased free T3 and T4 and decreased TSH levels, Skorkowska et al., 2020 in Mustieles et al., 2023) (N: No effects on TSH, free T3, T4 in developmentally exposed animals, Krzyzanowska et al., 2018 in Mustieles et al., 2023) |
| Thyroid stimulating hormone level (TSH) | 1Y, 1N (Y: Increased free T3 and T4 and decreased TSH levels, Skorkowska et al., 2020 in Mustieles et al., 2023) (N: No effects on TSH, free T3, T4 in developmentally exposed animals, Krzyzanowska et al., 2018 in Mustieles et al., 2023) |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Thyroid weight/histopathology | 1N (N: No changes in follicular cells in thyroid glands in 2-year carcinogenicity study, NTP, 2019 in Mustieles et al., 2023) |
| **"Sensitive to, but not diagnostic of, EATS" (ECHA/EFSA guidance)** | |

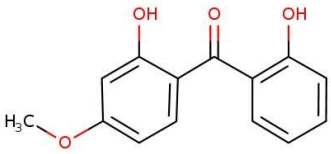
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-3 - CAS 131-57-7** | |
|---|---|
| Fetal development | 3N (3N: Only adverse developmental effects at doses causing maternal toxicity (Chapin et al., 1997, Nakamura et al., 2015, NTP, 2021 - all in Mustieles et al., 2023)) |
| Litter/pup weight | 1Y (Y: Small reductions in fetal and neonatal body weights in absence of maternal toxicity (Santamaria et al., 2020 in Mustieles et al., 2023) |
| Learning and memory in offspring | 1Y (Weakened offspring spatial memory (but no effect on short-term memory), Pomierny et al., 2019 in Mustieles et al., 2023) |
| Tumour types | 2Y (Y: Combination of high fat diet and BP-3 increased tumour cell proliferation and decreased tumour apoptosis, Kariagina et al., 2020 in Mustieles et al., 2023) 1Y, 2N (Y: Increased incidence of C-cell adenoma in thyroid glands in mid-dose females in 2-year carcinogenicity study, NTP, 2019 in Mustieles et al., 2023) |
| **Fish** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Thyroid hormones | 2Y (Y: decreased T4,T3 (Lee et al., 2018 (only strong trend for T4), Sun et al., 2021 (decreased T3 concentration and decreased T3/T4 ratio as well as downregulation of thyroid hormone system related genes) ) |
| **Other ED-related effects** | |
| Motility of human sperm cells | 1Y (Rehfeld et al., 2016,2018 in Mustieles et al., 2023) |
| Decreased PR transcription | 1Y (Schreurs et al., 2005 in Mustieles et al., 2023) |
| PPAR-gamma agonism | 1Y (Shin et al., 2020 in Mustieles et al., 2023) |
| AhR binding | 1Y (Sugihara et al., 2007) |
| Ex vivo neurotox | 1Y (Y: Ex vivo study in mice showed effects on neuronal gene and protein expression and epigenetics - BP3 induced apoptosis, caused neurotoxicity, and decreased ER-alpha and ER-beta expression levels in neocortical cells. Also, hypermethylation of estrogenic genes, Wnuk et al., 2018 in Mustieles et al., 2023) |

# Benzophenone-8, CAS no. 131-53-3

**Synonyms**: Dioxybenzone, BP-8, DHMB, 2,2'-Dihydroxy-4-methoxybenzophenone, (2-hydroxy-4-methoxyphenyl)-(2-hydroxyphenyl)methanone,

**Structure:**




**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu) **https://echa.europa.eu/da/registration-dossier/-/registered-dossier/23375**
Searched 26th of Sep 2023: CAS no 131-53-3
Latest update 29th of March 2018: 1-10 t/a

**3) SciFinder CAS**
26th September 2023
Search: 131-53-3
1799 Results
Filter: Freetext: Endocrine*
Filter: Language: English
**99 results**

| **Benzophenone-8 - CAS 131-53-3** | |
|---|---|
| **REACH registration status** | **1-10 t/a** |
| **OECD TG studies in REACH dossier** | Low tonnage: No repeated dose or reproductive toxicity info |
| **Metabolism** | |
| Rodents | M1 and M2 (Zhan et al., 2021a), M1 is 2,2',4-triOH BP |
| **EAS-relevant** | |
| **In vitro** | |
| Estrogenicity (E) | 1Y (Kawamura et al., 2005) |

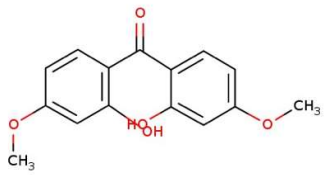
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-8 - CAS 131-53-3** | |
|---|---|
| Anti-A | 1Y (Kawamura et al., 2005) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Uterotrophic (E) | 1 N (Zhan et al., 2021a) |
| Other mechanistic (E) | 1 Y (Change in gene expression in mice uterus similar to estradiol, Zhan et al., 2021b) |
| **Fish** | |
| **Other in vivo mechanistic** | |
| Effects on gene transcription | 1Y (Y: agonists of the estrogen receptors α and β1 in zebrafish embryos (Meng et al., 2020)) |
| **Thyroid-relevant** | |
| **In vitro** | |
| TPO activation | 1Y (Increased TPO activity, Song et al., 2012) |
| TTR binding | 1Y (Weak interaction with TTR, Cotrina et al., 2023) |
| Altered gene expression | 1Y (Lee et al., 2018 ) |
| **Fish** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Thyroid hormones | 1Y (Decreased T3,T4 (Lee et al. (2018)) |

# Benzophenone-6, CAS no. 131-54-4

**Synonyms:** 2,2'-dihydroxy-4,4'-dimethoxybenzophenone, 2-(2-hydroxy-4-methoxybenzoyl)-5-methoxyphenol, BP-6

**Structure:**



**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu) **https://echa.europa.eu/da/registration-dossier/-/registered-dossier/25592**
Searched 26th of Sep 2023: CAS no 131-54-4
Latest update 30th of March 2018: 1-10 t/a

**3) SciFinder CAS**
26 September 2023
Search: 131-54-4
1035 Results
Filter: Freetext: Endocrine*
**43 results**

| **Benzophenone-6 - CAS 131-54-4** | |
|---|---|
| **REACH registration status** | **1-10 t/a** |
| **OECD TG studies in REACH dossier** | Low tonnage: No repeated dose or reproductive toxicity info |
| **Metabolism** | |
| Rodents | Demethylation, hydroxylation, sulfonation and glucuronidation leads to five main metabolites in rats (Li et al., 2006), including demethylation to 2,2',4-triOH-4'-MeO BP |
| **EAS-relevant** | |
| **In vitro** | |

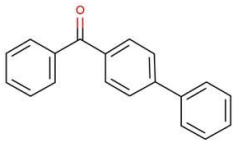
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Benzophenone-6 - CAS 131-54-4** | |
|---|---|
| Estrogenicity (E) | 1Y,2N (Y: Kawamura et al., 2005) (N: Suzuki et al., 2005, Kawamura et al., 2003) |
| Anti-A | 1Y,1N (Y: Suzuki et al., 2005) (N: Kawamura et al., 2005) |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Uterotrophic (E) | 1Y,1N (Y: Subcutaneous: Ohta et al., 2012) (N: Oral: Ohta et al., 2012) |
| Uterotrophic (anti-E) | 2N (Subcutaneous and orally: Ohta et al., 2012) |
| **Other ED-related effects** | |
| AhR binding | 1Y (Sugihara et al., 2007) |

# 4-phenylbenzophenone, CAS no. 2128-93-0

**Synonyms**: 4-Benzoylbiphenyl, [1,1'-biphenyl]-4-ylphenyl-methanone, biphenyl-4-yl(phenyl)methanone, Genocure PBZ, Methanone, [1,1'-biphenyl]-4-ylphenyl-, phenyl(4-phenylphenyl)methanone, {[1,1'-biphenyl]-4-yl}(phenyl)methanone,

**Structure:**



**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu) **https://echa.europa.eu/da/registration-dossier/-/registered-dossier/25482**
Searched 02nd of Nov 2023: CAS no 2128-93-0
Latest update November 2020: 10-100 t/a

**3) SciFinder CAS**
6th October 2023
Search: 2128-93-0
1451 Results
Filter: Freetext: Endocrine*
**9 results**

| **4-phenylbenzophenone - CAS 2128-93-0** | |
|---|---|
| **REACH registration status** | **10-100 t/a** |
| **OECD TG studies in REACH dossier** | Rat TG422 (2017) |
| **EAS-relevant** | |
| **Rodents** | |
| **"Sensitive to, but not diagnostic of, EATS" (ECHA/EFSA guidance)** | |
| Litter size | 1Y (decreased live litter size in TG4222 from 2018) |
| Implantation loss | 1Y (OECD TG 422 (2018): Decreased number of implantation sites + Reduced post-implantation survival) |

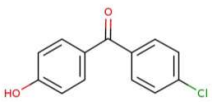
# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **4-phenylbenzophenone - CAS 2128-93-0** | |
|---|---|
| **Thyroid-relevant** | |
| **Rodents** | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Thyroid weight/histopathology | 1Y (OECD TG 422 (2018): Thyroid follicular cell hypertrophy in 300 mg/kg/d males and in 1000 mg/kg/d rats of both sexes, ECHA 2023) |

# 4-chloro-4-hydroxybenzophenone, CAS no. 42019-78-3

**Synonyms:** (4-chlorophenyl)(4-hydroxyphenyl)methanone; (4-chlorophenyl)-(4-hydroxyphenyl)methanone; 4-(4-chlorobenzoyl)phenol; 4-CHBP

**Structure:**




**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu) **https://echa.europa.eu/da/registration-dossier/-/registered-dossier/17952**
Searched 9th Sep 2023: 42019-78-3
Latest update Feb 2018: Intermediate use only

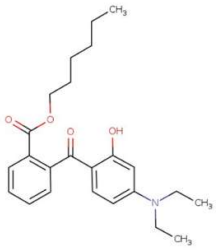
**3) SciFinder CAS**
Searched 9th Sep 2023: 42019-78-3
333 References
Filter Freetext: "Endocrine*"
**9 references**

| **4-chloro-4-hydroxybenzophenone - CAS 42019-78-3** | |
|---|---|
| **REACH registration status** | **Intermediate** |
| **OECD TG studies in REACH dossier** | None - but 4 in vitro studies |
| **EAS-relevant** | |
| **In vitro** | |
| Estrogenicity (E) | 4Y (Kawamura et al., 2003 + 3Y in ECHA reg dossier) |
| Anti-A | 1Y (2005 in REACH registration dossier, ECHA 2023) |

# DHHB, CAS no. 302776-68-7

**Synonyms:** Benzoic acid, 2-(4-(diethylamino)-2-hydroxybenzoyl)-, hexyl ester; Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, Hexyl ester; Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexyl ester; Diethylamino hydroxybenzoyl hexyl benzoate; Hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; Hexyl 2-(4-(diethylamino)-2-hydroxybenzoyl)benzoate; UVINUL A PLUS; Uvinul A Plus; Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexyl ester; DHHB; Hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; Hexyl 2-(4-(diethylamino)-2-hydroxybenzoyl)benzoate; Hexyl 2-[4-(Diethylamino)-2-hydroxybenzoyl]benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate; Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine

**Structure:**



**Searches and dates:**

**1) ECHA registration dossier**
Registered substances - ECHA (europa.eu) **https://echa.europa.eu/da/registration-dossier/-/registered-dossier/29413**
Searched 9th of October 2023: CAS no 302776-68-7
Latest update May 2023: 1000-10.000 t/a

**3) SciFinder CAS**
9th October 2023
Search: 302776-68-7
1284 Results
Filter: Freetext: Endocrine*
**1 result**

| **Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate - CAS 302776-68-7** | |
|---|---|
| **REACH registration status** | **1000-10.000 t/a** |

## Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate - CAS 302776-68-7** | |
|---|---|
| **OECD TG studies in REACH dossier** | Rat TG408 (2001), Rat TG416 (2002), Rat TG414 (2000), Uterotrophic assay (2001), Hershberger (2003) |
| **EAS-relevant** | |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Uterotrophic (E) | 1N (ECHA 2023) |
| Hershberger (A and anti-A) | 1N (ECHA 2023) |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Testis weight/histopathology (EAS) | 1Y (Increased mean relative weights of testes in TG408, ECHA 2023) |

# Blank table – for information

| **Substance name - CAS number** | |
|---|---|
| **REACH registration status** | |
| **OECD TG studies in REACH dossier** | |
| **Metabolism** | |
| Rodents | |
| Fish | |
| **EAS-relevant** | |
| **In vitro** | |
| Estrogenicity (E) | |
| Estrogen-like changes in gene expression, motility etc. | |
| Anti-E | |
| Androgenicity | |
| Anti-A | |
| Steroid synthesis changes | |
| **Rodents** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Estradiol level (EAS) | |
| Follicle stimulating hormone level (EAS) | |
| Luteinising hormone (LH) level (EAS) | |
| Testosterone level (EAS) | |
| Uterotrophic (E) | |
| Uterotrophic (anti-E) | |
| Hershberger (A and anti-A) | |
| **Other in vivo mechanistic** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Substance name - CAS number** | |
|---|---|
| Other mechanistic (E) | |
| Other mechanistic (A/anti-A) | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Age at puberty (EAS) | |
| Anogenital distance | |
| Epididymis weight/histopathology (EAS) | |
| Estrous cyclicity (EAS) | |
| Genital anomalies (EAS) | |
| Mammary gland histopathology (EAS) | |
| Nipple development (EAS) | |
| Ovary weight/histopathology (EAS) | |
| Prostate weight/histopathology (EAS) | |
| Seminal vesicle weight/histopathology(EAS) | |
| Testis weight/histopathology (EAS) | |
| Sperm parameters (EAS) | |
| Uterus weight/histopathology (EAS) | |
| **"Sensitive to, but not diagnostic of, EATS" (ECHA/EFSA guidance)** | |
| Fetal development | |
| Litter size | |
| Implantation loss | |
| Learning and memory in offspring | |
| Tumour types | |
| **Fish** | |
| **Other in vivo mechanistic** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Substance name - CAS number** | |
|---|---|
| Effects on gene transcription | |
| Estrogenicity (E) | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Vitellogenin | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| secondary sex characteristics | |
| sex ratio | |
| **"Sensitive to, but not diagnostic of, EATS" (ECHA/EFSA guidance)** | |
| other activities | |
| other adverse | |
| **Amphibians** | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| vitellogenin | |
| sex ratio | |
| Effects on testis | |
| **Thyroid-relevant** | |
| **In vitro** | |
| TPO activation | |
| TPO inhibition | |
| TTR binding | |
| TR agonist | |
| Altered gene expression | |
| Inhibition of Th synthesis | |
| **Rodents** | |

# Annex 5 - Preliminary literature screening of a group of 12 benzophenones

| **Substance name - CAS number** | |
|---|---|
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| T3 and/or T4 level | |
| Thyroid stimulating hormone level (TSH) | |
| **"EATS-mediated" (ECHA/EFSA guidance)** | |
| Thyroid weight/histopathology | |
| **"Sensitive to, but not diagnostic of, EATS" (ECHA/EFSA guidance)** | |
| Fetal development | |
| Litter/pup weight | |
| Learning and memory in offspring | |
| Tumour types | |
| **Fish** | |
| **"In vivo mechanistic" (ECHA/EFSA guidance)** | |
| Thyroid hormones | |
| **Other ED-related effects** | |
| Motility of human sperm cells | |
| Decreased PR transcription | |
| PPAR-gamma agonism | |
| ERR-gamma | |
| AhR binding | |
| Ex vivo neurotox | |

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## Annex 5 - Preliminary literature screening of a group of 12 benzophenones

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