

**Analysis of the most appropriate risk management option (RMOA)**

**Substance Name: Melamine**

**EC Number: 203-615-4**

**CAS Number: 108-78-1**

**Authority: FR CA**

**Date: 16/05/2023**

**Cover Note**

In the framework of the second National Strategy on Endocrine Disruptors (SNPE 2 2019-2022), the French Competent Authority requested ANSES to evaluate the endocrine disrupting profile of Melamine EC 203-615-4/CAS 108-78-1. This RMOA, specifically dedicated to the ED profile of Melamine, comes in addition to the german RMOA (https://www.reach-clp-biozid-helpdesk.de/DE/REACH/Verfahren/SVHC-Verfahren/Stoffliste-EN/Stoffliste-EN.html) published in 2022.

**Comments and additional relevant information are invited on this RMOA by 15 September 2023.**

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# identity of the substance

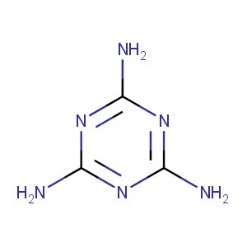
## Name and other identifiers of the substance

**Table 1: Substance identity**

|  |  |
| --- | --- |
| **EC name (public):** | Melamine |
| **EC number** | 203-615-4 |
| **CAS number** | 108-78-1 |
| **IUPAC name (public):** | 1,3,5-triazine-2,4,6-triamine |
| **Index number in Annex VI of the CLP Regulation:** | 613-345-00-2 |
| **Molecular formula:** | C3H6N6 |
| **Molecular weight or molecular weight range:** | 126,12 g/mol |
| **Synonyms:** | *Inter alia:*  *Cyanuramide*  *Cyanurotriamine*  *Triaminotriazine* |

**Type of substance**  Mono-constituent  Multi-constituent  UVCB

**Structural formula:**



# OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

**Table 2: Completed or ongoing processes**

|  |  |  |
| --- | --- | --- |
| RMOA |  | Risk Management Option Analysis (RMOA) other than this RMOA |

|  |  |  |
| --- | --- | --- |
| REACH Processes | Evaluation | Compliance check, Final decision |
| Testing proposal |
| CoRAP and Substance Evaluation |
| Authorisation | Candidate List |
| Annex XIV |
| Restri-ction | Annex XVII |
| Harmonised C&L |  | Annex VI (CLP) (see section 5.1) |
| Processes under other EU legislation |  | Plant Protection Products Regulation  Regulation (EC) No 1107/2009 |
|  | Biocidal Product Regulation  Regulation (EU) 528/2012 and amendments |
|  | Cosmetic Regulation  Regulation (EC) N° 1223/2009 |
| Previous legislation |  | Dangerous substances Directive  Directive 67/548/EEC (NONS) |
|  | Existing Substances Regulation  Regulation 793/93/EEC (RAR/RRS) |
| (UNEP) Stockholm convention (POPs Protocol) |  | Assessment |
|  | In relevant Annex |
| Other processes/ EU legislation |  | Other (provide further details below)  Plastic food contact materials  Regulation (EU) No 10/2011 (Annex I; to be used as  additive or monomer, specific migration limit: 2.5  mg/kg food) |

# CONCERN(S) SUBJECT TO EVALUATION

Melamine (EC 203-615-4/CAS 108-78-1) was assessed by DE CA for its concern regarding PMT/vPvM properties. A Risk Management Option Analysis (RMOA) Conclusion Document has been published[[1]](#footnote-1) in June 2022 to help the authorities decide whether further regulatory risk management activities were required for this substance and to identify the most appropriate instrument to address a concern. First, DE CA indicated his intention for an inclusion of Melamine as a substance of very high concern (SVHC) under article 57(f) (Equivalent level of concern having probable serious effects to human health and to the environment). The 15th of December 2022, an agreement was reached at the member state committee (MSC) on the identification of melamine as a substance of very high concern[[2]](#footnote-2) and melamine was included in the candidate list for eventual inclusion in Annex XIV as of 17th of January 2023.

In addition, DE CA indicates its intention to revise the actual harmonised classification of Melamine. A RAC opinion was previously adopted the 10th of December 2020 for harmonised classification of melamine as STOT RE 2, H373, and Carc. 2, H351. Following the dossier evaluation, a testing proposal for an extended one-generation reproductive toxicity study (EOGRTS; EU B.56./OECD TG 443) was submitted by industry (Submission Number: WS600383-16) and a decision was issued by ECHA (TPE-C-2114529505-49-01/F, 2017), supporting the conduct of the EOGRTS. Given that the outcome of the study was not available by the time the CLH-dossier was finalised, DE CA did not assessed this endpoint. To date, the results of the EOGRTS study have been made available, were assessed, and raised the concern for reproductive toxicity. Based on the histopathological changes and altered sperm cell morphology observed in P0/F1 animals, DE CA feels confident that classification for effects on sexual function is warranted.

In the framework of the second National Strategy on Endocrine Disruptor chemicals (SNPE 2)[[3]](#footnote-3) aiming at reducing the impact of Endocrine Disruptors Chemicals (EDCs) on the population and on the environment, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) was mandated on 8 October 2019 to identify and prioritize “chemicals that may present Endocrine Disruptor (ED) properties” for building a list that is scientifically robust, resulting from an inventory of published lists at European and international levels (Anses, 2021). This list aims, firstly, at informing on substances that may have potential endocrine properties regardless of their sectors of use and the sectorial regulations applicable, thus warranting an in depth evaluation of the ED data available.

In total, 27 initiatives have been identified. These initiatives were qualitatively compared and grouped according to their scope, selection criteria, processes, and information included. Based on this detailed analysis of the different initiatives and due to its specific strengths, the DEDuCT (Database of Endocrine Disrupting Chemicals and their Toxicity Profiles)-2019 methodology was selected as a starting list by the experts, in particular because it has a robust selection process to identify potential EDCs rather than substances having endocrine activities only.

The starting point for this prioritisation exercise is the list of substances of interest. This list covers all substances regardless of their sector of use or the sectoral regulations concerned, based on the methodology established by the ANSES ED-WG (DEDuCT-2019: 686 substances) together with the 81 substances used as co-formulants in PPP and BP identified by ANSES during their evaluation. It also contains the 197 substances ranked category I, II, III in the EU Impact Assessment (2016). As 13 co-formulants and 45 active substances were initially proposed by DEDuCT, the list of substances of interest as regards to their potential endocrine activity contains a total of 906 substances.

The list of substances of interest has been fine-tuned before being proposed for prioritisation. 12 are already identified as substances of very high concern (SVHC) due to their ED properties. In addition, 12 active substances are already banned in PPP under 79/117/EC12 and/or 850/2004/EC13 and prohibited in cosmetics. Among them, 7 are Persistent Organic Pollutants that are very strictly regulated. These 12 substances are already known hazardous substances. Independently of any ED property, they are regulated and do not need to be prioritized for further assessment and potential regulation. The 163 active substances regulated by PPPR and the 39 BPR (among which 27 are also planned under PPPR) will be evaluated with the calendar defined by these regulations and are therefore not entering the pool of prioritisation setting unless other uses exposing population can be demonstrated. Therefore, on the 906 substances identified initially, the remaining 705 substances are considered for further evaluation and are proposed for prioritisation

Among the 705 substances proposed for prioritisation, 266 only are registered under REACH. In order to identify the substances to be assessed by ANSES in the frame of its annual work program, the ministries have proposed selection criteria based on REACH registration status and on-going regulatory actions. After applying the criteria proposed by the ministries, 59 substances have been selected. Additional criteria were added aiming at selecting a target of around 20 substances for the scoring exercise, focusing on substances in the scope of its activities and for which a higher impact is expected. Melamine was one of the remaining 16 candidates substances due to his hazard criteria, exposure potential and uses, and societal concern.

Thus, based on the work performed on prioritisation, the French Ministry of Ecological and Solidarity Transition and the French Ministry of Solidarity and Health mandated the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) to evaluate the endocrine disrupting profile of melamine.

# USES

## Tonnage and registration status

**Table 3: Tonnage and registration status**

|  |  |
| --- | --- |
| **From ECHA dissemination site** | |
| **Registrations** | **Full registration(s)**  **(Art. 10)**  **Intermediate registration(s)**  **(Art. 17 and/or 18)** |
| **Total tonnage band for substance (excluding volume registered under Art 17 or Art 18, or directly exported)** | 100,000-1,000,000 tpa |

## Overview of uses

According to ECHA disseminated website[[4]](#footnote-4), melamine is used in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

Melamine is used in consumer and commercial paints and coatings (for the production of consumer goods such as tableware), in foam seating and bedding and in melamine-formaldehyde resin that is used by the woodworking industry to produce different types of wood based panels including laminate flooring and melamine-impregnated papers and foils for surface-coating panels.

Melamine and its salts with acids such as cyanuric acid, boric acid and phosphoric acids are available commercially for fire safety applications (EFSA, 2010). According to the Canadian assessment of the substance (Health Canada, 2020), melamine is used as a flame retardant mainly in polyurethane foams and is often used in combination with numerous other flame retardants such as bicyclic phosphate, decabromodiphenyl ether (decaBDE), antimony oxide, Dechlorane Plus (DP), and others, and in polyolefin formulations for use in plastics and elastomers. Melamine is also used in the production of other flame-retardants, such as melamine cyanurate (CAS RN 37640-57-6), melamine phosphate (CAS RN 20208-95-1), melamine polyphosphate (CAS RN 218768-84-4), and melamine pyrophosphate (CAS RN 15541-60-3).

Due to its high nitrogen content, melamine has applications in agriculture and can be used as a slow-release fertilizer (Health Canada, 2020). In addition, melamine may also occur in food as a metabolite and degradation product of cyromazine, which is an insect growth inhibitor used as a plant protection product (EFSA, 2010).

**Uses advised against**

Uses advised against consistently discourage applicants of melamine from using the substance for the production of /addition to food or feed products as a nitrogen source. Illegal adulteration of food and feed with melamine has resulted in illness and deaths of human infants and pet animals (cats and dogs), primarily as a result of kidney damage caused by crystals or stones in the urinary tract. One of the most famous case is the contamination incident of 2008 in China, where the compound was added illegally to powdered milk and baby formulas with the intention of falsifying protein content causing an outbreak of urinary tract stones and renal-failure. At least 6 children have died in China from severe kidney failure due to the melamine added to milk powder, and more than 200.000 infants and young children have been affected by kidney problems with more than 50.000 infants and young children hospitalized (EFSA, 2010).

# harmonised classification and labelling

## Harmonised Classification in Annex VI of the CLP

A proposal for harmonized classification of melamine was submitted in 2019. RAC opinion was adopted on December 2020 for harmonised classification of melamine as STOT RE 2, H373 (urinary tract system), and Carc. 2, H351. Melamine has been included in the 18th Adaptation to Technical Progress of 16 February 2022. The new harmonised classification and labelling shall apply from 23 November 2023.

## Self classification

Classification notified by industry to ECHA in addition to harmonized classification:

* In the registration:

Repr. 2; Suspected of damaging fertility H361f

* The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

**Table 4: Hazard classes notified as self-classifications**

|  |  |
| --- | --- |
| Hazard Class and Category Code(s) | Hazard Statement Code(s) |
| Repr. 2 | H361 |
| Aquatic Acute 1 | H400 |
| Aquatic Chronic 1 | H410 |
| Skin Corr. 1C | H314 |
| Skin Sens. 1 | H317 |
| Skin Irrit. 2 | H315 |
| Eye Irrit. 2 | H319 |
| Acute Tox. 4 | H332 |
| STOT SE 3 | H335 |

## CLP Notification Status

**Table 5: CLP Notifications**

|  |  |
| --- | --- |
|  | **CLP Notifications[[5]](#footnote-5)** |
| Number of aggregated notifications | 20 |
| Total number of notifiers | 863 |

# Environmental fate properties

Environmental fate properties have been analysed in-depth by DE CA in its proposal for identification as a substance of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) as there is scientific evidence of probable serious effects to the environment and human health which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of the REACH Regulation. A summary of ECHA disseminated website data as submitted by the registrant, the conclusion of the substance assessment as reported in the RMOA conclusion document (2022) and the support document for identification of melamine as a SVHC[[6]](#footnote-6) are reported in this section.

## Degradation

Hydrolysis is not expected to be an important fate pathway under environmentally relevant conditions due to the structural properties of melamine and abiotic degradation is not considered a relevant pathway for the removal of melamine in both air and water.

Melamine was found to be not readily biodegradable (OECD TG 301C test 0 % degradation (oxygen demand) after 14 days), and not inherently biodegradable (in two OECD TG 302: < 10 % degradation (DOC removal) was after 28 and 16 % degradation (DOC removal) after 20d). The surface water simulation study (OECD TG 309) indicated a calculated half-life (DT50) of over 60 days. For degradation in soil, based on a non-standard test on degradation of melamine (Hauck and Stephenson, 1964), it is concluded that the degradability of melamine is expected to be slow in the soil compartment (in the range of 2-3 years). According to the data, the DE CA concluded that the substance is persistent and very persistent in surface water according to the persistency criteria of REACH Annex XIII.

Melamine is known to be metabolized through three consecutive hydrolytic deamination reactions; Ammeline and ammelide, two intermediate transformation products, are converted into cyanuric acid (Health Canada, 2020).

## Environmental distribution

Melamine has a low tendency to adsorb to organic matter (results in the range of log Koc < 2 for the non-ionic forms of melamine whereas log KOC < 3.5 estimated for the ionic forms) and is expected to remain in the water phase once the substance is released to the environment.

Due to its high water solubility (3.48 g/L) and low log Koc melamine is not likely to be efficiently removed by adsorption to organic materials in sewage treatment plants or in drinking water production.

DE CA concluded that the substance is very mobile in aquatic environment. The consequence resulting from these substance properties in addition to the gathered information on the behavior of the substance in STP raises concern for drinking water resources, drinking water and the remediation. In addition, results of the TaPL3 model (2003) and the OECD POV and LRTP Screening Tool (OECD 2009) indicated a potential for long-range transport of the substance in water (Health Canada, 2020).

## Bioaccumulation

According to the registration dossier[[7]](#footnote-7), three experimental determinations of the bioconcentration factor (BCF) in 3 species of fishes were reported. The BCF was <1 in each case, except for the *Cyprinus carpio* study with the lower concentration of 0.2 ppm in water where the BCF was 3.8 L/kg ww. This BCF value is therefore taken as key value.

The calculated log Koa for the substance is 11.12 and the reported log Kow for melamine is -1.22. Considering these two parameters, terrestrial bioaccumulation is considered low although no experimental studies gives clear information of the bioaccumulative potential of melamine in terrestrial organisms.

The substance is therefore not considered as bioaccumulative in aquatic organisms according to the bioaccumulation criteria of REACH Annex XIII. No conclusion can be clearly drawn for terrestrial bioaccumulation.

## Summary and discussion of environmental fate properties

After assessment[[8]](#footnote-8), DE CA concludes that the substance is very persistent in the environment and is very mobile in aquatic environment. Melamine is considered to have a low bioaccumulation potential. However, the long residency time in water and soil compartment raise an important concern for environmental hazard and most specifically for the drinking water sources.

# Health hazard Assessment

Human health hazards relevant for endocrine disruption assessment presented in section 7 are based on available data from the chemical safety report (CSR OCI Nitrogen B.V., 2020) of melamine (EC 203-615-4/CAS 108-78-1), the ECHA registration dossier[[9]](#footnote-9), the DE CA assessment in the course of CLH report (2019)[[10]](#footnote-10) and the RAC opinion proposing harmonised classification and labelling at EU level of melamine (2020)[[11]](#footnote-11).

The scientific literature review assessing ED properties of melamine will be presented under section 9.

## Toxicokinetics (absorption, distribution, metabolisation and elimination).

Numerous studies in the registration dossier[[12]](#footnote-12) assessed the toxicokinetic, metabolism and distribution endpoints for melamine. These information are also reported and had been assessed by DE CA in the CLH report for the proposal for harmonised classification and labelling of melamine (BAuA, 2019).

According to the key study in rats (Mast *et al*., 1983), the oral absorption is fast and rather complete (73 to 98 %), with a distribution/absorption half-time of 0.25 h. The time of maximal plasma concentration were found within less than 60 minutes after dosing.

There are only a few studies available where metabolism of melamine had been investigated. In the key study performed by Mast *et al*. (1983) in rats, no metabolism of melamine was indicated and melamine was considered to be excreted unchanged. While detailed information concerning pharmacokinetic in humans is not available, melamine was, similarly to the observations in animals, detected unmetabolised in the urine of paediatric patients that had been exposed to melamine-tainted milk products (Cheng *et al*., 2009; Kong *et al*., 2011; Lam *et al*., 2009; Zhang *et al*., 2010b).

In this study from Mast *et al*. (1983) distribution was also investigated. The authors found a fast distribution to the tissues with highest concentrations detected in the kidney and the urinary bladder. The results further indicate a fast melamine excretion from tissues. At 96 h post-dosing, melamine was detectable in the liver and the kidney, but only at very low concentrations. Although kidney is one of the organs with the highest detected melamine concentration, many studies indicate a fast distribution of melamine to most tissues (including liver, stomach, spleen, heart, uterus, ovaries, testis, brain, bladder, and lungs). Evidence is also made that melamine passes the placental barrier (Partanen, 2012) and is detected in foetal tissues. This implies that melamine exposure can occur during critical periods of development (i.e., during foetal development) for both animals and humans.

Additionally, Mast *et al*. (1983) found a high clearance from plasma with a plasma half-life of 2.7 h and a fast excretion from the whole body. This finding is supported by high clearance from plasma in other supporting oral studies. About 90 % of the dose was excreted 24 h post-dosing. The urinary excretion was found to be the sole route of elimination with a fast elimination half-life of 3.0 h. At higher dose levels (Wu *et al*., 2011), excretion via faeces becomes predominant compared to excretion via urine (61 versus 25 % 24 h after dosing). Nevertheless, very low levels of melamine were still detectable at 168 h post-dosing in plasma, urine, and faeces. In a randomized crossover human study (Wu *et al*. 2013), urinary melamine excretion subsequent to low-dose melamine exposure (migration from melamine resin plastic bowls) was investigated and the half-life of urinary melamine elimination was of 6h, approximately. Hence, as melamine undergoes rapid renal clearance in multiple mammalian species, it appears likely that humans show a similar pharmacokinetic.

## Subchronic and chronic toxicity

CLH report (BAuA, 2019) mentioned that “*A substantial body of evidence concerning melamine-related toxicity following repeated oral exposure exists and a clear and consistent relationship between repeated melamine exposure and significant adverse health effects in humans can be established. [..]Significant adverse effects in experimental animals derived from sub-acute, sub-chronic, and chronic studies are mainly and consistently observed* ***in the urinary tract system, comprising urolithiasis and signs of nephrotoxicity such as chronic inflammation and renal injuries****. Consequently, the urinary tract system, specifically the kidney and the urinary bladder, was identified as the main target organ system (Bhat et al., 2010; Dalal and Goldfarb, 2011; Deng and Li, 2012; Early et al., 2013; Hau et al., 2009; NTP, 1983; WHO / FAO, 2009)*”. The proposed mechanism of melamine-induced nephrotoxicity involves induction of oxidative stress and an inflammatory response triggered by renal crystals (EFSA 2010; Chu and Wang 2013; Rai et al. 2014 in Health Canada 2020).

In the CLH dossier, studies provided by NTP technical report (1983) and Early *et al*. (2013) were identified as key information sources considered relevant for classification.

The data set concerning sub-chronic repeated dose toxicity (NTP, 1983) consists of three different 13-weeks (91-day) studies with repeated oral melamine administration in rats. The studies aimed at identifying the cumulative toxic effects of melamine, the primary target organ, and the appropriate concentration for a subsequent carcinogenicity study. The effective dose level based on the observed effects may be set at 72 and 84 mg/kg bw/d for **male (urolithiasis) and female (calcareous renal deposits)**, respectively. This study with dietary exposure of male rats to melamine provided the best basis for characterising the dose-response relationship in experimental animals. For a 10 % increase in the incidence of urinary bladder crystals, a benchmark dose (BMD10) of 41 mg/kg bw/d and its lower confidence limit (BMDL10) of 19 mg/kg bw/d (EFSA, 2010). A tolerable daily intake (TDI) was derived from this value. The EFSA Panel considered the factors that could contribute to inter and intra-species differences in the effects of melamine, such as the impact of urinary uric acid concentrations and pH on the formation of the melamine complexes with uric acid, and concluded that the default uncertainty factor of 100 was appropriate for deriving a tolerable daily intake (TDI). The Panel established a TDI of 0.2 mg/kg bw which is considered appropriate for infants, except for those born prematurely who have higher urinary uric acid levels and greater immaturity of kidney function.

The second key study was realized by Early *et al*. (2013). The effects of repeated oral (gavage) melamine administration (140, 700, 1400 (lowered to 1,000 mg/kg bw/d subsequently due to mortality) mg/kg bw/d) for 14 consecutive days in rats were investigated. The study was conducted according to GLP and can be assigned as similar to OECD guideline TG 407 with some deviations mostly in terms of the test duration. The study identified the kidney as the main target organ related to melamine-mediated repeated dose toxicity. Renal damages (necrosis/degeneration/hyperactive regeneration of distal nephron tubular epithelium of ♂/♀ rats, reduced renal function, crystal depositions) were observed in all animals subjected to ≥ 700 mg/kg bw/d, which, when extrapolated to a 90-day study design, would give an effect dose level of 108.9 mg/kg bw/d, which is not coherent with the NOAEL proposed in this study. Due to widely spaced dose selection (2- to 4-fold recommended by OECD TG 407/408 vs. 5-fold in the current study between the low and mid dose), uncertainty exists as to which exposure levels below the mid-dose are lacking effects. A NOAEL of 140 mg/kg bw/d was still derived by the authors of the study. At 700 mg/kg bw/d, the incidence of renal injuries was 100 %. It is expected that such effects would occur at doses < 700 mg/kg bw/d although at lower incidences. Indeed, it was observed a 33 % increase in the renal crystal incidence in females, which may be considered adverse at 140 mg/kg bw/d. The crystals are regarded to be nephrotoxic and a clear threshold concentration as to when crystals become toxic has yet not been established. Beyond that, renal crystal formation is regarded as initial key event in the MoA culminating in severe epithelial damages and cancer. Thus, from a conservative perspective, 140 mg/kg bw/d was set as the first effective dose level instead of a NOAEL. Benchmark was employed to better reflect the pattern of this dose-response relationship. The benchmark modelling revealed a BMD10 of 292.036 mg/kg bw/d (BMDL10=138.40) which, when extrapolated to a 90-day study design, would give an effective dose of 45.4 mg/kg bw/d.

The EOGRTS OECD TG 443 study (detailed in section 7.5-reproductive toxicity) was not available at the time of the CLH report. The effects observed in this study also confirmed that kidney is identified as a target organ of melamine in parental animals and progenies (Study report, 2020). The NOAEL is based on findings of retrograde nephropathy (a kidney damage due to urine flowing backward (reflux) from the bladder toward the kidneys) in both generations and sexes at 4000 ppm. In conclusion for this study, kidney is a target organs with macroscopic findings, this adverse effects appeared at 4000ppm. Based on adverse nephrotoxic effects, the NOAEL was set at 1000 ppm (65/87 mg/kg bw/d M/F) for P0 generation and 1000 ppm (89/93mg/kg bw/d M/F) for F1 generation.

## Genotoxicity as presented in the CLH report (2019)

“*Numerous in vitro and in vivo genotoxicity studies are available for melamine. […]However, none of the studies were performed according to the respective standardised OECD test guidelines (TG) without deviations. Hence, a key study (OECD TG without deviations) that would provide data to conclusively assess the mutagenic potential of melamine (e.g. with confidence in the presence or absence of an effect) in a corresponding test system, was not identified.*

*There is no evidence for melamine-induced genotoxic effects in vitro from the available data. There is no evidence for melamine-induced genotoxic effects in vivo (soma cells) from the available data. No human data are available. Available in vitro and in vivo genotoxicity tests performed with melamine which are considered relevant and reliable (with restrictions) are consistently negative for the respective genotoxic test system. Hence, there is no evidence of induction of gene mutations, clastogenic effects or aneuploidy. Classification criteria for germ cell mutagens are not fulfilled for melamine*.”

The risk assessment committee (RAC) gave also an opinion (2020)[[13]](#footnote-13) following the assessment for melamine classification mentioning that: “*Overall, based on negative results in vitro and mostly negative results in vivo, RAC agrees with the DS that* ***no classification for germ cell mutagenicity is warranted for melamine***.”

## Cancerogenicity as presented in the CLH report (2019)

“*Several studies concerning the carcinogenic potential of melamine have been conducted in experimental animals. Evidence for melamine-related carcinogenic effects is mainly derived from multiple studies with rats. There is limited evidence from mice as only two studies address tumorigenesis in this species. For the purpose of classification, four key (Hazleton, 1983; NTP, 1983; Ogasawara et al., 1995; Okumura et al., 1992) and three supporting studies were identified (Cremonezzi et al., 2004; Cremonezzi et al., 2001; Hazleton, 1953). Altogether, a dose-response relationship with respect to* ***urinary tumour formation*** *can be established when combining the data from the four key studies. In summary, the results listed […] constitute convincing and sufficient evidence of carcinogenic activity evoked by dietary melamine exposure in experimental animals. The results from several key studies in experimental animal models with oral exposure to pure melamine demonstrate strong evidence for neoplastic findings in the urinary bladder of male rats, thus providing sufficient evidence of melamine-mediated carcinogenicity in animals that may potentially justify the classification in category 1B. In addition to the clear effects in male rats, further supporting studies provide limited evidence of carcinogenic effects in female rats and mice. Considering the overall evidence for melamine-mediated carcinogenesis, classification in category 2 rather than category 1B is considered most appropriate for the following reasons: 1) Sufficient evidence of carcinogenicity (benign and malignant tumours) only in the urinary bladder of male rats (key studies in experimental animal studies), 2) Supporting studies demonstrate the induction of only benign tumours and preneoplastic lesions, 3) Non-genotoxic mode of action, 4) Secondary mechanism of action with a threshold, and 5) Sufficient evidence indicating relevance to human carcinogenicity*.”

Following this assessment, RAC (2020)[[14]](#footnote-14) emitted the opinion that“*[…] the critical issue is the ability of melamine to reach a threshold concentration in human urine in order for calculi to form. Such a threshold cannot be established based on the available human data as there are too many uncertainties on actual exposure levels in the available studies. RAC notes that, to date, there is no strong evidence that calculi could occur following low exposure of melamine. Due to the uncertainties on potential effect of melamine at low dose exposure, RAC agrees with the DS to classify melamine as Carc. 2 (H351).*”

The risk assessment committee concludes that the mode of action for melamine associated cancer is thought to be through a non-genotoxic threshold mechanism. It is commonly accepted that melamine-induced carcinogenicity acts through the formation of calculi. The postulated MoA is that the urinary tumours in rats may be due to the formation of urinary crystals or calculi producing persistent irritation/inflammation and consequent transitional cell epithelium proliferation and urinary tract tumours.

## Toxicity to reproduction and development

Relevant information on toxicity to reproduction was retrieved from DE competent authority assessment and registration dossier[[15]](#footnote-15). The literature studies from Khalil *et al*. (2017), Lv *et al*. (2013), Sun *et al*. (2016a), Yin *et al*. (2013), Huang *et al*. (2018), Chang *et al*. (2014), Sun *et al*. (2016b) and Dai *et al*. (2015) are relevant for ED assessment of melamine and are detailed in section 9.

The newly generated EOGRTS (Study report, 2020) which has been made available after the German evaluation, has been assessed in this section. Male and female Wistar Han rats were exposed via dietary administration (ad libitum) at dose levels 0, 1000, 4000 and 12500 ppm (12500 ppm corresponding to 833 mg/kg bw/d for P0 males and 1200 mg/kg bw/d F1 males). The study is of good quality, follows the guideline without limitation and is considered the key study regarding toxicity to reproduction and development.

**Regarding general toxicity and organ toxicity:**

No change in body weight was observed for P0-generation and F2-generation. A decrease in body weight was measured in males and females of F1 generation (Cohort 1B) at high dose (12500ppm) during mating and at termination.

Liver was not identified as a target organ of melamine. No significant changes were observed in organ weight, nor enzyme activities (ALP, ASAT, ALAT) and no histopathology findings were observed. Adrenals were not identified as target organs, no treatment related effects were observed.

The EOGRTS OECD TG 443 study confirmed that kidney is identified as a target organ of melamine in parental animals and progenies. The absolute and relative organ weight was significantly increased at high dose for P0-generation. The NOAEL for P0 and F1-generation was set at low dose (1000 ppm equivalent of 65 mg/kg bw/d (P0)) based on findings of retrograde nephropathy (as described in section 7.2). In conclusion, **kidney is a target organ with macroscopic findings, this adverse effect appeared at 4000ppm equivalent of 250 mg/kg bw/d.**

Significantly reduced white blood cells count (12 500ppm) in particular mean value for neutrophils (1000ppm and 12 500 ppm) were measured in P0 females. No statistically significant differences were observed for the other hematology parameters measured (monocytes, eosinophils, basophils…). The observed effects on neutrophils could be due to renal impairment. Biological link between the two effects are well-known and appear at the same dose.

**Regarding adverse effects on sexual function and fertility:**

**Significant decrease in percentage of sperm motility was measured at 4000 (-15.2%) and 12 500 ppm (-16.5%) for P0-generation. A significant decrease was also measured (-21.3%) at high-dose for Cohort 1A (F1).** No significant findings were noted in males of Cohort 1B.

**The number of sperm with detached head was significantly increased at high dose for both P0-generation (+260%) and Cohort 1A (F1) (+1100%) and the effects are considered as treatment-related and adverse.** Percentage of sperm cells with normal morphology was significantly decreased (-14%) at high dose in cohort 1A (F1).

**Histopathology analyses of the testis showed an increase in degeneration and atrophy of germ cells at 12500ppm in P0 and at 4000 and 12 500 ppm in F1-generation (Cohort 1A).** Cells debris in lumen of epididymis were also observed at low and high-dose in P0 generation and at high dose in F1-generation.

No adverse change was observed in any of the other parameters of the reproductive function (i.e. epididymal sperm count, and spermatogenic profiling).

Regarding the endocrine functions, androgeno-dependant organs weights (seminal vesicles and prostate gland) in males and oestrus cycle in females were unchanged in P0 and F1 generation.

The observed tubular degeneration/atrophy in the testis (and related minimal cell debris in the epididymis), and the increase in sperm with detached heads in rodents did not affect fertility of P0 and F1-generations exposed to doses up to 12500 ppm. Indeed, no changes were observed in any reproductive parameters (i.e. mating and fertility indices, precoital time, number of implantations). The absence of effects must be interpreted cautiously because, regarding rodents, a reduction by half of the spermatozoa production does not impact fertility (Forand *et al*., 2009). In contrast, even a small decrease of spermatozoa concentrations in semen in the human population could lead to lower couple fecundability and an increased demand for assisted reproduction (Leridon and Slama, 2008; Slama *et a*l., 2004; Skakkebæk *et al*. 2022). The mean concentrations of spermatozoa in human semen have declined over the last few decades, and are currently reaching a level below which hypofertility begins (Bonde *et al*., 1998; Le Moal *et al*., 2014; Levine *et al*., 2022).

**In conclusion, the effects on male reproduction observed here are considered harmful** on the basis of spermatic parameters (reduction in motile sperm, increased number of sperm cells with detached head, reduced number of sperm cells with normal morphology) and on the following testicular histology (degeneration/atrophy germ cells in testis, cell debris in lumen epididymis) which **justifies a classification for reproductive toxicity**. **No additional endocrine effect were observed either in males or in females in this study.**

**Regarding effects on developmental toxicity:**

No developmental toxicity was observed up to the highest dose level tested in F1 and F2 pups (i.e. viability indices, sex ratio, litter size, and early postnatal pup development consisting of mortality, clinical signs, body weight, areola/nipple retention, macroscopic examination and brain and spleen weight)

No treatment-related changes were observed in the developmental immunotoxicity endpoints tested. This is supported by the results from the T-cell dependent antibody response (TDAR) assay which indicated that the test item did not induce any immunotoxic effect in young Wistar Han rats. In accordance with this, no pathology findings in the lymphoid organs (i.e. histopathology and organ weight) and no test item-related changes in splenic lymphocyte subpopulations were noted. Due to the retrograde nephropathy with inflammatory context with reflective of the cells in the kidney, an increase of white blood cells are observed but are secondary to the recruitment of the inflammatory cells.

Additional data were monitored related to developmental neurotoxicity and adverse effects on thyroid were also investigated. These two endpoints are further developed in section 9.3.1 and 9.3.2 as regards to the adverse effects related to PE properties.

In conclusion, based on specific effects on male reproductive system (testis, sperm) **the reprotoxicity NOAEL is 4000 ppm (ca. 268 mg/kg bw/d) in males P0-generation and 1000 ppm (ca. 89 mg/kg bw/d) in males F1-generation**. The effects on sperm cannot be considered adverse on fertility for rodents, although these results need to be interpreted cautiously in regards to human relevance. **The no adverse effect level (NOAEL) for development in F1 and F2 generation was set at 1000 ppm (ca. 89 mg/kg bw/d) in males F1-generation** based on adverse histopathological changes in epididymis and testis, and nephrotoxicity at 4000 ppm.

**Table 6. Summary of guidelines experimental data for toxicity to reproduction and developmental studies from registration dossier[[16]](#footnote-16)**

|  |  |  |  |
| --- | --- | --- | --- |
| Method | Results | Remarks | Reference |
| Extended one generation study (OECD TG 443)  GLP compliance  Wistar Han rats (F0 28/sex/group, F1 )  Doses/concentration: 1000, 4000, 12500 ppm  corresponding to :  65, 268, 833 mg/kg bw/d P0 M  87, 355, 1124 mg/kg bw/d P0 F  89, 370, 1200 mg/kg bw/d F1 M  93, 377, 1227 mg/kg bw/d F1 F  Oral diet (2 weeks premating exposure) | Sexual function and fertility  (P0 and F1):  NOAEL 4000 ppm (P0 M)/1000 ppm (F1 M)  268 mg/kg bw/d (P0 M)/89 mg/kg bw/d (F1 M)  (↑ tub. deg./atrophy germ cells (testis); cell debris, lumen (epididymis);↑ sperms with detached heads (P0) at 12500/4000 ppm)  No significant weight change for seminal vesicles and prostate gland for P0 and F1 generation  Development (F1 and F2):  NOAEL 1000 ppm  89 mg/kg bw/d F1 males (based on adverse histopathological changes in epididymis and testis at 4000 ppm)  General toxicity (P):  NOAEL 1000 ppm  65 mg/kg bw/d (P0 M)/87 mg/kg bw/d (P0 F)  89 mg/kg bw/d (F1 M) /93 mg/kg bw/d (F1 F) (based on nephrotox in both generations  and sexes at 4000 ppm) | 1 (reliable without restriction)  Test material Melamine  Purity 99.8% | Study report, 2020 |
| OECD Guideline 414 (Prenatal Developmental Toxicity Study) ; according to EU Method B.31 (Prenatal Developmental Toxicity Study)  Wistar rats,  Doses / Concentrations: 1500; 4500; 15000 ppm Basis: nominal in diet corresponding to ca. 136; 400; 1060 mg/kg bw/d. | clinical signs body weight and weight gain  food consumption and compound intake urinalysis  DUNNETT-Test (Dunnett, 1955/1964)  Maternal animals:  no effects observed  NOAEL: 400 mg/kg bw/d  Fetuses:  non-treatment-related  NOAEL: 1060 mg/kg bw/d. | 1 (reliable without restriction)  Test material Melamine | Study report 1996 |
| OECD Guideline 414 (Prenatal Developmental Toxicity Study) ; according to EU Method B.31 (Prenatal Developmental Toxicity Study) ; according to EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study)  New Zealand White rabbit  Doses / Concentrations: 15, 50, 150mg/kg bw/d.  oral: gavage | Maternal general toxicity/fetal abnormalities Yes  Maternal animals:  No adverse effects observed NOAEL: 150 mg/kg bw/d  Fetuses:  Fetal abnormalities no effects observed  NOAEL: 150 mg/kg bw/d | 1 (reliable without restriction)  Test material Melamine | Study report 2019 |
| Equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)  Sprague-Dawley rat  Doses / Concentrations: 200, 400, 800 mg/kg bw/d Basis: nominal conc.  oral: gavage | Maternal general toxicity/fetal abnormalitiesone-way analysis of variance (ANOVA), and a Scheffe's multiple comparison test. chi-square test and Fisher's exact probability test. Maternal animals:  Maternal abnormalities no effects observed  NOAEL: 400 mg/kg bw/d (nominal) based on: (test mat.)  Fetuses:  Fetal abnormalities effects observed, treatment-related  Increase in the incidence of skeletal variations and a delay in fetal ossification at 800 mg/kg bw/d.  NOAEL: 400 mg/kg bw/d based on: (test mat.) | 4  Test material Melamine | Kim *et al*. 2011 |

# Environmental hazard assessment

Environmental hazards relevant for endocrine disruption assessment presented in section 8 are based on available data from the chemical safety report (CSR, 2020) of melamine (EC 203-615-4/CAS 108-78-1), the ECHA registration dossier[[17]](#footnote-17) and the assessment realised by DE CA as presented in the Annex XV report for identification of melamine as SVHC[[18]](#footnote-18). Relevant information on environmental hazard assessment was summarised and additional literature studies relevant for ED assessment of melamine are detailed in section 10.

In toxicity studies, transformation products of melamine are often found in concomitant exposure with this substance (Health Canada, 2020). Melamine is known to be metabolized through three consecutive hydrolytic deamination reactions. Ammeline and ammelide, two intermediate transformation products, are converted into cyanuric acid resulting in fact, in a mixture of several triazines found in the animal feed (Puschner and Reimschuessel, 2011) and increasing the nitrogen content in food.

## Fish

**Fish short term toxicity**

As reported in the Annex XV report (2022) for identification of melamine as SVHC, “*several studies on short term toxicity to fish are available on the ECHA dissemination website showing that melamine does not cause acute mortality in adult fish. This was demonstrated in two reliable studies from which, one study examined exposure via water, and one study exposed the fish via feed. In the exposure study via water over 96 h with O.mykiss (rainbow trout) the LC50 was > 3000 mg/L melamine (nominal) (Study report, 1984). In the feeding study by Pirarat et al. (2012) Clarius batrachus (walking catfish) were fed for 2 weeks at doses 5 or 20 g/kg melamine. In this study, darkening of skin was observed as well as the following histological effects: tubular degeneration in the kidneys (tubular epithelium with hyaline droplet accumulation or vacuolation of tubular epithelial cells) at the doses 5 and 20 g/kg, livers revealed single cell necrosis at 20 g/kg feed; at 20 g/kg the histopathology of gills was abnormal (hyperplasia of the epithelium, chondrocytes had degenerative changes, dilation and congestion of the capillaries of the gill lamellae were noted).*

*Another feeding study by Reimschuessel et al. (2008) with lower reliability (Klimisch 3) due to insufficient number of exposed fishes, examined four different fish species (tilapia, rainbow trout, channel catfish, Atlantic salmon). The fish were exposed for 3 days at doses of 300 to 479 mg/kg melamine and examined at days 1, 3, 6, 10 and 14 after exposure ceased.*

*In both feeding studies no mortality appeared. However, sublethal effects were seen in walking catfish (Clarius batrachus).*

*In conclusion, melamine has a low acute toxicity to fish, at higher concentrations sublethal effects appear that are related to nephrotoxicity.”*

**Fish long term toxicity**

As presented in the Annex XV report (2022), “*in a fish early life stage study available on the ECHA dissemination site performed with fathead minnow according to OECD TG 210 and duration of 36 d the LOECs for survival and growth were 10.1 mg/L (NOEC 5.25 mg/L), (Study report, 2015). The study is assessed with Klimisch 1. In a long-term toxicity study with egg-larval development with American flagfish the NOEC was > 1000 mg/L after 35 d exposure (Study report, 1982).*

*A juvenile growth test with rainbow trout and exposure over water showed decreased survival and weight after 28 days exposure (LOEC 3000 mg/L) (Study report, 1984).*

*In conclusion, melamine has a moderate chronic toxicity to fish based on the NOEC of 5.25 mg/L in the FELS study.* ”

In its Annex XV report, DE CA added also several literature studies relevant for aquatic chronic toxicity assessment. Although they did not allow to reduce the NOEC value, they are of relevance for possible ED adverse effects, thus they are discussed further in section 10.

## Amphibian

One publication (Rengel and Pisano, 1994)[[19]](#footnote-19) mentioned studying the “Teratogenic Effects and Cannabalism Caused by Melamine on *Bufo arenarum* Larvae”. As reported in the registration dossier, the chronic effects of melamine on *Bufo arenarum* development was analyzed using 0.25, 0.5, 1 and 2 g ‰ doses. Melamine showed a greater teratogenic action at higher concentrations and caused anomalous features on the mouth, an atypical differentiation of the digestive system switching from herbivorous to carnivorous, forcing larvae to cannibalism. Inner gills always reduced were formed with difficulties. **Gonad evidenced a lack of germinal cells and renal tubules showed anatomical modifications**. According to doses, melamine has more or less drastic effects on the mortality of larvae. This publication is however of low reliability (RI3) due to the following reasons: 1) although the method of ovocyte production is reported to be prone to developmental anomalies no precautionary measures by randomisation of allocation to groups was performed; 2) the cause of starving of the larvae is stated to be the malformations, without proof and not vice versa, although it is known from mammals that starving can produce malformations; 3) dosing caused a high mortality (without giving a time scale) so that malformations are not unlikely.

## Birds

No study available in the registration dossier

## Invertebrates

Daphnia magna Reproduction Test (OECD TG 211) is considered relevant for endocrine disruption assessment as level 4 *in vivo* assays providing data on adverse effects on endocrine-relevant endpoints.

As reported in the Annex XV report (2022), “*in a chronic Daphnia study according to OECD TG 211 available on the ECHA dissemination website no effects on mortality, reproduction or behaviour were seen up to 11 mg/L (measured). This test is rated as Klimisch 1.*

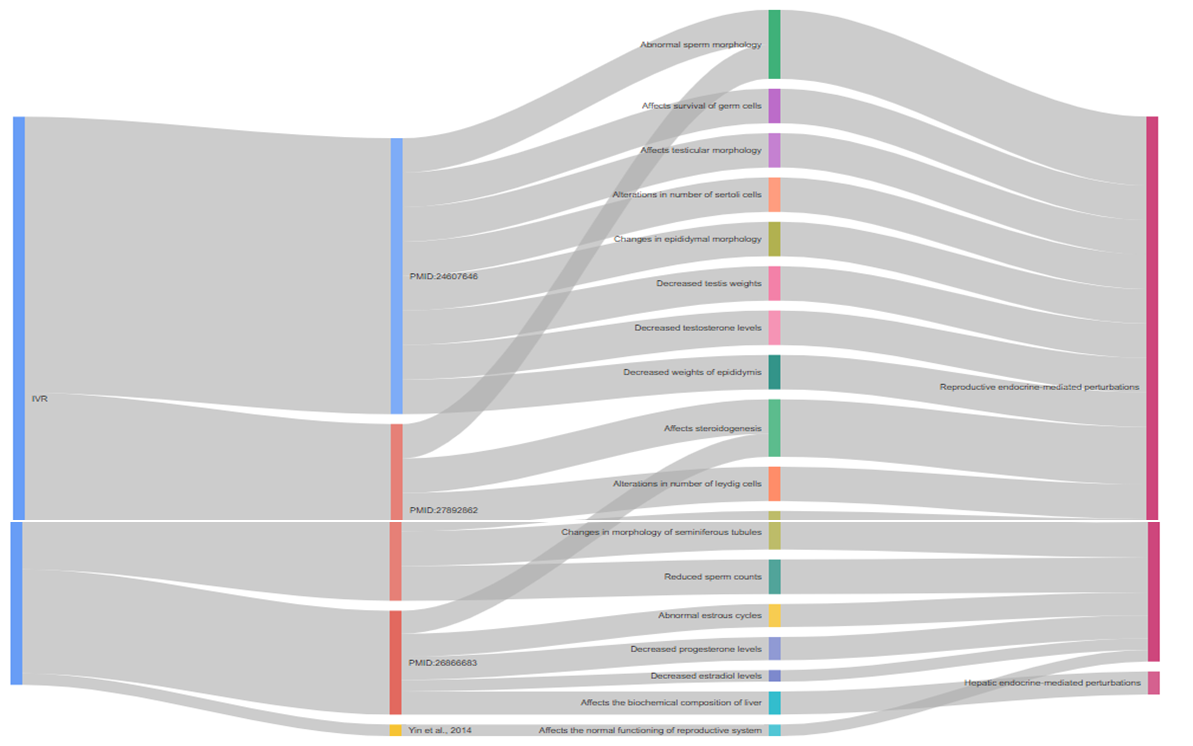
*In another chronic Daphnia study without analytics 100% mortality appeared at 56 mg/L after 7 and 21 days exposure (Study report, 1978). The LC50 was between 32 and 56 mg/L. The NOEC (21 d) for mortality was given by the registrant to be 18 mg/L. This part of test is rated as Klimisch 2. The NOEC (21 d) for reproduction was given to be 18 mg/L. However, the part of test on reproduction has the reliability Klimisch 3*

*In conclusion, melamine has a low chronic toxicity to invertebrates.”*

# assessment of the Endocrine disruption (Human Health)

## General approach

Priorisation work on substance melamine allowed to identify reproductive toxicity endpoint as a major target. DEDuCT (Database of Endocrine Disrupting Chemicals and their Toxicity Profiles) database[[20]](#footnote-20) mentioned 4 articles related to reproductive endocrine-mediated perturbations of melamine; three describing adverse effects in mice male reproductive system (Chang *et al.* 2014, Sun *et al.* 2016a and Yin *et al.* 2013) and one showing adverse effects related to toxicity in rats female reproductive system (Sun *et al*. 2016b) together with hepatic endocrine-mediated perturbations affecting the biochemical composition of liver.



**Figure 1: Systems-level perturbation and endocrine mediated endpoints base on the main literature identifier for substance melamine (source DEDuCT).**

Following dossier evaluation, a testing proposal for an extended one-generation reproductive toxicity study (EOGRTS; EU B.56./OECD TG 443) was submitted and conducted by the Registrants. The data had been made available in 2020 and the results, after analysis, confirmed adverse repro-developmental effects as described in section 7.5.

Moreover, a literature search, described in the following section, was performed to confirm the reproductive male toxicity as the specific endpoint to investigate in relation to the revealed adverse effects and to determine if the EAS modalities are relevant for an investigation of the lines of evidence for endocrine activity and adversity.

## Literature search

Data for assessing potential ED properties of melamine was gathered by a structured literature review based on the principles of systematic review methodology in the electronic databases Pubmed and Scopus. This literature review was realized in accordance to ECHA & EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (2018) and following the recommendation and methodology of ANSES’s GT ACCMER (2023).

A targeted search strategy was applied in electronic database Pubmed (2021/30/09) and Scopus (2021/30/09) by using terms related to the substance melamine (CAS number, IUPAC name and chemical name synonyms) and by adding specific terms related to the relevant endpoint for ED assessment. Keywords and equations used in the systematic literature review are detailed in Annexe I.

The individual studies from all databases were transferred into the electronic reference management software Endnote and reference duplicates were removed to obtain the preliminary dossier. Afterwards, the references were imported into the web-tool Rayyan to create, share and organize the systematic review.

For the specific endpoint “male reproductive data” a total of 743 references were screened for title-abstract review. Criteria for exclusion and inclusion of these articles are listed in the table below.

**Table 7: Literature search inclusion and exclusion criteria**

|  |  |  |
| --- | --- | --- |
| Publication type | IN | Primary research studies |
| OUT | Secondary studies (e.g. reviews, editorials, conference) |
| Language | IN | English |
| OUT | Other languages |
| Outcome | IN | Human/animals/*in vitro* (mammalian health endpoints) |
| OUT | Exposure assessment  Clinical cases (food adulteration)  Non toxicological study  Wrong drug  Wrong outcome  Measurement or detection techniques |

A total of 91 references, including the 9 studies retrieved from the registration dossier from reproductive toxicity studies, were considered for full text selection considering the same inclusion/exclusion criteria.

A total of 39 references were considered for eligibility. Reasons for not including the study, in the scope of this report, are the following: the article does not reveal adverse effects on EAS modalities or included methodology deficiencies. The results of the studies included are further described and analysed in section 9.4.

In addition to the systematic literature search and screening, ToxCast[[21]](#footnote-21) and EDSP (US EPA 2018) databases were queried for melamine bioactivity results using the CASRN to identify high-throughput in vitro screening assays that measured endocrine activity. Cross references of peer-reviewed research articles and grey literature (e.g., reports by national agencies) were also included in the literature search and identification of studies.

**Identification of studies via databases and registers**

Records removed before screening:

Duplicate records removed (n = 850)

Records identified from:

Pubmed Databases (n =622)

Scopus Database (n=960)

Other literature (n=2)

Registration dossier (n =9)

**Identification**

Records screened (title-abstract review)

(n =743)

Records excluded\* (n = 652)

Reports not retrieved\* (n =52)

Reports sought for retrieval (full text review)

(n =91)

**Screening**

Reports excluded (n =32)

Reason 1: does not reveal adverse effects on EATS modalities

Reason 2: methodology deficiencies

Reports assessed for eligibility

(n =39)

Reports of included studies

(n = 7)

**Included**

\* See Table 7: Literature search inclusion and exclusion criteria

**Figure 2: PRISMA 2020 flow chart diagram for systematic reviews of Melamine ED properties for human health.**

*From:*  Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

## Adverse effects related to ED properties

The gathered information as presented above allowed to identify reproductive toxicity of melamine as the most relevant endpoint that can be subsequently assessed for the lines of evidence for EAS modalities for adversity and endocrine activity. Other endpoints presented below (nervous system and thyroid) have been considered. The rational to dismiss them is reported here.

### Neurotoxicity

Literature studies were identified regarding the effects of melamine on the nervous system. Most of the studies on this endpoint were performed by one group of researchers and the results were synthesized in a literature review (An and Sun, 2017). According to these authors, melamine can alter the central nervous system (CNS) function and induce cognitive deficits. The most investigated region of the brain for neurotoxicity induced by melamine exposure was the hippocampus.

In addition to literature search, neurotoxicity was investigated in the OECD TG 443 study (Study report, 2020) with rats of the F1 generation being exposed to 1000, 4000 or 12500ppm melamine. There was no effect on brain weights, no change in brain dimensions greater than 2.5% and no concordance among cohorts or sexes of these subtle variations were observed. The thickness of the corpus callosum size decreased by 9% in males at both ages and increased in females at both ages (PND21-22=+6% and PND76-90=+3%). **Effect on size appeared statistically significant at the only -high dose- dose tested** (12500ppm corresponding to 1200 mg/kg bw/d in males and 1227 mg/kg bw/d in females) compared to controls and suggests a possible sex-dependent effect. The findings in Al Qattan study (Al Qattan *et al*., 2019) indicated gender-dependent neuroanatomical differences in the adult rat corpus callosum (myelinated, unmyelinated or the ratio of myelinated/total axons) which might be region-specific and could explain the observed differences for the corpus callosum.

Locomotor activity was tested in the F1 generation (PND 69-75) using the Kinder Scientific Motor Monitor System. Recording period was one hour under normal laboratory light conditions. All groups showed a similar motor activity habituation profile with a decreasing trend in activity over the duration of the test period.

In the acoustic startle response test performed once between PND 23-25 (F1 generation), **the average response amplitude (N) was significantly lower for males in the mid dose group (4000 ppm) in one of the 5 blocks tested**. Whereas for females the average response was the highest for this group (non-significant). The lack of methodological details and historical and positive controls, the absence of statistical significance and the high variability lead to inconclusive interpretation of this test.

The memory test using the Biel maze (F1 generation PND 62-68) was also inconclusive because of an inappropriate methodology and lack of specific description of the statistics. Many details of the methodology were lacking including water temperature and how potential external influences to the experiment were controlled, like handling, noise, odor, light, randomization of dose groups over the day, relative to motor activity testing and light/dark cycle.

Thus, no significant adverse effects regarding neurodevelopment based on this OECD guidance was considered relevant for endocrine disruption assessment.

### Thyroid toxicity

Adverse effects on thyroid were investigated in the EOGRTS OECD TG 443 study (Study report, 2020). **Significant reduction in T4 levels has been observed at high doses** in P0 male (833 mg/kg bw/d) and in F1 pups (1200/1227 mg/kg bw/d M/F) at PND4. A **significant increase in relative thyroid weight** in F1 animals (+22% in F1A males PND 89-95, +15% in F1B males PND 140-155 and +18% in F1A females) was observed at the highest dose and could be related at least in part to reduced body weight (-8.1% in F1A males, -7.3% in F1B males and -8.3% in females). However, although T4 decrease was observed, which *per se* constitutes an alert, no histopathological changes were observed in the thyroid gland which undermines this alert.

T modality was also considered and the literature search allowed retrieving only one article (Son *et al*., 2014) mentioning adverse effects of melamine related to the thyroid function. In this study, Sprague-Dawley male rats were exposed to 63 mg/kd bw/d of melamine administrated orally for 50 days and the **absolute and relative weights of the thyroid gland were significantly increased** as compared to controls. No histopathological analysis of the thyroid gland was available for this study. Thyroid hormones levels were not measured.

The level and quantity of information available is thus insufficient to conclude on an adverse effect or endocrine activity related to the thyroid function.

## Identification of the relevant ED effect and modality

Guidance for the identification of endocrine disruptors (ECHA & EFSA, 2018) indicate that the assembling of lines of evidence should take into consideration all the available evidence (positive and negative) that have been evaluated as relevant and reliable. Relevant and reliable parameters should be assembled to determine whether and how they contribute to the lines of evidence for adversity and/or endocrine activity.

### Why adverse effect on Thyroid are not considered relevant thereafter

There is a concern that an association may exist between T4 levels and neurodevelopmental alteration observed in F1 pups at PND4 in the OECD TG 443 study. This period is a critical window of development and T4 decrease could be associated with the changes in brain morphometry and ambiguous findings in Auditory Startle Response (ASR) in the developmental neurotoxicity (DNT) cohort (particularly at the highest dose 1200/1227 mg/kg bw/d M/F). However, the evidence is not strong enough to claim that melamine is a thyroid disruptor for the following reasons:

* In the EOGRTS OECD TG 443 study assessing both thyroid and neurodevelopmental/behavioral endpoints, the effects were observed at comparable high doses where general toxicity induced by melamine occurs (doses much higher than ones associated to nephrotoxicity). Alternative MOA have been proposed in the literature regarding neurotoxicity. Neurotoxicity studies, both in vitro and in vivo, indicated that melamine can induce apoptosis (Han *et al*., 2011), disrupt metabolism (Wang *et al*., 2011), enhance autophagy through increasing ROS levels (Wang *et al*., 2015), alter the synaptic transmission (Zhang *et al*. 2016) and induce learning and memory deficit (An *et al*., 2011), thus cognition and behavior could be impacted. Although adverse effects of high dose exposure to melamine are well described (unique dose of 300 mg/kg bw/d in in vivo studies), the mechanisms of melamine toxicity are not well understood and may be different with varying routes of exposure. The authors concluded that the underlying mechanisms of neurotoxicity may include impairment of hippocampal synaptic plasticity, generation of oxidative stress, impairment of the cholinergic system and local field potentials alteration.
* A neurotoxic effect secondary of renal distress is also possible. Such a hypothesis was however not comforted by biochemical parameters (urea and creatinine) not modified in melamine-treated animals. In contrast, the results show a histology impairment with retrograde nephropathy at the highest dose.
* Concerning the thyroid effects, there is a bidirectional, multi-layered interplay between the kidneys and the thyroid gland explaining how pathologies in one organ may affect the other and vice versa (Echterdiek *et al*., 2022). It is noteworthy that a thyroid impact of melamine was observed at doses usually associated to renal dysfunction. As reported in the EOGRTS OECD TG 443 (Study report, 2020), based on adverse nephrotoxic effects, the NOAEL was set at the lowest dose tested 1000 ppm (65/87 mg/kg bw/d M/F) for P0 generation and 1000 ppm (89/93mg/kg bw/d M/F) for F1 generation which is much lower than the doses for which modifications of T4 were reported. In these conditions, even though there is clearly an effect of high doses of melamine on thyroid hormone profiles, it is difficult to discriminate a true endocrine disrupting effect from a physiopathological consequence of severe renal dysfunction.

The level and quantity of information available is thus insufficient to conclude on an adverse effect or endocrine activity related to T-modality. In addition, on the basis of available information on neurotoxicity, there is no strong evidence that melamine shall be considered as having properties meeting ED criteria; the adverse effects could hardly be the consequence of an endocrine mode of action.

### ED modalities concerning reprotoxicity

**Table 8: Summary table of studies considered for the determination of lines of evidence for adversity and endocrine activity**

|  |  |  |  |
| --- | --- | --- | --- |
| Method | Results | Remarks | Reference |
| **Male reproductive function** | | | |
| Extended one generation study (OECD TG 443)  GLP compliance  1000, 4000, 12500 ppm corresponding to :  65, 268, 833 mg/kg bw/d P0 M  87, 355, 1124 mg/kg bw/d P0 F  89, 370, 1200 mg/kg bw/d F1 M  93, 377, 1227 mg/kg bw/d F1 F  Wistar Han rats (F0 28/sex/group, F1 )  Oral diet (2 weeks premating exposure) | Sexual function and fertility  (P0 and F1):  NOAEL 4000 ppm (P0 M)/1000 ppm (F1 M)  268 mg/kg bw/d (P0 M)/89 mg/kg bw/d (F1 M)  (↑ tub. deg./atrophy germ cells (testis); cell debris, lumen (epididymis);↑ sperms with detached heads (P0) at 12500/4000 ppm)  No significant weight change of seminal vesicles and prostate gland for P0 and F1 generation  Development (F1 and F2):  NOAEL 1000 ppm  89 mg/kg bw/d F1 males (based on adverse histopathological changes in epididymis and testis at 4000 ppm)  General toxicity (P):  NOAEL 1000 ppm  65 mg/kg bw/d (P0 M)/87 mg/kg bw/d (P0 F)  89 mg/kg bw/d (F1 M) /93 mg/kg bw/d (F1 F) (based on nephrotox in both generations  and sexes at 4000 ppm) | 1 (reliable without restriction)  Test material Melamine  Purity 99.8% | Study report, 2020 |
| Acute study on the male reproductive system  30, 140 or 700 mg/kg bw/d  Male Kunming mice 8 weeks (12/group)  Oral gavage (once/day for 3 days) | Testicular and epididymis histopathological modifications (700 mg/kg bw/d)  Germinal cells apoptosis-TUNEL method (30 mg/kg bw/d)  Spermatozoid with abnormal form (for all groups)  Alteration of testosterone (Increase after 5 d at 140 mg/kg bw/d) | 2 (reliable with restriction)  Test material Melamine  Purity >99% | Chang *et al*. 2014 |
| Reprotoxicity study in male mice  400, 800 or 1600 mg/kg bw/d  Male Kunming mice 6-8 weeks (10/group)  Oral gavage intragastrically (once/day for 5 days) | Sperm parameters alterations (400, 800 and 1600 mg/kg bw/d)  Increase in serum testosterone level (400, 800 and 1600 mg/kg bw/d)  Decrease SOD activity in testicular tissues (400, 800 and 1600 mg/kg bw/d)  Increase in apoptotic rates of spermatogenic cells (1600 mg/kg bw/d)  Decreased expression of Bcl-2 (400, 800 and 1600 mg/kg bw/d), and increased expression of Bax and caspase-3 (800 and 1600 mg/kg bw/d) in germ and interstitial cells | 3 (not reliable)  Test material Melamine  Purity not mentioned | Huang *et al*. 2018 |
| Reprotoxicity study in male mice  50 mg/kg bw/d  Male Swiss mice adults (10/group)  Oral gavage feeding needle (once/day for 65 days) | Reduction in the number of spermatogonial cells and spermatids  Sperm parameters degradation (reduction of number and motility, increase of abnormal forms)  Presence of apoptotic Sertoli cells  Alteration of the Leydig cells morphology (vacuolation)  Inhibition of testicular enzyme activities involved in energetic metabolism and in steroidogenic pathway  Reduction in plasma testosterone and luteinizing hormone levels | 2 (reliable with restriction)  Test material Melamine  Purity 99.5% | Khalil *et al*. 2017 |
| Reprotoxicity study in male mice  2, 10, 50 mg/kg bw/d  Male ICR mice 4 weeks (10/group)  Oral gavage (once/day for 28 days) | Sperm parameters alterations (2, 10, 50 mg/kg bw/d)  Decrease in sperm number (2, 10, 50 mg/kg bw/d)  Altered testis morphology (10, 50 mg/kg bw/d)  Decrease in testosterone production (10, 50 mg/kg bw/d)  Decrease in Leydig cell number (10, 50 mg/kg bw/d) | 2 (reliable with restriction)  Test material Melamine  Purity not mentionned | Sun *et al*. 2016a |
| Reprotoxicity study in male mice  1, 5, 25 mg/kg bw/d melamine in combination with 0, 1, 5, 25 mg/kg bw/d cyanuric acid  Male ICR mice 4 weeks (10/group)  Oral gavage (13 weeks, nb/day not specified) | Altered testis morphology (all doses)  Induction of oxidative stress (all doses)  Decrease in enzymatic activities involved in energetic metabolism (all doses) | 2 (reliable with restriction)  Test materials Melamine and cyanuric acid  Purity not mentionned | Lv *et al*. 2013 |
| Reprotoxicity study in male mice  Exp.1: 2, 10 and 50 mg/kg bw/d  Exp.2: 412, 824, and 1648 mg/kg bw/d  Male Kunming mice adults (8/group)  Oral gavage intragastrically (once/day for 14 days) | Abnormalities in testicular histology (2, 10, 50 mg/kg bw/d)  Increase in apoptotic index of spermatogenic cells (50 mg/kg bw/d)  Multiple sperm abnormalities (412, 824, 1648 mg/kg bw/d) | 2 (reliable with restriction)  Test material Melamine  Purity 99% | Yin *et al*. 2013 |
| **Femelle reproductive function** | | | |
| Reprotoxicity study in female rats  10, 20, or 40 mg/kg bw/d  Female Sprague Dawley rats 28d old (10/group)  Oral gavage (once/day for 28 days) | Ovarian lesion and apoptosis of granulosa cells and oocytes (40 mg/kg bw/d)  Decreased expressions of genes associated with steroidogenesis (20 and 40 mg/kg bw/d) | 2 (reliable with restriction)  Test material Melamine  Purity 99% | Sun *et al*. 2016b |
| Reprotoxicity study in female mice  10 or 50 mg/kg bw/d  Female ICR mice 3 weeks (120/group)  Oral water (8 weeks) | Decreased in ovary weight and reduced oocytes development (with regard to meiotic maturation) (10 and 50 mg/kg bw/d)  Early apoptosis among oocytes and increased Caspase 9 mARN expression (50 mg/kg bw/d)  Reduced average number of offspring (50 mg/kg bw/d) | 2 (reliable with restriction)  Test material Melamine  Purity not mentioned | Duan *et al*. 2015 |

The most relevant observations are the adverse effects affecting the male reproduction function in rodent experimental studies because they appear several times. The key study regarding to the adverse effects of melamine on the reproductive function is the OECD TG 443 and has been presented and discussed in section 7.5. In addition, 8 reliable and of good quality studies from literature showing similar effects on the reproductive function are also presented in the table above and in the text below. No epidemiological data was available regarding to the human reproductive function.

*Male reproductive studies reporting both adverse effect and MOA*

Testicular acute toxicity of melamine was investigated in **Chang *et al*. (2014)**. Male Kunming mice were given a suspension of melamine in 1% Carboxymethylcellulose (CMC) once a day by oral gavage for 3 consecutive days. Control animals received 1% CMC in the same manner. The animals were administered melamine at doses of 30, 140, and 700 mg/kg bw/d (12 mice/group). Animals were euthanized 1 or 5 days after the last dose (n=6 in each group).   
No general toxicity (normal general state, body weight and behavior) was detected in any treated groups, although white precipitates in the bladders of one mouse in the two lower MA dose groups (30 and 140 mg/kg bw/d) and in half of the mice in the highest MA dose group (700 mg/kg bw/d) at 1 day after exposure. Plasma urea and creatinine levels were never changed. The percentage of abnormal spermatozoa in the epidydimis was increased for all doses 1 and 5 days after the treatment. Serum testosterone level was unchanged except a significant increase 5 days after the treatment at 140 mg/kg bw/d. A destruction of the blood-testis barrier was clearly evidenced by electronic microscopy and histology after immunostaining for vimentin.

**In conclusion, this study shows that 30 mg/kg bw/d melamine causes a clear alteration of the spermatogenesis and blood-testis barrier, without nephrotoxic effects and without reduction of testosterone production.**

The study from **Huang *et al*. (2018)** was indicated as Klimish 3 due to the shortcomings in the description of the methodology. Analysis were realized 35 days after exposure of relatively high doses of melamine (400, 800 or 1600 mg/kg bw/d), and the studied parameters were serum testosterone detection, change of visceral index, sperm parameters (motility, sperm count, morphology), LDH and SDH in testicular homogenate, oxidative stress indices (SOD, MDA), histopathological change in testicular tissue and apoptosis-related proteins. Melamine showed reproductive toxicity in male mice by affecting the normal formation and maturation of sperms, damaging testicular tissue structures, decreasing sperm quality and quantity, antioxidant capacity and LDH and SDH enzymatic activities involved in glucose metabolism, and promoting the apoptosis of spermatogenic cells. At these dose levels (400, 800 or 1600 mg/kg bw/d) serum testosterone level was increased from 2.2 nmol/L (corresponding to 633 pg/mL) to 3.5 nmol/L (corresponding to 1008 pg/mL).

**In conclusion, available data do not plead in favor of testosterone-mediated effects of melamine on spermatogenesis. The mode of action might possibly involve general cellular biology alterations (reduction in energetic metabolism, oxidative stress and apoptosis).**

In **Khalil *et al*. (2017),** the authors demonstrated that melamine induced reproductive toxic effects at the unique dose tested (50 mg/kg bw/d *per os* for 65 consecutive days). General toxicity was observed (lethargy, rough hair coat & anorexia) in the melamine-treated group. Body weight was 28.17 g and 19.53 g in control and treated groups respectively, but the difference was not statistically significant. Lesions in the testes (structural and functional alterations of Leydig cells through cystic dilatation of the seminiferous tubules with a marked reduction in the spermatocyte percentage of most seminiferous tubules) were observed in treated group. The sperm analysis showed a deterioration of sperm characteristics. Testicular enzyme activities involved in energetic metabolism were strongly reduced (by three-fold) in the melamine-exposed group. Testosterone and luteinizing hormone levels were significantly reduced (from 2.5 ng/mL to 0.5 ng/mL for testosterone) in the melamine-exposed group, which correlated with decreases in mRNA transcript levels for the key steroidogenic enzymes.

**This study shows a reduction of the cellular metabolism and testosterone level. Because of melamine-induced general toxicity, the reduction of testosterone level observed here does not appear as the main pathway for the effects of melamine on spermatogenesis.**

In **Sun *et al*. (2016a)** no general toxicity (body weight or food intake) were noticed at the tested doses (2, 10, 50 mg/kg bw/d orally given for 28 days to male mice 5 weeks-old). Although testis weight did not change, the sperm count was dose-dependently decreased in all the treated groups (around -20 % at 2 mg/kg bw/d). The rate of abnormal sperm was dose-dependently increased in all the treated groups (four-fold at 2mg/kg bw/d). Disruption of the seminiferous tubule structure, decreased spermatogenic cell series, nuclei pyknosis and decreased sperm number were found at 50 mg/kg bw/d. Looseness of the seminiferous tubule structure was observed at 10 mg/kg bw/d**.** The quantitative analysis showed that the number of Leydig cells was significantly lower with 10 and 50 mg/kg bw/d.In the same way, testosterone level decreased (10 and 50 mg/kg bw/d) with increasing melamine dose but was unchanged with 2 mg/kg bw/d.

**In conclusion, a dose as low as 2 mg/kg bw/d alters spermatogenesis and, at this dose, testosterone production is not affected. A decline in the number of Leydig cells, is not observed at 2 mg/kg bw/d but at higher doses, indicating that Leydig cells are another, but less sensitive, target than germ cells.**

**In Lv *et al*. (2013),** in combination with cyanuric acid, general toxicity is observed at 5 and 25 mg/kg bw/d. Animals were lethargic, with rough hair, and reduction of food and water intake and of body and absolute but not relative testis weight, were observed. Alteration of testis morphology and of the seminiferous tubule structure were noted. In addition, decreases in the spermatogenic cell series and coagulation necrosis were found in combination treatment groups. Oxidative stress was investigated. The activities of TAC and SOD (for 5 and 25 mg/kg bw/d) and the concentration of GSH (for 1, 5 and 25 mg/kg bw/d) decreased with increased dosage. Higher concentration of MDA (effects observed as low as 1 mg/kg bw/d) were measured although no statistically significant differences were measured with respect to the concentration of PSH. Finally, the authors investigated the enzymes related to energy metabolism and reported that Na+/K+-ATPase (5 and 25 mg/kg bw/d) and the activities of MDH and LDH (1, 5 and 25 mg/kg bw/d) were significantly lower in treated groups than in controls.   
**The critical effect emerging here is the oxidative stress linked to an insufficient renal function and a reduction of food intake.**

In **Yin *et al*. (2013)**, at doses levels 2, 10 and 50 mg/kg bw/d tested for 14 days, testis histology and TUNEL assay of spermatogenic cell apoptosis were performed. No apparent general toxicity, but 3 deaths in the high-dose group, were observed. The authors observed the ultrastructural changes in testis of male mice treated with different doses of melamine in a dose-dependent manner. Testicular histopathology examination showed irregular nucleus shape in some cells at 2 mg/kg bw/d. At exposure dose 10 mg/kg bw/d, seminiferous tubules had indistinct basement membrane, and spermatogenic cells at all stages exhibited a slightly loosened organization with decreased cell layers. At 50 mg/kg bw/d, damage of basement membrane of seminiferous tubules was observed, with decreased layers of epithelial cell and structural damage found in many spermatogenic cells at all stages. Also, swelling and lysis of nucleus occurs in the primary spermatocytes, secondary spermatocytes and the sperm cells with the uneven size and staining of varying intensity. In addition, a reduction in the number of sperm or even no mature sperm was observed in some Sertoli cells of seminiferous tubule.

In a second experiment 412, 824, and 1648 mg/kg bw/d were administred for 5 days to study the epididymal sperm. No mortality was observed but body weight was reduced at the higher dose (1648 mg/kg bw/d). The percentage of spermatozoa with morphological alteration was dose-dependently increased from 1.93% in controls to 5.63% for the highest dose.

**In conclusion, this paper shows that the lower dose used of 2 mg/kg bw/d alters spermatogenesis. No endocrine parameter was evaluated.**

*Supportive data - Female reproductive studies*

Additionally, in one study female rats Sun *et al*. (2016a), the authors concluded that melamine caused ovarian lesions, including **increased numbers of atretic follicles and necrosis of granulosa cells and oocytes** (40 mg/kg bw/d). Moreover, ovaries of melamine-treated rats showed apoptosis of granulosa cells, oxidative damages, and decreased expressions of genes associated with steroidogenesis, indicating that melamine is toxic to rat ovaries. The apoptosis and oxidative stress contributed to ovarian lesions, and the decreased expression of steroidogenic enzymes although the decreased serum E2 and P levels is non-significant.

Melamine’s adverse effects on oocyte quality was also investigated in Duan *et al*. (2015) study. Female 3-week-old ICR mice were exposed to 0, 10 and 50 mg/kg bw/d (n=120/group) of melamine given in drinking water for 8 weeks. In this study, the authors showed that melamine had toxic effects on oocyte quality and fertility due to its effects on the **oocyte cytoskeleton, apoptosis and autophagy induction, and epigenetic modification**s in an *in vivo* mouse model. The abnormal oocyte rate is significantly higher in melamine-treated mice at 50 mg/kg bw/d than in control mice. However, the numbers of ovulated oocytes were not significantly different. The rate of polar body extrusion after oocytes cultured for 12 h was significantly higher for control oocytes than those for treated oocytes at 50 mg/kg bw/d showing a reduced developmental competence with regard to meiotic maturation. The protein expression for the actin nucleation factor ROCK was significantly reduced in both treated groups when compared with control. The early apoptosis rate of GV oocytes and caspase9 mRNA level were significantly higher at 50 mg/kg bw/d when compared with controls. Melamine also significantly affects histone methylation in mouse oocytes at both doses. Finally, results showed that exposure during pregnancy at 50 mg/kg bw/d significantly reduced the average of offspring (11.5+0.86, n = 4 for control vs 15.3+0.63, n = 4 for 50 mg/kg bw/d, n = dam mouse number). The article mention that the treatment period was covering pregnancy although no other detail on the methodology is mentioned.

## Overall conclusion on endocrine disruption with regards to human health

### Reprotoxicity

**The present set of data clearly shows that melamine is able to alter reproductive functions by impairing spermatogenesis.**

In the EOGRTS, the extent of reduced sperm quality was weak and had no consequences for the rat fertility in accordance with the fact that, in rodents, the reduction in spermatozoa production must be higher than 55% to affect fertility (Forand *et al*., 2009). Limits of this conclusion for human has been discussed above (Section 7.5).

Importantly, the deleterious effect was observed with 2 mg/kg bw/d (Yin *et al*., 2013, Sun *et al*., 2016a) which is lower than the BMDL10 (19 mg/kg bw/d) used for the derivation of tolerable daily intake for melamine (EFSA, 2010). Thus, the current Tolerable Daily Intake (TDI) of 0.2 mg/kg bw (EFSA, 2010) should be revised in the light of the information gathered in the present assessment.

### Mode of action

**The present set of data does not allow to postulate for an ED MoA for the following reasons:**

* No *in vitro* mechanistic studies are available.
* *In vivo* effects of melamine on endocrine regulations have been poorly studied for all functions. The best studied endocrine regulation was the control of spermatogenesis with one EOGRTS study and 4 academic papers (Chang *et al*., 2014, Sun *et al*., 2016a, Khalil *et al*., 2017, Huang *et al*., 2018). It was limited to the investigation of one type of spermatogenesis control that is exerted by testosterone. Two studies observed a reduction of serum testosterone levels (Sun *et al*., 2016a for 10 and 50 mg/kg bw/d and Khalil *et al*., 2017 at 50 mg/kg bw/d). On the contrary, four studies described negative effect of melamine on spermatogenesis whereas serum testosterone level was increased (Chang *et al*., 2014 at 140 mg/kg bw/d, Huang *et al*., 2018 at 400, 800 and 1600 mg/kg bw/d), unchanged (Chang *et al*., 2014 at 30 and 700 mg/kg bw/d, Sun *et al*., 2016a with 2mg/kg bw/d), or whereas the weights of androgenic-dependent organs were unchanged (EOGRTS study report, 2020, from 65 to 1200 mg/kg bw/d). The differences observed could be explained either by the doses, the route of exposure or the duration of the study protocol. Based on the only dose tested in Khalil *et al*. (2017), and considering the general toxicity observed in this study, the reduction of testosterone level does not appear as the main pathway for the effects of melamine on spermatogenesis but rather a consequence of spermato-toxicity. Indeed, the causal association between testosterone reduction and spermatogenesis alteration is not demonstrated.
* An increase in oxidative stress and a reduction of the activities of enzymes involved in energy metabolism in the testis were observed in all the studies that investigated these parameters, and where melamine-induced general toxicity is observed (Lv *et al.* 2013, Khalil *et al.* 2017, Huang *et al.* 2018).
* Female reproductive studies (Sun *et al*. 2016a, Duan *et al*. 2015) are supporting this conclusion. The reduced oocytes development related to meiotic maturation and oocytes abnormalities in female mice are evidence for similar effects of melamine among both sex. Comparable oxidatives effects on gametogenesis (ovogenesis and spermatogenesis) were observed in both sexes.

* Finally, based on the evidence reported in Kuczera *et al*. (2022), the hypothesis that renal toxicity of melamine could have reprotoxic incidence could be explored. It should occur independently of the sex. It would be coherent with the fact that effects were reported in female rodents. Indeed, at the same exposure dose than the one used in male experiments (10, 20 and 40 mg/kg bw/d), Sun *et al*. (2016b) observed a decrease non-significant estrogen level correlated to an effect on ovarian function and oogenesis in rat. In addition, the authors explored the renal status and observed structural and biochemical alterations that could be linked to the observed non-significant decrease in estrogen level. The non-sex specific effects reported, although non statistically significant, could therefore support a non-specific secondary consequence of the renal toxicity rather than an endocrine disruption type of effect. These results are in line with the study of Dai *et al.* (2015), linking the reprotoxic effects to oxidative stress and non ED-related. Further histological data or biochemical markers expression for renal damage could allow to confirm the link between the reprotoxic effects observed and the upstream nephrotoxic activity of melamine.

**Taken together, the ANSES expert working group on endocrine disruptors concluded that spermatogenesis alteration occurred primarily in the absence of an endocrine MoA (testosterone decrease) and could involve general cellular biology alterations (reduction in energy metabolism, oxidative stress, apoptosis). Testosterone decrease may amplify this direct effect at higher doses in some protocols.**

**The available data today investigating possible ED mode of action of melamine for male reproduction lead to conclude that other MoA are responsible for reprotoxic effects.**

**Effects were also observed on the thyroid function and T modality was considered. The level and quantity of information available was insufficient to warrant further investigation of adverse effects or endocrine activity related to the thyroid function. ED MoA for thyroid effects is not demonstrated today.**

**For the other modalities, the observed effects appear at doses above those leading to nephrotoxicity and general toxicities. Therefore, it is concluded that ED properties are not confirmed for human health**

# assessment of the Endocrine disruption (Environment)

## General approach

Human health assessment identifies the reproductive toxicity of melamine as the most relevant endpoint for the endocrine disruption properties assessment and to subsequently assess the lines of evidence for EAS modalities for adversity and endocrine activity. Although it was concluded that the substance melamine is not considered to have endocrine disruption properties considering the mammalian species, additional literature review was realized to gather all the relevant information regarding the potential endocrine disrupting properties of melamine on non-mammalian species.

On a populational level it was mentioned in the EOGRTS OECD 443 study that the reproductive adverse effects do not have an effect on fertility. Thus, based on this study, melamine should not have an effect on the populational or subpopulational level on rodents.

## Literature search

Data for assessing potential ED properties of melamine was gathered by a structured literature review based on the principles of systematic review methodology in the electronic databases Pubmed and Scopus. According to the ECHA/EFSA guidance and following the recommendation and methodology of ANSES’s GT ACCMER (2023).

A single concept approach by using the substance search terms (CAS number, IUPAC name and chemical name synonyms) was applied in electronic database Pubmed as a first step (November 12th 2021). Since the number of hits retrieved by the single concept search was not excessively large (3440), additional search refinement was not required for further screening steps.

Based on the previous literature research and initial concern raised from Deduct[[22]](#footnote-22) database, an additional targeted search strategy on specific endpoint reproductive/developmental function for non-mammalian species was realised on Scopus (June 6th 2022):

TITLE-ABS-KEY (Melamine OR (6-triamino-s-triazine) OR (triaminotriazine\*) OR (triaminostriazine\*) OR (triazine-triamine) OR (triazinetriamine) OR (isomelamines) OR (108-78-1)) AND (tox\* OR hazard OR adverse OR health OR effect\* OR chronic\* OR "in vivo" OR "in vitro" OR mechanis\*) AND (bird\* OR mallard OR duck OR quail OR bobwhite OR anas\* OR colinus\* OR vertebrat\* OR mammal\* OR rat OR mouse OR mice OR rabbit OR hare OR invertebrat\* OR aquatic OR fish OR fathead OR medaka OR zebrafish OR stickleback OR sheephead OR minnow OR daphni\* OR chiron\* OR crusta\* OR gastropod\* OR mollusc OR reptile OR amphib\* OR bee\* OR api\* OR bumble\* OR arthropod\* OR typhlodromus OR aphidius OR insect\* OR worm\* OR \*worm OR eisenia OR collembol\* OR macroorganism OR folsomia OR springtail OR mite\* OR hypoaspis) AND (reproduct\* OR development\* OR malformation\* OR anomal\* OR fertil\* OR fecund\*)

An additional 277 hits were added to the 3440 records identified from Pubmed. The individual studies from all databases were transferred into the electronic reference management software Rayyan and reference duplicates were removed to obtain the preliminary dossier.

A total of 242 references were kept after title-abstract review without distinction between mammalian or non-mammalian species. Criteria for exclusion and inclusion of these articles are listed in the table below.

**Table 9: Literature search inclusion and exclusion criteria**

|  |  |  |
| --- | --- | --- |
| Publication type | IN | Primary research studies |
| OUT | Secondary studies (e.g. reviews, editorials, conference) |
| Language | IN | English |
| OUT | Other languages |
| Outcome | IN | Human/animals (including non-mammalian species) health endpoints |
| OUT | Microscopy techniques  Measurements or detection in the environment or media Measurement or detection techniques |

A total of 49 references related to environmental hazard (non-mammalian species) were further investigated. In addition to the 7 studies retrieved from the registration dossier[[23]](#footnote-23) from chronic toxicity studies on fish, invertebrates and amphibian, additional references on fish (19), birds (17), and invertebrates (6) were analysed.

On this number only 24 articles were included in the analysis of ED properties of melamine for the environment. Reasons for report exclusion are related to the unavailability of a full text article (n= 8), the article does not reveal adverse effects on EATS modalities (n= 14) or included methodology deficiencies (n= 3). The results of the studies included are further described and analysed in section 11.3.

**Identification of studies via databases and registers**

Records removed before screening:

Duplicate records removed (n = 106)

Records identified from:

Pubmed Databases (n = 3440)

Scopus Database (n= 277)

Registration dossier (n =7)

**Identification**

Records screened (title-abstract review)

(n = 3618)

Records excluded\*

(n = 3376)

Reports sought for retrieval (full text review)

(n = 242)

Reports not retrieved\*

(n = 193)

**Screening**

Reports assessed for eligibility

(n = 49)

Reports excluded:

Reason 1 full text not available (n = 8)

Reason 2 does not reveal adverse effects on EATS modalities (n = 14)

Reason 3 methodology deficiencies (n = 3)

etc.

Reports of included studies

(n = 24)

**Included**

\* See Table 9: Literature search inclusion and exclusion criteria

**Figure 3:** **PRISMA 2020 flow chart diagram for systematic reviews of Melamine ED properties for the environment.**

*From:*  Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

## Assessment of information on endocrine disruption properties

**Invertebrate data**

Data related to adverse effects of melamine in insects show a sterilisation with important effects on egg production and eclosion (Borkovec 1967, Labrecque 1968, Matsumoto 2019). The articles are relatively old, with a summary description. In addition, one publication (Chen, 2012) used the Drosophila model for the study of renal stone disease and the publication was not considered ED-related.

Melamine was also illegally used for shrimp in aquaculture to elevate the nitrogen content of in the food. One study (Lightner *et al*., 2009) on Shrimp (*Penaeus monodon* and *P. vannamei*) confirmed that the melamine-contaminated feed induced prominent granulomas in the antennal gland with the characteristic crystals within 10 d of the first feeding, experimentally confirming the direct relationship of melamine-adulterated feed to the unique pathology observed. This article did not bring any relevant information related to the endocrine disrupting properties of melamine. A study by Nuntapong *et al*. (2019) with Pacific white shrimp showed that survival, growth and specific growth rate were significantly reduced after a 10-weeks exposure to melamine over food (dose 10.1 g/kg feed). As described in Annex XV (2022), “*histological observations showed degenerated and shrunken hepatopancreatic tubules. The immune system was negatively affected as shown by decreased hemocyte count and decreased lysozyme activity. Antioxidant enzymes in gill and hepatopancreas were significantly decreased. Further sign of oxidative stress and damage was the elevated content of malondialdehyde (main lipid peroxidation indicator) in hepatopancreas. The effects on antioxidant enzymes and malondialdehyde are to a large extent comparable to those seen in fish.*”

**Amphibian data**

The study from Rengel and Pisano (1994) is mentioned in the registration dossier and full text document was not available. However, the abstract revealed the chronic effects of melamine on *Bufo arenarum* development using 0.25, 0.5, 1 and 2 g ‰ doses. According to the author, melamine showed a greater teratogenic action at higher concentrations and caused anomalous features on the mouth, an atypcial differentiation of the digestive system switching from herbivorous to carnivorous, forcing larvae to cannibalism. Inner gills always reduced were formed with difficulties. **Gonad evidenced a lack of germinal cells and renal tubules showed anatomical modifications**. According to doses, melamine has more or less drastic effects on the mortality of larvae. This publication is however of low reliability (RI3) according to the registration dossier and the absence of the full text article prevented to use these data for the ED assessment.

**Fish data**

One FELS study (OECD TG 210) is available in the registration dossier[[24]](#footnote-24) and is considered a key study in this assessment. This study is a level 4 *in vivo* assays providing data on adverse effects on endocrine-relevant endpoints and is considered a key study. The test was conducted in a continuous flow through system. Fertilized eggs (embryos) and larvae of *Pimephales promelas* were exposed in cylindrical glass vessels to melamine at nominal concentrations of 0, 0.625, 1.25, 2.5, 5.0 and 10 mg/L. In the OECD TG 210 study, the most sensitive endpoint was growth (total length). There was no statistically significant decrease in hatching success (i.e. embryo survival until end of hatch) in any of the treatment groups in comparison to the control group. There was no statistically significant decrease in survival from hatch until the end of swim up in any of the treatment groups in comparison to the control group. However, there was a statistically significant decrease in fish survival from end of swim-up (day 6) until the end of exposure (day 36) in the test group 10.0 mg/L in comparison to the control group. Overall survival was statistically significantly decreased in the treatment group 10.0 mg/L in comparison to the control group. No test substance-related effect on survival was seen in the test concentrations ≤5.0 mg/L (nominal). A toxic effect is seen at dose 10 mg/L, however this toxicity cannot be attributed to EAST modalities.

In addition, in the registration dossier (and also reported in DEDuct), there is a description of a study of interest on *Salmo gairdneri* (Ramusino, 1982). In this study, the fish were exposed to melamine concentrations 125, 250, 500 and 1000 mg/L in a flow-through system. The duration of exposure is unknown (between 18-26 days) and no statistical analysis was reported. The full article was not available and was not analysed further for this reason, although the data retrieved from the registration dossier mentioned information of interest on adverse effect of melamine (increased the incidence of malformation in exposed larvae).

Many of the literature data retrieved were also previously indicated in the ANNEX XV report (2022), and the adverse effects are reported here as assessed by the DE CA. “*Six feeding studies similar to juvenile growth tests with five fish species (red tilapia (Phromkunthong et al., 2013), Asian sea bass (Phromkunthong et al., 2015), darkbarbel catfish (Jipeng et al., 2011), African catfish (Iheanacho et al., 2020; Iheanacho et al., 2021) and humpback grouper (Mahardika et al., 2017) are available. The exposure durations were in the range of 6.4 to 12 weeks. Mortality significantly increased at the dose 4 g/kg feed in red tilapia and 167 mg/kg feed in humpback grouper; no effect in the other juvenile feeding studies on mortality were seen. In all species the body weight and/or the specific growth rate were significantly decreased (except humpback grouper, not assessed). The LOECs for specific growth rate are 4 g/kg and 2 g/kg feed for red tilapia and darkbarbel catfish, respectively; the LOECs for reduced weight are 3 g/kg feed for African catfish and 4 g/kg feed for red tilapia. Asian seabass exposed to the single concentration of 10 g/kg feed had only 42 % body weight compared to control. The values were the lowest tested doses; hence the NOEC is below these effect values.*

*In two studies (red tilapia and darkbarbel catfish) effects on skin colouration were seen. In red tilapia discolouration increased with increasing doses, at 10 g/kg (10-25 %), at 15 g/kg (>25 %). In darkbarbel catfish discolouration was increased at ≥ 5 g/kg feed, additional in this study the biological pigment melanin in the skin was measured and seen that it was dose-dependently decreased. Effects on the skin were also seen in the acute study with walking catfish (see above).*

*In two studies effects on hematology were examined: In Asian sea bass and African catfish erythrocytes, leukocytes and hemoglobin were significantly decreased; the effect on hematocrit/packed cell volume was inconsistent.*

*Histological effects: in red tilapia dose-dependent effects appeared, as enlargement of renal tubules, degenerated tubular epithelium, vacuolisation of liver hepatocytes, hyperplasia of gill lamellae, blood clotting and lamellar disorganisation in gills; in Asian sea bass enlargement of the renal tubules, degenerated tubular epithelium, pyknotic nuclei, melano-macrophages in the kidneys, distinctive vacuolisation in the liver were seen. The effects partly resemble histological effects in the acute study with walking catfish. In humpback grouper swollen kidneys and necrosis of renal tubules were seen.*

*In African catfish a neurotoxicological effect was seen, as acetylcholinesterase activity in the brain was significantly decreased.*

*Signs for oxidative stress in different organs were seen in three species: Asian sea bass, African catfish and rainbow trout. For example, in African catfish the antioxidant enzymes SOD, GPx, CAT (superoxide dismutase, glutathione peroxidase, catalase) activities were significantly decreased in the brain, pointing to enhanced formation of ROS or free radicals following exposure to melamine.”*

**Birds data**

In birds, most of the article studied the toxicokinetic of melamine (i.e. melamine concentration and deposition in tissues, elimination of melamine in eggs and tissues).

The effect of a 5-week melamine administration in feed on the egg quality and blood variables of laying hens was investigated (Suchý *et al*., 2014). Ten 36 weeks old laying hens were in the exposure group, as well as in the control group. During the exposure period the hens received a diet containing 100 mg/kg feed. No effects on mortality, production of eggs and body weight were observed. Egg shell weight and egg shell strength were significantly decreased after 3 and 4 weeks, although not significantly decreased after 5 weeks. No measurement was available for oestradiol, testosterone or thyroid hormone level. The number of erythrocytes increased significantly, but the mean corpuscular haemoglobin concentration (MCH) decreased significantly. Gross pathology findings are not reported.

In additional literature studies, a toxicity (growth inhibition, damage to liver and kidney, mortality) was notable at doses ≥100 mg/kg feed (Kim *et al*., 2019; Brand *et al*., 2012; Ding *et al*., 2012; Wang *et al*., 2012). This toxicity observed could not be linked to ED activity or adverse effects.

Among all the revised articles of interest from fish and birds species, adverse effects were related to oxidative stress nephrotoxicity, kidney and liver lesion. Notably also in non-mammal studies, co-exposure to melamine and cyanuric acid shows higher toxicity compared with melamine or cyanuric acid alone.

## Identification of the relevant ED effect and modality

### Lines of evidence (LoE)- EAST modalities

Guidance for the identification of endocrine disruptors (ECHA & EFSA, 2018) indicate that the assembling of lines of evidence should take into consideration all the available evidence (positive and negative) that have been evaluated as relevant and reliable. Relevant and reliable parameters should be assembled to determine whether and how they contribute to the lines of evidence for adversity and/or endocrine activity.

When considering all the available information, from registration dossier and literature review, several data indicated the toxic effect of melamine on non-mammals species. However, no adverse effect or mechanistic data related to ED properties has emerged from this assessment.

Therefore, the line of evidence for adversity and endocrine activity were not further developed.

#### LoE Adversity – EAST

To have the EAS-mediated adversity for non-target organisms sufficiently investigated, the ‘EAS-mediated’ parameters foreseen to be measured in the Medaka extended one-generation test (MEOGRT, OECD TG 240 (OECD, 2015c)) should have been investigated and the results included in the dossier. Alternatively, a FLCTT covering all the ‘EAS mediated’ parameters foreseen to be measured in the MEOGRT is acceptable. Those studies were not available in the registration dossier of melamine.

#### Population relevance of MoA

Adverses effects have been illustrated in the Human Health section on EAS modality. On a populational level it was demonstrated in the EOGRTS OECD 443 study that the reproductive adverse effects observed in male rodents did not impact their fertility, thus had no impact on the populational or subpopulational level. The assessment indicates that the spermatic effects do not impact the fertility in rats. No additional information was retrieved related to the populational effect of melamine on non-mammalian species.

## Overall conclusion on endocrine disruption with regards to environment

It was concluded that, in the present state of knowledge, the substance melamine is not considered to have endocrine disruption properties with regards to environmental (non-mammalian) species. The information shows that the substance does not meet the criteria for endocrine disruption as defined by the WHO/IPCS (2002).

# Main Conclusion

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) was mandated to evaluate the endocrine disrupting profile of melamine. The substance was identified in DEDuCT database as a category III based on the analysis of existing scientific literature data containing experimental evidence on endocrine-specific perturbations. According to the DEDuCT methodology (Karthikeyan *et al*., 2019), a substance is in category III when its effects were observed solely *in vivo*, in rodents, and data on mode of action might be missing. DEDuCT mentioned 4 articles related to reproductive endocrine-mediated perturbations of melamine in rodents. Priorisation work on this substance identify the reproductive toxicity endpoint as a major target.

After in depth investigation, the ANSES expert working group on endocrine disruptors concluded that spermatogenesis alteration occurred primarily in the **absence of a demonstrated endocrine MoA** and could involve general cellular biology alterations. Thus, **melamine is not considered to have endocrine disrupting properties based on these effects**. The available data on male reproduction allows us to conclude on a non-ED mode of action. For the other modalities, the effect observed appear at doses above those leading to nephrotoxicity or general toxicity. The level and quantity of information available was insufficient to warrant further investigation of adverse effects or endocrine activity related to the thyroid function. ED MoA for thyroid effects is not demonstrated today. Therefore, it is concluded that ED properties are not confirmed for human health.

In addition to the 4 articles mentioned in DEDuCT, several other publications revealed the severity and repeatability of the effects caused by melamine on the male and female reproductive system. **A classification for the toxic effects of melamine on the sexual function is warranted** and ANSES is in line with the DE CA intention to revise the harmonised classification of melamine for this specific endpoint. Moreover, the deleterious effect was observed at 2 mg/kg bw/d (Yin *et al*., 2013, Sun *et al*., 2016a), which is lower than the BMDL10 (19 mg/kg bw/d used for the derivation of tolerable daily intake for melamine (EFSA, 2010). **The toxicological value of reference for melamine, based on the adverse effects on the reproductive system, needs to be reconsidered.**

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# ANNEXE I

List of keywords and equations used for systemic literature review in Pubmed and Scopus (TITLE-ABS-KEY):

1. Substance: melamine OR 108-78-1[EC/RN Number]
2. Human and epidemiological data: Epidemiology OR "epidemiological data" OR human
3. In vivo data: "in vivo" OR animal\* OR mice OR mouse OR rat OR dog OR rabbit OR “guinea pig”
4. Mechanistic data: “in vitro" OR cell\* OR "ex vivo" OR culture OR "mechanistic assays" OR "mechanistic study" OR "Adverse Outcome Pathways" OR AOPs
5. Endocrine disruptors: endocrine\* OR hormon\* OR “endocrine disrupting substances” OR “Endocrine Disrupters” OR "Endocrine System" OR "Endocrine Glands" OR "Endocrine System Diseases" OR "Gonadal Hormones" OR "Placental Hormones" OR "Pituitary Hormones" OR "Growth Hormone" OR "Thyroid Hormones" OR "Gastrointestinal Hormones" OR "Sex Hormone-Binding Globulin" OR "Adrenocorticotropic Hormone" OR "Adrenal Cortex Hormones" OR hypothyroidism OR hyperthyroidism OR adrenal
6. Male reproductive data: reproduct\* OR development\* OR growth\* OR malformation\* OR anomal\* OR fertil\* OR infertility OR foet\* OR matern\* OR pregnan\* OR embryo\* OR puberty OR infertility OR placenta OR “anogenital distance” OR hypospadia OR cryptorchidism OR "Reproductive Health" OR Testes OR testicular OR sperm\* OR spermatogenesis OR epididymis\* OR "semen quality" OR testosterone OR fetus\* OR fetal\* OR LH OR FSH OR testis\* OR germ\* OR leydig OR sertoli OR prostate OR "seminal vesicle" OR mating
7. Female reproductive data: reproduct\* OR development\* OR growth\* OR malformation\* OR anomal\* OR fertil\* OR infertility OR foet\* OR matern\* OR pregnan\* OR embryo\* OR puberty OR infertility OR placenta OR "anogenital distance" OR "Reproductive Health" OR ovaries OR uterus OR estrogen OR progesterone OR fetus\* OR fetal\* OR LH OR FSH OR germ\* OR mating
8. Metabolism/obesity: "Metabolic Syndrome" OR "Nutritional and Metabolic Diseases" OR "Metabolic Diseases" OR Metabolism OR metabolite\* OR metabolic OR distribution OR adsorption OR excretion OR elimination OR kinetic OR PBPK OR glucagon OR GLP1 OR insulin OR pancreas OR liver OR "Diabetes Mellitus Type 2" OR "diabetes mellitus experimental" OR "Glucose Metabolism Disorders" OR "glucose homeostasis" OR lipid\* OR triglyceride\* OR Hyperlipidemia OR "metabolism disorder" OR Acidosis OR Metabolome OR Metabolomics OR Lipolysis OR Obesity OR "Pediatric Obesity" OR "Obesity Abdominal" OR obesogen\* OR adiposity OR "adipose tissue" OR "Abdominal obesity metabolic syndrome" OR adypokine OR "visceral fat" OR "body fat" OR overweight OR "pregnane X receptor" OR PXR OR "Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha" OR PPAR
9. Thyroid: Thyroid OR thyroxine OR T4 OR triiodothyronine OR T3 OR "Thyroid-stimulating hormone" OR thyrotropin OR TSH OR hypothyroxinemia OR "thyroid hormones"
10. Immunological endocrine-mediated perturbations: "immune system" OR "immune response mechanism" OR allergy OR "multiple chemical sensitivity” OR "autoimmune disease" OR cytokines OR “white cells”
11. Neurological endocrine-mediated perturbations: "nervous system" OR "neurodevelopment effects" OR Neurotoxicity OR "developmental neurotoxicity" OR DNT OR brain OR "mental ability" OR intelligence OR cognition OR motor OR language OR behavior OR autism development OR prenatal OR postnatal OR infant OR child

**Table 11: Pubmed and Scopus systematic reviews number of references (30/09/2021)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Number of references (Pubmed + Scopus)** | | |
| **Melamine + Endocrine disruptors** | 77+84 | | |
| **Endpoint** | **Epidemiological data**  Equations: 1+2+endpoint | **In vivo data**  Equations: 1+3+endpoint | **Mechanistic data**  Equations: 1+4+endpoint |
| **Male reproductive data** | 189+287 | 273+309 | 160+364 |
| **Female reproductive data** | 190+286 | 259+299 | 155+366 |
| **Metabolism/obesity** | 275+349 | 370+409 | 233+475 |
| **Thyroid** | 6+11 | 8+12 | 2+7 |
| **Immunological endocrine-mediated perturbations** | 38+58 | 23+13 | 19+13 |
| **Neurological endocrine-mediated perturbations** | 325+36 | 238+41 | 95+40 |

1. https://echa.europa.eu/documents/10162/9b7c2612-f481-a249-50e0-9e61c06e5e36 [↑](#footnote-ref-1)
2. https://echa.europa.eu/documents/10162/470b1aed-0ba9-c47f-c2ba-1a078c5c41a8 [↑](#footnote-ref-2)
3. https://www.ecologie.gouv.fr/sites/default/files/SNPE%202%20english%20-%20Action%20plan.pdf [↑](#footnote-ref-3)
4. https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.003.288 [↑](#footnote-ref-4)
5. C&L Inventory database, <http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database> (accessed 17 January 2022) [↑](#footnote-ref-5)
6. https://echa.europa.eu/documents/10162/49f9ee64-f18d-778a-3052-1a292a3f32a0 [↑](#footnote-ref-6)
7. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978/5/4/2 [↑](#footnote-ref-7)
8. https://echa.europa.eu/documents/10162/49f9ee64-f18d-778a-3052-1a292a3f32a0 [↑](#footnote-ref-8)
9. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978 [↑](#footnote-ref-9)
10. https://echa.europa.eu/documents/10162/3bdeab03-8147-b308-acd0-4ed5848340c0 [↑](#footnote-ref-10)
11. https://echa.europa.eu/documents/10162/bfeec668-edf2-d959-3af9-861020103a4d [↑](#footnote-ref-11)
12. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978/7/2/1 [↑](#footnote-ref-12)
13. https://echa.europa.eu/documents/10162/bfeec668-edf2-d959-3af9-861020103a4d [↑](#footnote-ref-13)
14. https://echa.europa.eu/documents/10162/bfeec668-edf2-d959-3af9-861020103a4d [↑](#footnote-ref-14)
15. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978/7/9/2 [↑](#footnote-ref-15)
16. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978/7/9/3 [↑](#footnote-ref-16)
17. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978/6/1 [↑](#footnote-ref-17)
18. https://echa.europa.eu/documents/10162/ea0f4b42-6c6c-bca2-29ee-fded8a55f7fa [↑](#footnote-ref-18)
19. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978/6/7 [↑](#footnote-ref-19)
20. DEDuCT <https://cb.imsc.res.in/deduct/experimentalevidence/eJaFhpZybG4> [↑](#footnote-ref-20)
21. https://actor.epa.gov/dashboard/, accessed on October 8th 2018 [↑](#footnote-ref-21)
22. Deduct database of Endocrine Disrupting Chemicals and their toxicity profiles https://cb.imsc.res.in/deduct/home [↑](#footnote-ref-22)
23. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978/6/2/2 [↑](#footnote-ref-23)
24. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15978/6/2/3 [↑](#footnote-ref-24)