

Annex XV report

PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance Name: melamine; 1,3,5-triazine-2,4,6-triamine;

EC Number: 203-615-4

CAS Number: 108-78-1

Submitted by: Germany

Date: August 2022

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ABBREVIATIONS

B	bioaccumulative
vB	very bioaccumulative
BCF	bioconcentration factor
c _{max}	maximum concentration
CAKE	Computer Assisted Kinetic Evaluation
CTD	characteristic travel distance
DOC	dissolved organic carbon
DFOP	Double first-order in parallel
DT50	Half-live
DegT50	Degradation Half-live
EC10	10% effect concentration
EC50	50% effect concentration
EUSES	European Union System for the Evaluation of Substances
FOMC	First-order multi-compartment
HS	Hockey-Stick
K _{oc}	normalised organic carbon to water partition coefficient
LC50	50% lethal concentration
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LOD	limit of detection
LOEC	Lowest Observed Effect Concentration
LOQ	limit of quantification
LRTP	long range transport potential
NOEC	no observed effect concentration
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
P	persistent
vP	very persistent
P _{ov}	overall environmental persistence
PBT	persistent, bioaccumulative, toxic
POP	persistent organic pollutant
PMT	persistent, mobile, toxic
P _{ov}	overall persistence
QSAR	quantitative structure-activity relationship
RAC	Committee for Risk Assessment
SFO	Single first-order
STP	sewage treatment plant
SVHC	substance of very high concern
vPvB	very persistent, very bioaccumulative
vPvM	very Persistent, very mobile
WWTP	wastewater treatment plant

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Substance name: melamine; 1,3,5-triazine-2,4,6-triamine

EC number: 203-615-4

CAS number: 108-78-1

- It is proposed to identify the substance as a substance of equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of Regulation (EC) No 1907/2006 (REACH) according to Article 57(f) of REACH Regulation.

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation

Melamine is identified 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.

Intrinsic properties

Degradation:

Abiotic degradation of melamine by hydrolysis and phototransformation in air and water is regarded as negligible.

Based on modelling data, melamine is predicted to be not readily biodegradable and hence potentially persistent. A reliable OECD TG 301C study shows that melamine is not readily biodegradable (0% degradation after 14 days). Biotic degradation of melamine was investigated in a surface water over 60 days according to OECD TG 309, showing no degradation of the substance. Therefore, the degradation half-life of melamine is longer than 60 days. Considering the data on abiotic and biotic degradation, melamine fulfils the P and vP criteria (half-life in water >60 days) of REACH Annex XIII.

Volatility, water solubility, adsorption:

The substance has an experimentally derived log K_{oc} of 1.81 indicating a low potential for adsorption on organic matter and clay minerals in the environment. As melamine has a pK_{b1} of 7.3, non-ionic and ionic species of the molecule occur under environmentally relevant conditions. It has to be considered, that the QSAR calculations normally refer to the non-ionic (undissociated) form of a substance. For the cationic species of melamine, a log K_{oc} of around 3.41 is assumed based on a study investigating the experimental and calculated log K_{oc} of a model cationic substance. Thus, also for the ionic melamine species, adsorption on organic matter and clay minerals is still expected to be low.

The substance's intrinsic behaviour of low volatility from water (calculated Henry's law constant $2.0e-08$ Pa*m³/mol) together with its low potential to adsorb to organic matter result in a high mobility in water. Additionally, the intrinsic substance properties indicate

that the substance will partition primarily to the water compartment and will undergo environmental distribution via aqueous media, easily reaching groundwaters.

High water solubility (3.48 g/L at 20°C and pH 7.7 using EU Method A.6) and low adsorption potential of melamine makes it difficult to remove the substance from water by commonly applied sewage treatment and water purification techniques as it only has a low potential to adsorb to materials and prefers to remain in the water phase.

Because of melamine's high water solubility, low volatility from water and low potential for adsorption, water will be the dominant transport medium in the environment once the substance is released. In combination with its long environmental half-life, there is a potential for widespread contamination of the water environment.

The OECD tool for Long-Range Transport Potential (LRTP) predicts a characteristic travel distance (CTD) of 3530 km together with an overall environmental persistence (P_{ov}) of 2181 days for melamine. This indicates that melamine is capable of reaching regions far away from the point of initial emission.

Toxicity:

Urinary tract toxicity after oral melamine exposure has been shown both in experimental animal studies and observational studies in humans. Following oral exposure, melamine is rapidly excreted mostly unchanged via the urine. Once the urinary concentration of melamine exceeds a certain threshold, precipitation occurs within the urinary tract leading to the formation of crystals and calculi. Melamine-related uroliths have been linked to the formation of rare urinary tract tumours in rodents (with an unusually short latency) and nephrotoxicity in humans. Epidemiological data show that the intake of high amounts of melamine leads to precipitation in the lower urinary tract and to melamine-associated formation of urinary stones in humans.

Based on these effects, the Committee for Risk Assessment (RAC) has concluded that melamine fulfils the criteria for classification as STOT RE 2 (urinary tract) and Carc. 2. The harmonised classification has been included in the 18th ATP to CLP¹. This classification is of relevance for the assessment of melamine as a substance of very high concern according to Article 57 (f), i.e., applying the T-criterion of REACH Annex XIII; Section 1.1.3 c).

Melamine fulfils the T-criteria of REACH Annex XIII Section 1.1.3 c) through its harmonised classification as STOT RE 2 (urinary tract) as of 23 November 2023. Melamine therefore has the potential to produce the same adverse toxic effects as a PBT substance.

According to the registrants, the observed testicular and sperm effects of melamine identified in experimental animals are of concern and warrant self-classification in Repr. category 2 (H361f).

Concerns arising from the substance properties

The concern raised by melamine is triggered by individual properties as well as by combination of its properties.

¹ Commission Delegated Regulation (EU) 2022/692 of 16 February 2022 amending, for the purposes of its adaptation to technical and scientific progress, Part 3 of Annex VI to Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (the 18th ATP to CLP). Pursuant to the second paragraph of Article 2 of this Regulation, this new harmonised classification applies from 23 November 2023. However, pursuant to the third paragraph of that provision substances and mixtures may already be classified, labelled and packaged in accordance with this classification.

Concern for an irreversible and increasing presence in the environment

The combination of the substance intrinsic properties persistency, mobility and potential for being transported in the water phase over long distances lead to an irreversible presence in the environment, together with a widespread contamination of the aquatic environment.

Due to its low tendency for adsorption, the substance will not attach to suspended or organic matter in the environment to any significant degree.

Due to the lack of abiotic and biotic degradation of melamine, it is expected that there is no significant removal of melamine by biological processes in conventional sewage treatment plants dealing with mixed sewage. Thus, the overall amount of melamine emitted from production and use is, if at all, only marginally reduced in sewage treatment plants. Once released, melamine remains in the environment due to its persistency.

Monitoring already confirmed occurrence of melamine in various rivers (e.g. Rhine, Meuse, Mulde, Danube, Nau), where sampling points that do not influence each. Concentrations found exceeded the value of 0.1 µg/L. Even while not being subject to the list of substances for regular groundwater monitoring, recent projects were able to identify and quantify melamine in groundwater samples in Germany and Switzerland, too.

Due to the global water cycle and the fact that the aqueous compartments are all well connected, the high persistency and the high mobility of melamine lead to long distance transport processes in the environment. The properties of melamine are likely to cause a transport across water bodies to pristine oceans and groundwaters, raising the concern that any effects caused by the substance might also occur at remote locations from the origin of release.

The substance stays in the environment even if releases of the substance have already ceased, as can be concluded from the substance properties and the recurring findings in groundwater samples.

Decontamination from the aquatic environment and from drinking water resources

Sources of emission in the vicinity are seldom apparent. Once, melamine is released into the aquatic compartment and widely distributed in the environment, it is difficult to be removed from there. This results from the recalcitrant degradation potential of melamine against abiotic and biological processes, leading to very slow or negligible removal from the water phase.

Due to its intrinsic properties melamine will not be easily removed with natural processes or conventional water purification techniques. This includes procedures such as river bank filtration or soil infiltration for groundwater enrichment, which are commonly applied on European level. Therefore, there is substantial evidence that melamine may not be removed from the water cycle, once a contamination of aquatic resources occurs. The same applies for the drinking water purification, as melamine will bypass these commonly used drinking water purification techniques and has been already found in drinking water.

Human Health effects

Melamine is classified as STOT RE 2, H373 (urinary tract), and Carc. 2, H351, and therefore its presence in drinking water may become a concern if critical dose levels are exceeded. A substance classified as STOT RE 2 fulfils the T criterion for PBT substances according to REACH Annex XIII.

Aside from effects caused by exposure to melamine only, combined effects due to co-exposure with other chemicals, e.g. cyanuric acid, have been reported in rats, pets and livestock (WHO / FAO, 2009) Dorne et al., 2013). Effects occur at lower melamine concentrations following co-exposure to melamine and cyanuric acid. Potential combined

effects as a consequence of co-exposure of melamine and cyanuric acid prompted the Dutch competent authority (RIVM) to lower their derived limit values for drinking water (Smit, 2018; Smit, 2019).

According to the registrants, the observed testicular and sperm effects of melamine identified in experimental animals are of concern and warrant self-classification. However, the severity of the testicular findings was generally low and adverse effects on fertility parameters were not observed. Therefore self-classification in Repr. category 2 (H361f) is considered most appropriate.

Human exposure

The most significant sources of human exposure to melamine are food contact materials and, with several uncertainties, articles containing melamine as flame retardant. Apart from cases of criminal food alteration, the contribution of nutrition and drinking water to human exposure to melamine is several orders of magnitude lower than the current health-based guidance value (EFSA 2010).

Melamine has been repeatedly detected in human urine samples from the general population in the USA and in East Asia (see(Health Canada, 2020), for an overview of these biomonitoring studies).

Melamine can also be ingested by infants through breast milk (Zhu and Kannan, 2019; Zhu and Kannan, 2020). Lactational and/or placental transfer was shown by (Chan et al., 2011; Cruywagen et al., 2009; Kim et al., 2011) following exposure to melamine in animal studies.

Water is used for drinking and cooking each day and it is the basis of all food and meals throughout the human life. Water taken from environmental sources is not only used for drinking water production but also for applications like irrigation in agriculture or cattle watering tanks. According to the German Association for Nutrition (DGE), the intake of water as such for adolescents and adults is approximately 1500 mL per day (DGE, 2020), not envisaging the incidental co-intake via nutrition. EFSA has established the tolerable daily intake (TDI) for melamine at 0.2 mg/kg bw/day in 2010 (EFSA, 2010). At the current stage the TDI is not exceeded via nutrition including beverages and tap water, and there are currently no risks for human health via this exposure pathway.

Due to the persistence and mobility of melamine, it is able to reach the resources of drinking water. Continued emissions and the limits of retrievability from the aqueous phase may result in increasing concentrations in raw water in the future and humans may be exposed to increasing concentrations in drinking water, too.

Environmental effects

Due to the properties of melamine (persistency, mobility, potential for being transported in the water phase over long distances and carcinogenicity) it is not possible to derive a safe concentration limit for the environment by the means of standardised acute and chronic ecotoxicological tests and the assessment criteria investigated within.

A concern observed for environmental organisms are sublethal effects in fish. Although available acute aquatic studies in fish do not show effects on mortality, sub-lethal effects were seen. In chronic fish studies mortality appeared and growth decreased (effect value in one study: NOEC 5.25 mg/L). Sub-lethal effects appeared in the long-term fish studies on the same organs as in the acute study: Kidney, liver and gills were affected with dose-dependent histological effects. Furthermore, in several fish studies there were effects on skin coloration and on blood cells, additionally the antioxidant system was impaired. In aquatic invertebrates, the following effects were observed as well. In a chronic Daphnia test, the NOEC for reproduction was 18 mg/L. In a feeding study with Pacific white shrimp, effects on survival, growth and histological effects on the hepatopancreas, as well as

effects on the antioxidant system were observed at 10 g/kg feed. Therefore, the substance properties raise the concern that these effects or yet unknown effects could appear in the environment and lead to population-relevant effects, due to long-term exposure over the whole life and over several generations, keeping in mind continuous exposure via water and potentially increasing concentrations.

Societal concern

Efforts to purify drinking water from surface water or groundwater bodies should be as low as reasonably possible. Therefore, Member States shall introduce measures to protect the water bodies with the aim of avoiding deterioration in their quality (see Article 7.3 of the Water Framework Directive (2000/60/EC)).

The combination of melamine's intrinsic properties of persistence and mobility results in a little effectivity of common and widely applied drinking water purification techniques, such as river bank filtration or soil infiltration for groundwater enrichment. Decontamination may only be achieved by advanced water purification techniques, if at all and at high costs that have to be paid predominantly by the society, not by the body that is responsible for the initial emission of melamine into the environment.

Exposure to humans might occur via consumption and use of contaminated drinking water. Furthermore, melamine received a harmonised classification for target organ toxicity after repeated exposure (STOT RE 2 (urinary tract)) and is likely carcinogenic to humans (Carc. 2). In case that humans may be harmed due to this potential effects, costs for medical treatment will be the result and those will be handed over predominantly to the society.

Consequently, there is societal concern regarding increasing concentrations of melamine in sources of drinking water, which requires action based on precautionary considerations.

Increasing presence in the aquatic environment results in increasing exposure of humans and environmental organisms

Melamine is already present in surface waters. Computational models predict, that the substance is able to remain in the environment for several years or maybe even decades in the environment and, once released, is able to reach remote regions because it can be distributed in the aquatic environment over several thousand kilometres. This means, findings and undesirable effects may occur far away from the point of initial release.

Due to its mobility, melamine can widely distribute in the water bodies, making it difficult to control the arising concentrations and conclude on appropriate, effective measures to remove the substance from environmental aquatic media.

Depending on the level of continuation of emissions into the environment and resulting from persistency and mobility of melamine, overall concentration in the environment is expected to increase over time. This will result in an increase of the concentrations in drinking water, too.

Consequently, exposure of environmental organisms to melamine via "natural" waters and exposure of humans via drinking water is expected to increase over time due to persistency and mobility. For these reasons and because the substance has a harmonised classification as STOT RE 2 (urinary tract) and Carc. 2, the release of melamine to the environment should be prevented.

Concern related to co-exposure and combined effects and inability to derive a safe concentration

Melamine and other melamine analogues occur in the different environmental compartments. Therefore co-exposure cannot be excluded. These substances can act jointly, so that exposures at comparatively low concentrations may lead to health and environmental effects. In addition, co-exposure and combined effects with other

substances are likely. Therefore, appearance of undesirable effects due to additive or even synergistic Mode of Action cannot be excluded and no safe concentration can be derived.

Overall evaluation of the concerns

The persistency, mobility and toxicity (specific target organ toxicity after repeated exposure and carcinogenicity) and in addition the irreversibility of the contamination of the aquatic compartment compromise the quality of drinking water resources and give rise to the concern of increasing exposure to wildlife and man via environment due to contaminated water.

Consequently, there is societal concern regarding increasing concentrations of melamine in sources of drinking water, which requires action, based on precautionary considerations. The environment provides natural drinking water sources, whose integrity needs to be ensured for future generations.

Equivalent level of concern

The level of concern is considered very high in particular due to the combination of the following concern elements:

- Irreversible and increasing presence in the environment, due to limited potential for removal of melamine from the aquatic environment and drinking water resources, which are leading to a potential for:
 - Human health effects, potentially long-lasting and more likely in susceptible populations such as infants, which may occur in the future due to increasing exposure over time
 - Societal concern through its ubiquitous presence in, and difficulty to remove from, surface water, groundwater and processing of raw water
- Increasing presence in the aquatic environment results in increasing exposure of humans and environmental organisms
- Concern about other potential effects (e.g., combined effects from concomitant exposure to similar substances)

Conclusion

The combination of melamine's substance properties causes high concern to the environment and human health (man via environment). The combined intrinsic properties which demonstrate scientific evidence of probable serious effects to human health and the environment and which give rise to an equivalent level of concern are the following: very high persistence, high mobility in water, potential for being transported in the water phase over long distances, difficulty of remediation and water purification. There is a potential for serious effects for human health and the environment that may occur with increasing concentrations in the environment. Thus, melamine may cause damage to the urinary tract organs through prolonged or repeated exposure, is suspected of causing cancer and yet unknown environmental effects. Together, these elements lead to a high potential for irreversible effects.

Registration dossiers submitted for the substance: Yes

PART I

Justification

1. Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	203-615-4
EC name:	melamine
CAS number (in the EC inventory):	108-78-1
CAS number:	108-78-1
IUPAC name:	1,3,5-triazine-2,4,6-triamine
Index number in Annex VI of the CLP Regulation	
Molecular formula:	C ₃ H ₆ N ₆
Molecular weight range:	126.1199 g/mol
Synonyms:	

Structural formula:



1.2 Composition of the substance

Name: melamine

Substance type: mono-constituent

Table 2: Constituents other than impurities/additives

Constituents	Typical concentration	Concentration range	Remarks
1,3,5-triazine-2,4,6-triamine, EC no 203-615-4	>99.8 % w/w	80-100 % w/w	

1.3 Physicochemical properties

Table 3: Overview of physicochemical properties

Property	Description of key information	Value [Unit]	Reference/source of information
Physical state at 20°C and 101.3 kPa	Visual observation	Solid white powder	(Reuse and Halzschuh, 2009)
Melting/freezing point	EU Method A.1	361°C	(Reuse and Halzschuh, 2009)
Boiling point	Data waiving according to Annex VII 7.3	Decomposition and sublimation occur at temperatures close to and above the melting temperature.	
Vapour pressure	Data waiving according to Annex VII 7.5	The study does not need to be conducted if the melting point is above 300 °C.	
	QSAR estimate	3.59e-10 mm Hg (25°C); equals 4.79e-08 Pa	database value of EPIsuite (v4.10); used for further assessment
Density	EU Method A.3	Relative density: 1.57 at 20°C	(Reuse and Halzschuh, 2009)
Water solubility	EU Method A.6	3.48 g/L at 20°C and pH 7.7	(Reuse and Halzschuh, 2009)
Partition coefficient n-octanol/water (log value)	EU Method A.8	Log Pow -1.22 at 22°C, pH 8 Log Pow -1.3 to -1.18 at pH 6-8	(Junghans, 2009) pH 6-8 (ACD/Labs, 2019)
Granulometry	Inter alia sieve analysis, dry powder laser diffraction,	The various products under the joint submission are fine powders with a mass median diameter below 100 µm, except for the Cytec product (Rich 2010) with a mass median diameter of 120 µm.	
Dissociation constant	OECD Guideline 112	pK _{b1} = 7.3 and pK _{b2} = 11.4.	(Reuse and Halzschuh, 2009)
Under strong acidic conditions it is likely that melamine is twofold protonated resulting in a corresponding high pK _b value and a log Pow value <-1.22. A single protonation with a corresponding lower pK _b value and a log Pow value closer to -1.22 can be expected under moderate acidic to neutral conditions. The inflexion points observed in the determination of the dissociation constant underlines these expectations.			

2. Harmonised classification and labelling

For melamine a proposal for harmonised classification was submitted in 2019.

In its opinion of December 2020 RAC agreed to the proposal to classify melamine as STOT RE 2 (urinary tract) and Carc. 2².

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 (EC, 2022).

² <https://echa.europa.eu/documents/10162/bfeec668-edf2-d959-3af9-861020103a4d>

3. Environmental fate properties

3.1 Degradation

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

There is no information available from standard tests about the potential for hydrolytic degradation of melamine but information is available from secondary sources.

In a publication of Crews et al. (2005) the degradation pathway and the identity of observed transformation products under influence of mineral acid, respectively inorganic alkali was described. Ammeline, ammelide and cyanuric acid were identified as the most relevant transformation products. However, information about degradation rate constants or environmental degradation half-life is lacking in the publication.

In a handbook article the measured half-lives and degradation rate constants at 100 °C and at extreme acidic and alkaline conditions were mentioned. No specific information about test conditions beyond pH and test temperature are provided. For pH 1.7 a half-life of 0.23 days, and for pH 12 a half-life of 0.04 days was shown in that article. The authors conclude, that *"No data for relevant environmental conditions are available, probably because the substance hydrolyses only in the extreme pH ranges."* (Gmelin et al., 1971).

A QSAR estimate by applying EPIsuite's Hydrowin model (US EPA, 2002-2012) was not possible because the substance does not fall into the applicability domain of the model.

Another trial for a QSAR estimation was undertaken with the Vega Hub models³. According to these prediction models the degradation half-life for hydrolysis is 29.2 days. However, reliability of this result is limited because a prominent number of atom centered fragments of melamine have not been found in the compounds of the training set or are rare fragments.

3.1.1.2 Oxidation

Melamine does not contain oxygen or halogen atoms. Therefore, it is unlikely that melamine has oxidizing properties.

3.1.1.3 Phototransformation/photolysis

3.1.1.3.1 Phototransformation in air

No experimental data on direct or indirect phototransformation in air is available on the ECHA dissemination site⁴ for melamine.

The QSAR model AOPWIN v1.92 of the tool EPISuite v4.10 (US EPA, 2002-2012) which predicts degradation rates and half-lives for indirect photolytic degradation in the atmosphere was applied and predictions were run for melamine.

For the substance a half-life of 24.3 days in the gas-phase was calculated under the assumption of indirect photolysis via OH-radicals, 0.5×10^6 OH/cm³, 24 h daylight. Assuming a 12 h daylight and an OH-radical concentration of 1.5×10^6 OH/cm³, the half-

³ QSARs available at <https://www.vegahub.eu/>

Date of access 19.08.2022

⁴ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/2/2;>

date of access 18.08.2022

life decreases to 16.22 days.

The AOPwin model also provides the information, that a fraction of melamine will adsorb to airborne particles and that the sorbed fraction may be resistant to atmospheric oxidation. Depending on the method applied the model states that between 52.7% (Koa method) and 61.1% (acc. to Junge-Pankow, MacKay avg.) of melamine in the atmosphere will sorb to airborne particulates. As a consequence, the atmospheric half-life of 24.3 days is considered to be an underestimate for melamine.

3.1.1.3.2 Phototransformation in water

No experimental data on phototransformation in water is available on the ECHA dissemination site⁵ for melamine.

The technical guidance document R.11 (ECHA, 2017) states regarding the consideration of photochemical degradation processes in water: *"Due to the large variation in the light available in different environmental compartments, the use of photolysis data is not generally recognised for persistence assessment."*

In conclusion, the contribution of photolytic degradation in the water phase to the total degradation in this compartment is expected to be negligible. In addition, due to the lack of UV-adsorbing functional groups in the molecular structure, melamine is not expected to be directly photolysed in water. Major releases during the life cycle of melamine (manufacture and application of the substance) are expected to occur predominantly into surface waters that show only little potential for aquatic photolytic degradation due to depth of the water column, turbidity and presence of suspended matter that hamper the photolytic degradation potential.

3.1.1.3.3 Phototransformation in soil

No experimental or calculated data on phototransformation in soil is available on the ECHA dissemination website⁶ for melamine.

3.1.1.4 Summary on abiotic degradation

Due to the structural properties, hydrolysis is not expected to be an important fate pathway under environmentally relevant conditions.

After evaporation or exposure to air, melamine undergoes relatively slow degradation by photochemical processes. However, volatilisation to the air is not a major pathway for removal of melamine from the water phase (see section 3.2.2 Volatilisation). Thus, abiotic degradation of the substance by phototransformation in air is regarded as negligible.

Photolytic degradation in the aquatic compartment is also expected to be negligible.

Overall, abiotic degradation is not a relevant pathway for removal of melamine from air or the aquatic environment.

⁵ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978>;
date of access 18.08.2022

⁶ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978>;
date of access 18.08.2022

3.1.2 Biodegradation

3.1.2.1 Biodegradation in aqueous media or aqueous environment

3.1.2.1.1 Estimated data

The QSAR model BIOWIN v4.10 of the EPISuite tool (US EPA, 2002-2012) includes several QSARs for estimating intrinsic substance properties and environmental fate and behaviour of chemicals, providing degradation timeframes for primary and ultimate degradation of chemicals.

BIOWIN also provides an estimate whether a substance fulfils the criteria of being rated as “readily biodegradable”. According to the estimation of biodegradation, melamine “does not biodegrade fast” because the probability is lower than 0.5 (Biowin2 = 0.0000). Ultimate biodegradation timeframes for melamine indicate ultimate degradation within months (Biowin3 = 2.2697). MITI non-linear model prediction results (Biowin6) indicates that melamine is not readily biodegradable, because the probability is lower than 0.5 (estimated value = 0.0000).

Based on the above information, QSAR data are considered sufficiently reliable to conclude that melamine is recalcitrant against biotic degradation processes and screens as potentially P/vP according to REACH guidance ECHA (2017).

3.1.2.1.2 Screening tests

Several standard and non-standard screening studies on biodegradability are available on the ECHA dissemination website for melamine⁷:

An OECD 301 C test (report dated 2010) using activated sludge (adaption not specified) showed 0% degradation (oxygen demand) after 14 days of incubation at a concentration of 100 ppm equivalent to 100 mg/L. The reliability of the data is considered to be high. From this test result it is concluded that the substance is not readily biodegradable.

In an OECD 302 B test for inherent biodegradability (report dated 1993) using melamine at a test concentration of 100 mg/L together with industrial, activated sludge with unknown adaptation, < 10 % degradation (DOC removal) was observed after 28 days of incubation. In a second available OECD 302 B test for inherent biodegradability (report dated 1991) using melamine at a test concentration of 400 mg/L and inoculum of an industrial sewage treatment plant, 16 % degradation (DOC removal) was observed after 20 days of incubation.

In a test on the toxicity of melamine to microorganisms according to test guideline DIN 38412-8 (bacterial inhibition test with *Pseudomonas putida*), EC10 value for microorganisms amounted > 10,000 mg/L. The reliability of the data is considered to be high.

Based on the results from the toxicity test, it can be concluded that the test concentrations used in the OECD screening test on ready biodegradation and both OECD tests on inherent biodegradability have no impact on the vitality of the inoculums in the test and do not hamper the degradation process in these tests.

The reliability of the data of both tests on inherent biodegradation are considered to be limited as the adaption of the inoculum cannot be excluded because it was sampled from an industrial sewage treatment plant. Nevertheless, despite possible adaption of the

⁷ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/3/2>
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inoculum used in these tests on inherent biodegradability, there is an obvious lack of degradation (<20% degradation) to be observed which is resulting in the conclusion, that the substance is not inherently biodegradable. This, together with the inclusion of the considerations about the potential adaption of the inoculum applied in both tests provide evidence that degradation of melamine in the environment is expected to be slow.

In a non-guideline study using melamine (unknown test concentration), < 1 % degradation (O₂ consumption) after 5 days with adapted inoculum was observed (Niemi et al., 1987).

The registration also utilises a non-guideline study performed by Fimberger (1997) using melamine in concentrations of 20 mg/L in activated sludge sampled in communal sewage treatment plant shows that microorganisms cannot use melamine as carbon source (test mat. analysis) within 28 days test duration. Activated sludge sampled in an industrial sewage treatment plant at a location, where melamine is produced, is able to degrade melamine rapidly. However, applying adapted inoculum in a screening test for the purpose of concluding about persistency of a substance is not accepted according to the recent ECHA Guidelines (R.7b and R.11: ECHA, 2017 and 2017b).

From these results it is concluded that degradation of melamine in the presence of activated sludge is slow or neglectable.

In conclusion, melamine is evaluated to be not readily biodegradable and not inherently biodegradable.

3.1.2.1.3 Simulation tests

3.1.2.1.3.1 Biodegradation in water

No standard simulation tests with melamine in water are available on the ECHA dissemination website⁸.

In a study by Hofman-Caris and Claßen (2020) according to OECD TG 309, degradation of unlabelled melamine was investigated in surface water without the addition of suspended sediment. The surface water was collected at the location Schalterberg (NL), a sub-surface springs which is used for the production of drinking water. The test was performed using stationary biometer test systems using 300 mL of surface water. After collection, the surface water was cooled at 13 °C for 7 days prior to the test.

Melamine with a purity of 99.8 % was dissolved in water resulting in a concentration of 5.21 mg/L and was added in a concentration of 5 µg/L to the test system. Duplicate samples were incubated for 0, 7, 15, 30, 45, and 60 days, respectively at 13 ± 1 °C in darkness. As reference substance, unlabelled aniline was used. The application rate of aniline was 1.0 µg/L. Besides the reference substance, the microbial activity of the water was also investigated by determining the amount of adenosine triphosphate (ATP). Degradation was evaluated based on the concentration of reference substance and melamine detected in the water phase using LC-MS/MS. The amount of ATP in the surface water without the addition of melamine at day 0 was 86 ± 1.2 pg/mL, after autoclaving ATP concentration was reduced (<1 ± 0.44 pg/mL), indicating that the ATP assay is able to reflect the microbial activity of the surface water. In the presence of melamine, the ATP concentration was slightly higher (92 ± 16 pg/mL) to those measured in surface water without any addition after 60 days, meaning that melamine has no negative effect on the microbial population. The concentration of the reference substance aniline decreased over the course of the study. After 15 days of incubation, the concentration was 0.418 ± 0.1 µg/L. At the end of the study (day 60), the aniline concentration amounted to < 0.01 µg/L

⁸ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978>

in the surface water. Degradation of aniline based on the concentration observed amounted to 52 % and > 98 % after 15 and 30 days of incubation. Based on the results it is concluded that the surface water used in the test contained an active microbial population.

Recovery of the melamine concentration applied to test ranged between 100-107 %. According to OECD 309, initial recovery should be between 70 % and 110 % for non-labelled substances. Thus, the study is regarded as valid.

The melamine concentration remained stable over the course of time and ranged between a minimum value of $5.0 \pm 0.0 \mu\text{g/L}$ at day 45 and a maximum value of $5.35 \pm 0.7 \mu\text{g/L}$ at day 15 (Figure 1). At the end of the study (day 60), $5.25 \pm 0.7 \mu\text{g/L}$ melamine could be detected in the surface water. As unlabelled melamine was used in this study, ultimate degradation of the substance could not be determined. Nevertheless, as no decline in test concentration was detected during the study, no ultimate or primary degradation of melamine took place in test system.

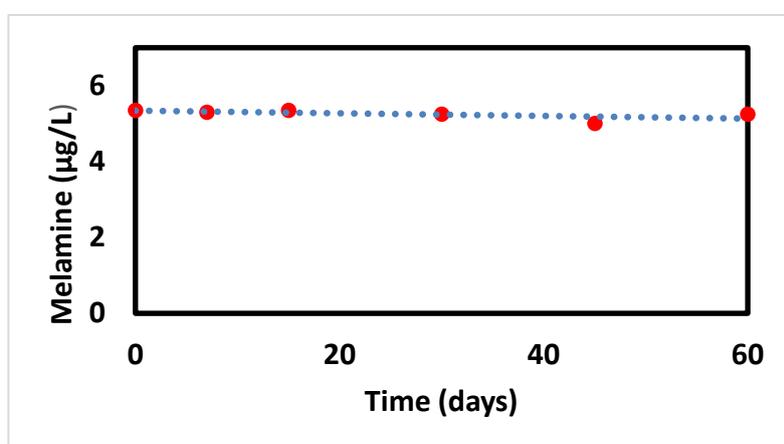


Figure 1: Concentration of melamine ($\mu\text{g/L}$) in a surface water test system in course of time (days). Data were shown as mean value of two individual bottles for each sampling point. Please note: Error bars indicating standard deviation are included in this figure. However, as the standard deviation resulted in values up to $0.071 \mu\text{g/L}$ only, the error bars are hardly noticeable in the plot.

The determination of half-life of melamine was calculated based on the amount of melamine detected in the water phase using the software Computer Assisted Kinetic Evaluation (CAKE, Tessella, Version 3.1). The software CAKE applies the kinetic models SFO (Single first-order), FOMC (First-order multi-compartment), DFOP (Double first-order in parallel) and HS (Hockey-Stick) to deviate half-lives whereas the latter ones describe biphasic kinetics characterised by a quick initial decrease in test compound concentration (k_1) followed by a slow phase (k_2).

Calculated half-lives (DT_{50}) of melamine are shown in Table 4. The SFO model is the best fit model (lowest χ^2 error value which is below 15 %). Results of the t-test reveal that the parameter k of the SFO model, as well as the k_1 and k_2 of the DFOP and HS models, are not significantly different from zero at the significance level of 5 % ($p < 0.05$). Therefore, it is concluded that there is no significant measurable degradation over 60 days. A half-life $> 10,000$ days (default value) was derived from the SFO model. However, as no degradation of the substance appears during the study, the calculated half-lives are considered uncertain. Thus, the respective DT_{50} should be interpreted with care. Nevertheless, it is possible to conclude on reaching certain trigger values, even though it is impossible to define exact values. Thus, the DT_{50} of melamine is regarded to be over 60 days.

Table 4: Kinetic calculations of half-lives (DT50) based on the amount of parent detected in the water phase

Kinetic model	DT ₅₀ (days)	Chi2 Error	Result t-test	Visual fit
SFO	>10,000	1%	k = 0.09306	medium
DFOP	DT _{50,overall days} = >10,000 DT _{50, k1} = 24.3 DT _{50, k2} = >10,000	2%	k1 =0.4856 k2=0.5	medium
HS	DT _{50,overall days} = >10,000 DT _{50, k1} = 874 DT _{50, k2} = >10,000	2%	k1 =0.2874 k2=0.4982	medium
FOMC	>10,000	2%	Not applicable	medium

The reliability of the data is considered to be high. In conclusion, melamine is evaluated as not degradable in surface water.

3.1.2.1.3.2 Biodegradation in sediment

No standard simulation tests with melamine in sediments are available on ECHA's dissemination website⁹.

3.1.2.2 Biodegradation in soil

3.1.2.2.1 Simulation tests in soil

No standard simulation tests with melamine in soil are available on ECHA's dissemination website, but additional data was obtained from non-standard tests.

In a non-standard study by Hauck and Stephenson (1964), degradation of melamine was followed in Webster silty clay loam soil (pH 8.2). Samples of 100 g soil were mixed with 200 mg/kg melamine and were incubated for 196 days at 32 °C and 60 % soil humidity. Degradation was followed by measuring the nitrate formation (NO₃⁻). The extent of nitrate formation from melamine in soil was low in the study. Only 0.7 % of organic N was found as NO₃⁻ in 70 days and 0 % after 196 days.

Based on these results, ECETOC estimated in their later report that the degradation half-life of melamine in soil is in the range of 2-3 years (ECETOC, 1983).

The reliability of the data is considered to be limited as there are information missing in the description of the study conditions (e.g., darkness during incubation, microbial activity of the soil used, negative control, soil history with regard to adaption). Nevertheless, the data provide evidence that degradation of melamine in the soil is expected to be slow,

⁹ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978>
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even if elevated temperatures (32 °C) were applied.

3.1.2.3 Summary and discussion on biodegradation

Low degradation in water is shown in the screening test on ready biodegradation, the two inherent tests available and other studies on biodegradation (Fimberger, 1997; Niemi et al., 1987), including the OECD TG 309 surface water simulation test. From this information, it is concluded that the biodegradation of melamine in the aquatic environment is very slow or negligible.

For degradation in soil, no studies following (or equivalent to) OECD test guidelines are available. Based on a non-standard test on degradation of melamine in Webster silty clay loam soil (Hauck and Stephenson, 1964) it is concluded, that the degradability of melamine is expected to be slow in the soil compartment.

3.1.3 Field data

Alhelou et al. (2019) investigated the concentration of melamine in four WWTPs. The authors report melamine with a median concentration of 0.5 – 1.7 µg/L in the influents of the four WWTPs. It increases by more than one order of magnitude to 8 µg/L in the effluent of one WWTP compared to the influent concentration, while this increase was lower (factor 2 - 5) in the other three WWTPs. A reason for this increase might be, that the precursors of melamine, which are better degradable, were predominantly degraded by forming melamine as recalcitrant degradation product.

Seitz and Winzenbacher (2017) investigated influent and effluent of four WWTPs. The median concentration of melamine in the influent was between 0.52 µg/L to 2.4 µg/L, whereas the median effluent concentrations ranged between 0.64 µg/L and 1.5 µg/L. The authors calculated an elimination rate of less than 40 % in a conventional wastewater treatment.

An et al. (2017) investigated the behaviour of melamine in a biological wastewater treatment plant (WWTP). By comparing the melamine concentrations in influent and effluent, the reported removal efficiencies amounted > 90 % in the systems with melamine concentrations 0.001, 0.01 and 0.10 mg/L. Removal of melamine from wastewater was lower at higher concentrations (influent concentrations 1 and 5 mg/L, effluent concentrations 0.840 and 4.763 mg/L) and are in line with the reported removal rates for melamine in WWTP. However, determination of melamine in the liquid and solid phase of all influent concentrations came to the result, that melamine in the liquid phase were mainly transferred to the solid phase. According to the authors, the removal of melamine was thus caused by biomass adsorption instead of biodegradation of the substance.

Henninge et al. (2020) identified melamine in 14 out of 14 effluent samples of wastewater treatment plants collected in Norway. The concentration ranged between 0.7 – 2.9 µg/L. Further the authors observed “no indication of reduction over the treatment steps of the plant.”

3.1.4 Summary and discussion of degradation

Abiotic degradation of melamine by hydrolysis and phototransformation in air as well as water is expected to be negligible under environmental conditions based on the chemical structure and QSAR predictions.

In a screening test investigating ready biodegradability no degradation with regard to oxygen demand occurred. Further studies (Fimberger, 1997; Niemi et al., 1987) on degradation of melamine in the presence of activated sludge show, that degradation is

slow or negligible. This data demonstrates that the substance can be evaluated as potentially persistent / potentially very persistent in water according to the screening criteria of REACH Annex XIII.

In two tests for inherent biodegradation using various concentrations, low degradation (less than 16 %) of melamine according to DOC removal was observed. Lack of degradation (<20% degradation) in the tests on inherently degradability equivalent to the OECD TG 302 series together with the provisions of the ECHA R.11 (ECHA, 2017) already allow to demonstrate that the substance is persistent in the environment according to the persistency criteria of REACH Annex XIII.

On the basis of a test according to test guideline OECD 309 (simulation test on ultimate degradation) with surface water Hofman-Caris and Claßen showed no degradation of the substance within 60 days. Therefore, the DT₅₀ of melamine is concluded to be over 60 days. This result confirms that the substance qualifies as very persistent in water according to the persistency criteria of REACH Annex XIII (Hofman-Caris and Claßen, 2020).

For degradation in soil, no studies following (or equivalent to) OECD test guidelines are available. Based on a non-standard test on degradation of melamine in Webster silty clay loam soil it is concluded, that the degradability of melamine is expected to be slow in the soil compartment (Hauck and Stephenson, 1964). In their report ECETOC concluded, that the degradation half-life of melamine in soil is in the range of 2-3 years (ECETOC, 1983).

Considering the data on abiotic and biotic degradation, it is concluded that melamine is persistent and very persistent in the aquatic compartment.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

Information included on the ECHA dissemination website¹⁰ report two adsorption coefficients for organic carbon / water: The first is based on a QSAR using the programme KOCWIN v2.00 the log K_{oc} = 1.51 was calculated (ECHA dissemination website, report dated 2009). The second one is based on an equation provided in the ECB Technical Guidance Document of 2000 for calculating the log K_{oc} from the log K_{ow} (partitioning coefficient octanol/water) the calculated log K_{oc} equals 1.13.

Additional information about the adsorption behaviour from standard tests or databases is scarce.

The INERIS database¹¹ provides data for an estimated K_{oc} of 14.4 L/kg, equals log K_{oc} = 1.16, which is referenced to the FOOTPRINT Pesticides Properties Database. INERIS database does also provide data for an experimentally determined value of K_{oc} = 64 L/kg, which equals log K_{oc} = 1.81. The database entry does not contain information about the test method applied or the pH-value, at which the value was measured.

The CompTox Chemicals Dashboard¹² provides an estimated range for the K_{oc} of 1.00 to 45.8 L/kg with an average value for the K_{oc} of 23.4 L/kg, which equals log K_{oc} = 1.37.

It has to be considered, that the QSAR calculations normally refer to the non-ionic (undissociated) form of a substance.

¹⁰ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/5/2>

¹¹ <https://substances.ineris.fr/fr/substance/1176>

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¹² <https://comptox.epa.gov/dashboard/chemical/env-fate-transport/DTXSID6020802>

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As melamine has a pK_{b1} of 7.3, non-ionic and ionic species of the molecule occur under environmentally relevant conditions. For the ionic species of melamine, a $\log K_{oc}$ of around 3.41 is expected based on a study investigating the experimental and calculated $\log K_{oc}$ of a cationic substance (Claßen, 2019). Here the author shows, that the experimental determined $\log K_{oc}$ value for the ionic species of the model cation was 1.6 log units higher compared to the calculated $\log K_{oc}$ for the non-ionic species of the molecule using EPISuite Software (EPIWEB Version 4.1).

In summary, the available data consistently shows an adsorption coefficient $\log K_{oc}$ below 2 for the non-ionic species of melamine and below 3.5 for the ionic species. The information substantiates that the substance has a low tendency to adsorb to organic matter or sewage sludge. For further assessments, the value of $\log K_{oc} = 1.81$ for the non-ionic form of melamine will be applied.

3.2.2 Volatilisation

Volatilisation describes the tendency of a substance to evaporate from the water phase, described by the Henry's Law Constant.

The ECHA dissemination website for melamine¹³ contains the result from a QSAR estimate from the module HenryWIN, being part of the QSAR collection EPI Suite. The registrants accounted the Henry's Law constant being about $2.0e-14 \text{ atm}\cdot\text{m}^3/\text{mol}$ at $25 \text{ }^\circ\text{C}$, which according to him equals ca. $2.0e-08 \text{ Pa}\cdot\text{m}^3/\text{mol}$. Being part of their assessment the registrants also state, that an EPA database contains an experimentally derived Henry's Law constant for melamine of $1.84e-14 \text{ atm}\cdot\text{m}^3/\text{mol}$ ($@20 \text{ }^\circ\text{C}$). This seems to be the initial value for their assessment.

Attempts were made to verify this value by applying HenryWIN from EPISuite, too. EPI suite v4.10 internal QSAR estimates for melting point and water solubility were replaced by the respective measured values from Table 3. The output of HenryWIN resulted in a value of $1.89e-13 \text{ atm}\cdot\text{m}^3/\text{mol}$ at $25 \text{ }^\circ\text{C}$, which equals ca. $1.89e-08 \text{ Pa}\cdot\text{m}^3/\text{mol}$.

Volatility can also be calculated by using the equation R.16-4 for the Henry's Law constant according to Guidance Document R.16 (ECHA, 2016):

$$HENRY = \frac{VP * MOLW}{SOL}$$

Table 5: calculated Henry's Law constant

Variable	Expression	Unit	Value
VP(*)	Vapour pressure	[Pa]	2.69e-08
MOLW	Molecular weight	[g/mol]	126.12
SOL(*)	Water solubility	[g/L]	3.101
HENRY	Henry's law constant	[Pa*m ³ /mol]	1.094e-09

(*) values taken from Table 3 and extrapolated from the individual test temperature of the specific endpoint to European average environmental temperature of $12 \text{ }^\circ\text{C}$ by applying the Arrhenius equation.

Commonly, slightly volatile substances are defined by falling below the threshold of

¹³ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/5/3>
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0.1 Pa*m³/mol, whereas highly volatile substance are expected to exceed the threshold of 100 Pa*m³/mol, as reported in OECD TG 309 "Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test".

The calculated values consistently provide Henry's Law constants in the range of 10⁻⁰⁸ to 10⁻⁰⁹ Pa*m³/mol. Therefore, the substance is judged being of very low volatility. It will remain in the water phase under environmental conditions.

Another parameter describing the distribution of a substance between air phase and water phase is the partition coefficient air-water (K_{AW}). This can easily be calculated by using the equation R.16-5 as set out in ECHA Guidance Document R.16 (ECHA, 2016):

$$K_{AW} = \frac{HENRY}{R*T} = -12.34 \text{ (@12 °C; displayed here as log } K_{AW}\text{)}$$

with R being the gas constant (8.314 Pa*m³*mol⁻¹*k⁻¹) and T the temperature at the air-water interface (285 K; European average temperature).

In conclusion, the available data clearly indicates that volatilisation is not a major pathway of removal of melamine from the water phase. For further assessments the value of 2.0e-08 Pa*m³/mol was applied.

3.2.3 Distribution modelling

Distribution in sewage treatment plants

The Simple Treat model predicts the share of degradation of a substance in a municipal STP together with the ratio of substance being released to surface waters, respectively the ratio emitted to air or retained in sewage sludge. The Simple Treat distribution model is generally accepted and included in various standard computerised models, such as EPISuite, EUSES and Chesar.

Using water solubility, vapour pressure from Table 3 together with the data on adsorption behaviour from section 3.2.1 and applying an elimination rate constant k=0/h for biodegradation of "not readily biodegradable" substances in the STP following the requirements of Table R.16-10 set out in ECHA Guidance R.16 (ECHA, 2016). Simple Treat provides the following distribution pattern for a municipal STP:

Table 6: Distribution pattern for a municipal STP

Summary of distribution (*)	(%)
to air	0.0
to water	99.2
via primary sludge	0.6
via surplus sludge	0.2
degraded	0.0
total	100.0

(*) Distribution according to SimpleTreat 3.0 (debugged version, Feb 1997); difference to 100 percent for sum of the single values caused by rounding errors.

The outcome of the distribution modelling suggests that currently applied standard wastewater treatment processes in principle have no influence on the concentration of melamine in sewage water. Virtually the whole amount of melamine entering the sewer system is emitted to surface water in the follow-up. Only a small amount (0.8%) is

removed by adsorption to sewage sludge.

In conclusion, the available distribution information indicates that standard sewage treatment plant treating predominantly waters from municipalities will not retain any melamine in the influent.

The small amount of melamine adsorbed to sewage sludge is capable to be emitted into soil. This is because application of sludge from municipal sewage treatment plants on agricultural soil without further specific treatment is still a common practice within various Member States of the EU.

Distribution processes between environmental compartments

The ECHA dissemination website¹⁴ contains the outcome of an environmental distribution prediction according to MacKay, Level I. The results of the registrants' QSAR estimates are presented in Table 7.

Table 7: environmental distribution according to MacKay, Level I

	Percent distribution in media (*)
Air (%)	5
Water (%)	94
Soil (%)	0
Sediment (%)	0
Susp. Sediment (%)	0
Biota (%)	0
Aerosol (%)	0

(*) Rounding error results in aggregated figure <100%

The QSAR of this model (here: MacKay Level I, v2.11) predicts the distribution of a substance between the compartments air, water, sediment and soil at equilibrium state based on the substance intrinsic properties but does not consider the impact of the compartment of initial release.

For estimating the environmental distribution under consideration of the compartment of the initial release the QSAR model MacKay Level III, which is part of EPISuite (v4.1) was applied. For all of the subsequent calculations the water solubility 3.48 g/L and the melting point 361°C taken from Table 3 were applied instead of the internal database values / the internal predictions of EPISuite. The assumed emission rate for the calculation was set being 1000 kg per hour and per selected compartment.

For the Multi Level distribution estimate (Fugacity Output Model runs 7 times per EPISuite run using permutations of initial release rates to air, water and soil compartment as either 0 or 1000 kg per hour) the Model according to MacKay predicts the following distribution:

¹⁴ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/5/4>
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Table 8: environmental distribution according to MacKay, Level III (Multi Level estimate) (scenario equal release to all compartments)

	Mass amount (%)	Predicted Half Life (hours)
Air:	1.85e-07	584
(surface)Water:	25	900
Soil:	74.9	1800
Sediment:	0.086	8100

Separate predictions for initial release into the single environmental compartments were conducted in addition. The outcomes are presented in the subsequent tables. The outcomes for the predicted half-lives in the different environmental compartments were identical to the ones in Table 8 **Table 8**, therefore these were not displayed again.

Table 9: environmental distribution according to MacKay, Level III (exclusive release to air)

	Mass amount (%)
Air:	4.45e-07
(surface)Water:	14.5
Soil:	85.4
Sediment:	0.05

Table 10: environmental distribution according to MacKay, Level III (exclusive release to water)

	Mass amount (%)
Air:	1.42e-16
(surface)Water:	99.7
Soil:	2.73e-08
Sediment:	0.343

Table 11: environmental distribution according to MacKay, Level III (exclusive release to soil)

	Mass amount (%)
Air:	1.18e-14
(surface)Water:	11.6
Soil:	88.3

Sediment:	0.04
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In conclusion of the distribution information above, it is obvious that in case melamine reaches the water compartment, it will remain there (please see Table 10). The high rate of occurrence for melamine in soil that is predicted by the fugacity model in EPISuite as shown in Table 8, Table 9 and Table 11 cannot be interpreted as “the soil compartment is a safe sink for emissions of melamine”.

In fact, it has to be seen as route of exposure for groundwater because of the low potential for adsorption of melamine resulting in a leaching process through the different layers of the soil into groundwater.

Sludge from municipal sewage treatment plants is applied on agricultural soil as common practice in the EU. It is known that this sludge contains plastic particles, which can consist of melamine based polymers, too, having a wide range of degrees of polymerisation and being able to emit unreacted melamine. Another burden is expected to result from the slow but steady decay of those polymer particles, resulting in melamine as a recalcitrant substance of the degradation of those polymers.

3.2.4 Field data

Findings in drinking water

Zhu and Kannan (2020) reported the occurrence and spatial distribution of melamine and its derivatives in waters in New York State, USA. For this study 223 water samples were analysed for melamine and derivatives. For tap water samples ($n=70$), C_{median} amounted 19 ng/l whereas C_{max} was 188 ng/L with a detection frequency of 100 %.

In a monitoring study performed by Kolkman et al. (2021), melamine in concentrations > 1.0 µg/L were found in drinking water samples ($n=10$). The samples were taken in The Netherlands and in Flanders (Belgium), without disclosure of the exact sampling locations.

Findings in the aquatic environment

Kiefer et al. (2021) reported melamine concentrations up to 690 ng/L in ground water samples (samples $s=60$; findings $n=14$) from various locations in Switzerland.

Melamine is regularly monitored by Dutch drinking water companies at drinking water intake points along rivers Rhine and Meuse and is reported by RIVM (Smit, 2018): For the Rhine area the annual average concentration for the year 2016 were 1.36 µg/L at Lobith, 1.33 µg/L at Nieuwegein, and 0.97 µg/L at Andijk, while maximum were 2.3 µg/L at Lobith from October to November, 2.8 µg/L at Nieuwegein in December and 1.6 µg/L at Andijk in December.

For the Meuse Area, Smit (2018) summarises maximum concentrations of melamine of 5.8 µg/L at Heel, 0.98 µg/L at Brakel, 4 µg/L at Keizersveer and 3.8 µg/L at Stevensweert. Concentrations were around 1 µg/L from January to June, highest concentrations were measured between September and December 2016.

Been et al. (2021) analysed raw water samples taken at monitoring station of Keizersveer (i.e., raw water from the river Meuse) and from the abstraction point Haringvliet (i.e., raw water characterised by a mixture of Meuse and Rhine) in 2018 and 2019. Melamine was found at both sampling locations at all three sampling timepoints (September 2018, February 2019 and May 2019) at concentrations between 0.7 – 2.3 µg/L.

Alhelou et al. (2019) conducted four samplings at river Mulde, downstream of WWTP discharges between November 2016 and May 2017. Concentrations between 0.4 µg/L (May 2017) and 19.90 µg/L were reported. Further, the authors investigated the behaviour of melamine in bank filtration besides other melamine derivatives. In this study, a bank filtration site was investigated which consisted of two sampling wells between river and production wells. Three additional wells beyond the production well were sampled to assess the influence of background water. It needs to be noted that the raw water used at this site is a mix of 80 % bank filtrate and 20 % background water. For melamine no decrease in concentration was visible for the bank filtration passage. The authors reflect that *"concentrations in the range of several µg/L were found in the raw water for drinking water production. Although the concentration profile suggests that melamine is formed as a TP [Transformation Product] during subsurface passage."*

Seitz and Winzenbacher (2017) did a comprehensive monitoring programme for 84 anthropogenic compounds at 20 sampling sites, including influents and effluents of four WWTPs, runoff waters, groundwater (hotspot sites) and surface water. Seven sampling campaigns were done between April 2012 and February 2014. The authors report the presence of melamine in run-off water, in a hotspot groundwater site, and stream water (Nau, Danube) in mean concentrations between 0.25 µg/L and 0.61 µg/L, while the presence of the substances in groundwater is attributed to the influence by disposed wastes at a landfill site.

A study by Park and Jeon (2021) analysed water samples taken from Nakdong (ND) river (Korea), 50 km downstream of Daegu Metropolitan City between August 2016 to August 2017. Melamine was found with a detection frequency of 100 % on a median concentration of 11 µg/L ($C_{max} = 94 \mu\text{g/L}$).

In a monitoring study performed by Kolkman et al. (2021), melamine in concentrations above 1.0 µg/L were found in surface water samples ($n=7$) in the Netherlands and in Flanders (Belgium). The exact locations of the sampling points were not disclosed in the publication.

Zhu and Kannan (2020) reported the occurrence and spatial distribution of melamine and its derivatives in waters in New York State, USA. For this study a total of 223 water samples were analysed for melamine and derivatives. The water samples covered within this study were not only samples from surface water bodies, but also sampled from tap water and marine waters. The following melamine concentrations were obtained in this study for surface water:

Table 12: Analysis of melamine in aqueous samples

Source of samples	C_{median}	C_{max}	Detection frequency
River water ($n = 35$)	235 ng/L	2650 ng/L	100 %
Lake water ($n = 38$)	205 ng/L	1750 ng/L	100 %
Sea water ($n = 10$)	28 ng/L	86 ng/L	100 %

3.2.5 Summary and discussion of environmental distribution

The available information on adsorption/desorption behaviour of the substance predominantly consists of QSAR information, supported by one measured value. The results are consistently in the range of $\log K_{oc} < 2$. The data is sufficient to prove that the substance will not adsorb to organic matter and clay minerals in relevant quantities.

With a Henry's Law constant in the range of 10^{-08} to 10^{-09} Pa*m³/mol the substance has a very low volatility from aqueous media.

Once reaching the water compartment, the substance will predominantly remain in it as adsorption, volatilisation and - as explained in section 3.1 - degradation processes have only little effect on the substance concentration once it is emitted into the environment.

Distribution modelling with SimpleTreat indicates that there will be in principle no retention of the substance in sewage treatment plants. Fugacity level III distribution modelling indicates that the substance to a large extent may remain in the compartment of its initial release. However, due to the high water solubility (3.48 g/L) and the very low adsorption potential the substance will be washed out from the atmosphere and from soil and is finally expected to remain in the water compartment.

Based on this evidence it becomes obvious that the substance is capable to accumulate not only in surface waters but also in groundwater. Both are sources of raw water used for drinking water production. Indeed, the available monitoring data show that melamine has been detected in groundwater, surface water and drinking water in Europe.

3.3 Data indicating potential for long-range transport

The intrinsic substance behaviour of mobility in aquatic environments is already evaluated in the previous section.

For deciding about the Long-Range Transport Potential (LRTP) of a substance, different information can be used. This includes data about the overall half-life in the environment (P_{ov}) and the characteristic travel distance (CTD). For decision making whether melamine is capable to reach remote areas the OECD Pov and LRTP Screening Tool (version 2.2; OECD 2009) was utilised.

The P_{ov} boundary is 195 days (P_{ov} of α -HCH) and the LRTP boundaries are 5097 km (CTD of PCB 28) and 2.248 % (Transfer Efficiency, TE of PCB-28) as defined by Scheringer et al. (2009). The Transfer Efficiency (TE) is calculated from the ratio of the deposition mass flux from air to surface media in a region adjacent to the region to which the chemical is released and the mass flux of the chemical emitted to air in the release region.

In their report, (Crookes and Fisk, 2018) point out that substances with $\log K_{ow} < 5$ or $\log K_{oc} < 5$ and $\log K_{aw}$ in the range < -3 tend to be mobile in water. Furthermore, they found that the overall CTD for substances with $\log K_{aw}$ down to around -3 is governed mainly by transport via the atmosphere, but at lower $\log K_{aw}$ values the overall CTD is increasingly governed by transport via the water phase.

Since no distinct information about degradation half-lives in each of the environmental compartments is available from simulation studies, an estimate was undertaken by utilising the tables R.16-11 and R.16-12 from the REACH technical guidance R.16 (ECHA, 2016) for environmental exposure assessment. These tables provide half-lives for biodegradation in surface water, bulk soil and sediment respectively. The predicted half-lives are based on results of standardised (screening) tests on biodegradability.

Both above mentioned tables from REACH guidance R.16 do not provide any specific half-life values for substances concluded to be "not biodegradable" on the basis of screening biodegradation tests. Since no value equals "infinite" in the tables the guidance suggests to use various values for the prediction and provides information that the upper boundary for half-lives, that should be applied is 10,000 days (boundary of the inputs for the EUSES model).

Two different calculations were conducted. The first calculation is expected to represent a 'pragmatic worst-case' for the substance being "not biodegradable" according to the results from screening tests only. For this calculation, the generic half-lives from tables R.16-11 and R.16-12 for a substance that would fulfil the criteria for being "inherently biodegradable" were multiplied with a factor of 10.

The second calculation ('upper limit') takes into account the degradation half-life (DegT₅₀) extrapolated by SFO kinetics from the result of the OECD 309 test for the water compartment. Because no definite half-life could be derived from this test, the upper boundary from EUSES was applied as value. The DegT₅₀ of 3 years (equals 1095 days) applied for soil is the upper end of the range presented in the ECETOC report (ECETOC, 1983). Input parameters needed for both calculations and being identically applied were:

- log K_{ow} = -1.22 (see Table 3);
- distribution coefficient air-water log K_{AW} = -12.34 (see section 3.2.2 Volatilisation);
- degradation half-life in the atmosphere 16.22 days (see section 3.1.1.3 Phototransformation/photolysis).

The individual half-lives applied in the two different scenarios for the prediction of long range transport behaviour and the results of the calculations are presented in the subsequent table.

Table 13: results of LRTP modelling applying different scenarios

Scenario	DegT ₅₀ (water) for calculation	DegT ₅₀ (soil) for calculation	Characteristic travel distance	Overall half-life (P _{ov})	Transfer efficiency (%)
'pragmatic worst-case': "not biodegradable"	1500 days (extrapolated)	3000 days (extrapolated)	3530.43 km	2180.91 days	3.8e-06
'upper limit': DegT ₅₀ (water) / DegT ₅₀ (soil)	10000 days (upper limit of EUSES; SFO extrapolation from study acc. to OECD 309)	1095 days (ECETOC, 1983)	17874.37 km	14426.95 days	3.8e-06

With an estimated atmospheric half-life of 16.22 days (or 389.28 hours) the substance does induce a concern for long-range transport via air at screening level in the initial assessment step. Applying the overall half-life (P_{ov}), melamine exceeds the corresponding boundary for POP substances (195 days) in every single scenario. The Characteristic Travel Distance (CTD) of melamine only exceeds the LRTP boundary of 5097 km (CTD of PCB 28) in the scenario, where the upper limits of the EUSES degradation half-lives are applied for the water and soil compartment.

Bearing in mind the low tendency for volatilisation from water, the results of the modelling show that the substance is capable to be transported over long distances in aquatic media and that it might be found far away from the point of release into the environment.

The OECD LRTP Tool also provides a graphical output for the relationship of the calculated

values CTD and overall environmental Persistence (P_{OV}), but also for the relationship of Transfer Efficiency and P_{OV} . Therefore, the tool compares the outcome with the values of the reference chemicals that are known as persistent organic chemicals. In case the substance fits in the lower left sector of the graph, the substance clearly does not show POP-like behaviour. If the substance fits in the upper right sector, then it would show POP-like behaviour, whereas fitting into the two other remaining sectors would require further expert judgement. The graphical output of the three runs for melamine are provided in the subsequent figures below.

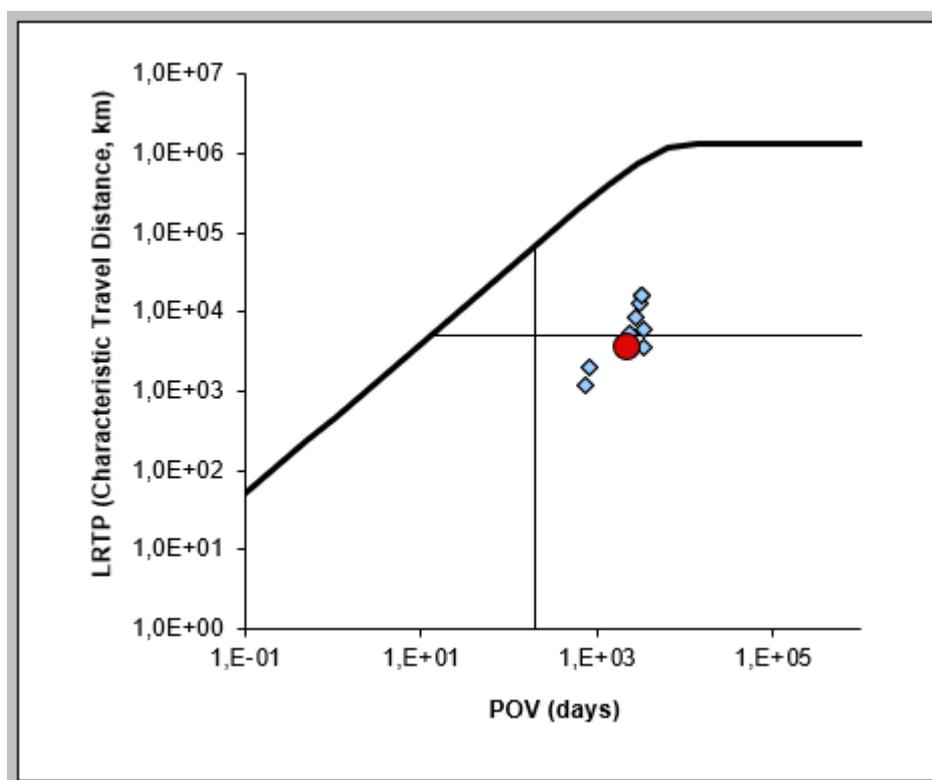


Figure 2: Graphical output for CTD of melamine assuming substance being "not biodegradable"; output in comparison to 'Generic PCB homologues'.

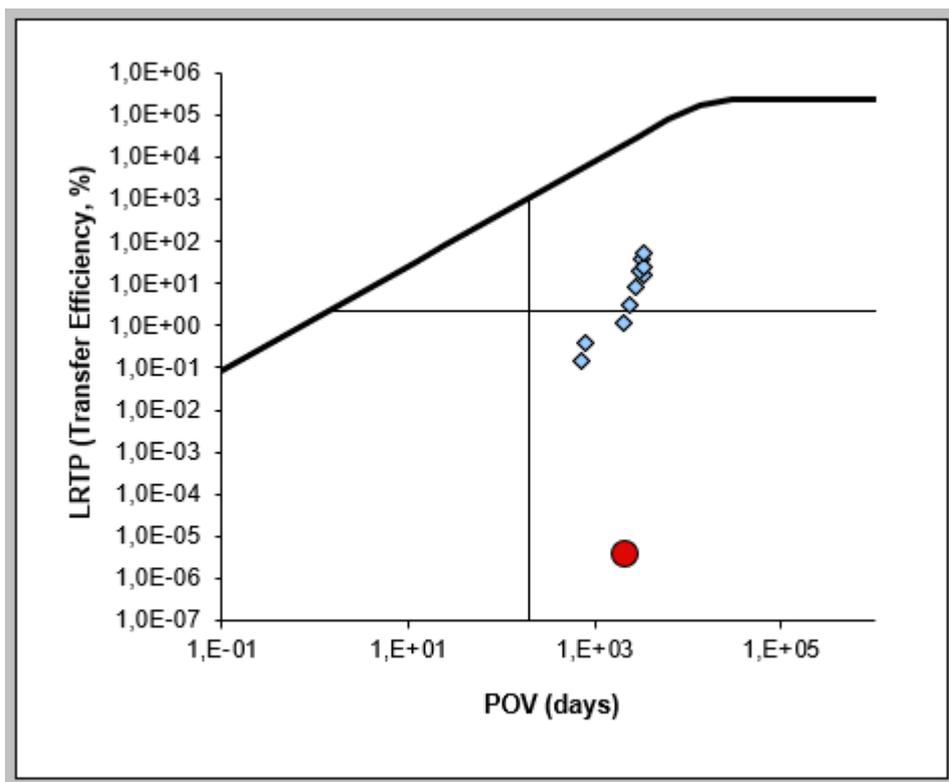


Figure 3: Graphical output for Transfer Efficiency of melamine assuming substance being "not biodegradable"; output in comparison to 'Generic PCB homologues'

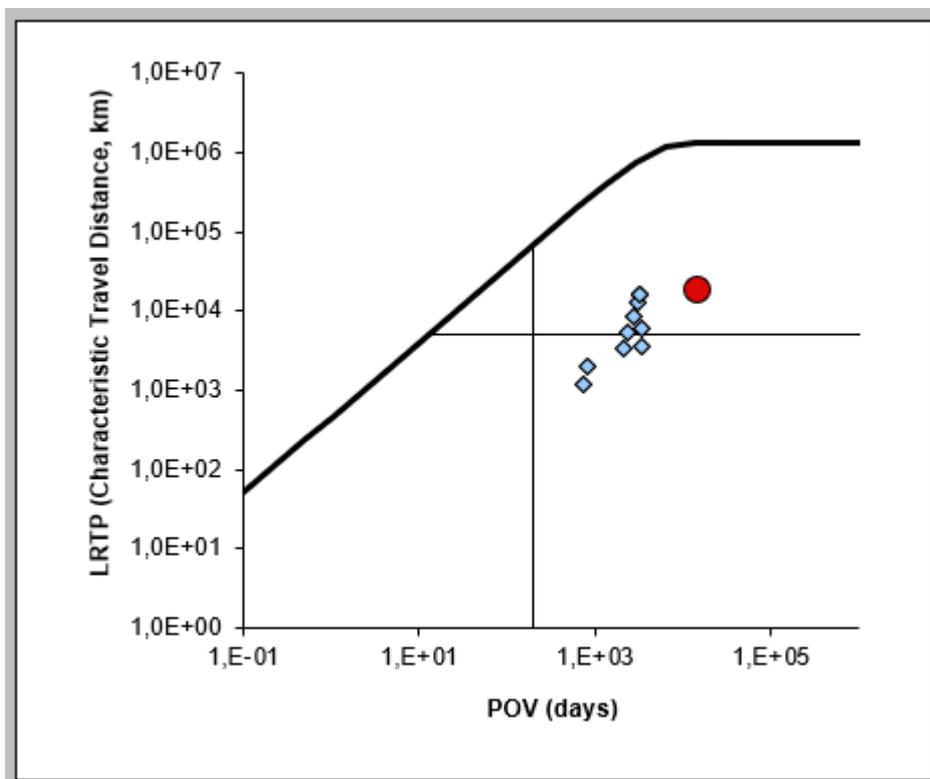


Figure 4: Graphical output for CTD of melamine assuming substance for scenario "upper limit"; output in comparison to 'Generic PCB homologues'.

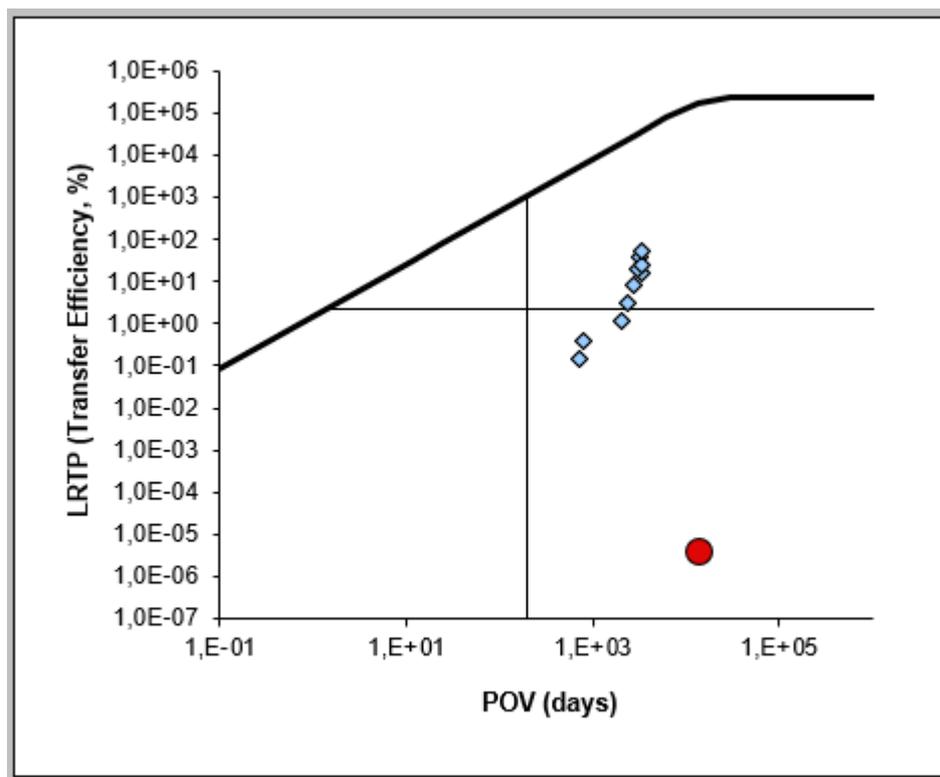


Figure 5: Graphical output for Transfer Efficiency of melamine assuming substance for scenario "upper limit"; output in comparison to 'Generic PCB homologues'

For the scenario 'pragmatic worst-case' (see Figure 2) the red dot gets close to the border of the upper right sector, indicating that melamine might have some serious potential for long-range transport. In fact, the calculated CTD for melamine in this scenario clearly exceeds the CTDs of monochloro PCBs (1205 km) and dichloro PCBs (1986 km). In the 'upper limit' scenario (see Figure 4) the red dot is situated in the upper right sector, clearly exceeding all of the CTD results for the Generic PCBs of the OECD LRTP Tool. In this scenario melamine will clearly qualify to undergo long-range transport.

In Figure 3 and Figure 5, which represent the relationship of P_{OV} and Transfer Efficiency, the red dot obviously moves further to the right side of the diagram, but remains at a constant low level at the Y-axis in the lower right sector. The graphical results in these two earlier mentioned figures show, that melamine has a low TE compared to known POP substances, thus suggesting that its deposition from air to surface media is low.

By evaluating the results of the OECD tool for prediction of long-range transport potential, it becomes obvious, that melamine does not behave like the "classic" POPs, which typically are "hoppers". For those "hoppers" the long-range transport is the result of a repetitive process of volatilisation and deposition allowing them to reach remote areas.

However, the data from the prediction model shows that melamine might be able to travel several thousand kilometres while predominantly staying in the water phase.

Summary

Melamine has an atmospheric half-life in the vapour phase of 16.22 days, thus indicating a potential for long-range transport in air.

Because of the substance's high water solubility, the low volatility from water and the low potential for adsorption, the water body is expected to be the dominant transport medium

in the environment once the substance is released. Data on degradation behaviour (see section 3.1.4 Summary and discussion of degradation) shows a lack of degradation potential for melamine under environmentally relevant conditions. By combining these considerations under the approach of the OECD tool for Long-Range Transport Potential (LRTP), there is clear evidence that the substance is able to travel long distances in the water phase. The OECD LRTP tool predicts a characteristic travel distance (CTD) of 3530 km together with an overall environmental persistence (P_{ov}) of 2181 days for melamine, even under preconditions that do not represent an unrealistic threshold situation. It is a conclusion from the available test data on degradation (here scenario 'pragmatic worst-case' being based on information from screening tests on biodegradation and extrapolated degradation half-lives for the environmental compartments, taken from REACH Guidance R.16 (ECHA, 2016)).

Even when considering this LRTP prediction as a rough and conservative estimate (because no distinct degradation half-life for surface water can be derived from the available simulation study and the report of ECETOC (1983) only provides a rough estimate for half-life in soil being 2-3 years), the results clearly indicate that melamine is capable to reach regions far away from the point of initial emission.

3.4 Bioaccumulation

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

The results of four different experimental studies are available on the ECHA dissemination website¹⁵.

The first one is a standard study on bioaccumulation of melamine in *Cyprinus carpio* according to OECD TG 305C. The test was performed as flow-through study. Uptake duration amounted to 42 days. The test concentrations were 2 and 0.2 mg/L. Depending on the test concentration the BCF value ranged from < 0.38 (2 mg/L) and < 3.8 (0.2 mg/L), indicating that melamine is not bioaccumulative. However, reliability of the study is limited due to the lack of study details, for example missing data about duration of depuration.

In a non-standard study on bioaccumulation of ¹⁴C-melamine in *Pimephales promelas*, ¹⁴C-melamine in a concentration of 0.082 mg/L was used in a static test system. Steady state was reached at ca. 48 h. Uptake duration amounted to 96 h, depuration duration amounted to 72 h. BCF values based on viscera was 0.11, indicating that melamine is not bioaccumulative.

In a non-standard study on bioaccumulation of melamine in *Oncorhynchus mykiss*, ¹⁴C-melamine in concentration of 0.087 mg/L was used in the static test system. Steady state was reached at ca. 48 h. Uptake duration amounted 72 h, depuration duration amounted 72 h. BCF values based on viscera was 0.32, indicating that melamine is not bioaccumulative.

3.4.2 Bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates)

No standard study on bioaccumulation of melamine in terrestrial organisms is available.

Based on the information on partition coefficient octanol-water (K_{ow}) and air-water (K_{aw}) on ECHA dissemination site, octanol-air partition coefficient (K_{oa}) for melamine was

¹⁵ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/4/2>
Date of access 18.08.2022

calculated to assess the bioaccumulation in terrestrial organism combination with log K_{ow} on screening level. According to ECHA R.11, substances with a log K_{oa} ≥ 5 in combination with a log K_{ow} ≥ 2 have the potential to biomagnify, while log K_{ow} below 2 indicates a low biomagnification potential as the substances are quickly eliminated by the urinary excretion.

The calculated log K_{oa} for the substance amounted 11.12, the reported log K_{ow} for melamine is -1.22. Considering the log K_{oa} and log K_{ow} for melamine, terrestrial bioaccumulation is considered low. However, as indicated by the dissociation constant of melamine it can be concluded that the substance is ionisable under environmentally relevant pH conditions and low log K_{ow} should be used with caution. Thus, it is not clear if melamine accumulates in terrestrial organisms.

3.4.3 Field data

No standard field data on bioaccumulation of melamine in terrestrial organisms are available.

3.4.4 Summary and discussion of bioaccumulation

According to the obtained BCF values, bioaccumulation in fish is low. However, details on the studies are lacking. The log K_{ow} of melamine is -1.22, indicating that the substance has a low bioaccumulation potential. However, as melamine is ionisable, log K_{ow} cannot be used as descriptor for assessing aquatic bioaccumulation on screening level according to ECHA R.11. Melamine has a calculated log K_{oa} of 11.12 and a reported log K_{ow} of -1.22, indicating a low potential for terrestrial bioaccumulation. However, as melamine is ionisable under environmentally relevant pH conditions, low K_{ow} should be treated with caution.

In conclusion, melamine is assessed as being not bioaccumulative in aquatic organisms. On terrestrial bioaccumulation, no conclusion can be drawn.

3.5 Summary and discussion of environmental fate properties

Melamine is recalcitrant against abiotic and biotic degradation processes to be found under environmentally relevant conditions. A simulation test on degradation in surface waters resulted in a calculated half-life exceeding the trigger for vP-substances (DegT₅₀ = 60 days) by several orders of magnitude. A lack of degradation can be observed in soil, too. Here, a half-life of 2-3 years was estimated, supporting the conclusion for the very persistent nature of the substance.

With the data available at the moment, there is currently no indication that melamine is prone to accumulate in aquatic organisms and to exceed values that trigger concerns for bioaccumulation.

The log K_{oc} < 2, a Henry's Law constant of about 2.0e-08 Pa*m³/mol and a water solubility of 3.48 g/L already indicate the importance of the water cycle for distribution of melamine in the environment. This is supported by distribution modelling applying the Fugacity multimedia models of MacKay, Level I and Level III. According to them, melamine virtually completely remains in aquatic compartment once it is released to the water phase. The estimate of the OECD tool for prediction of the Long Range Transport Potential of substances predict characteristic travel distances of several thousand kilometres in the environment, even when not applying ultimate worst-case assumptions in the model. This indicates that melamine is able to reach regions which are far away from the point of initial release into the (aquatic) environment.

Behaviour of melamine in sewage treatment plants is hard to predict for real conditions. Whereas distribution modelling with SimpleTreat predicts virtually no retention in municipal sewage treatment plants, the results of some single non-standard screening studies on biodegradation behaviour could suggest that degradation is a relevant removal pathway in sewage treatment plants. However, these screening studies on biodegradation predominantly apply inoculum, that is pre-adapted to melamine. Therefore, conclusions from these studies can only be rated being relevant for industrial sewage treatment plants with consistent and biased wastewater composition. In contrast, monitoring from municipal sewage treatment plants revealed that effluent concentrations of melamine exceed the findings in the catchment at the influent of the sewage treatment plant. It is assumed that this results from the degradation melamine precursors being easier degradable than melamine itself.

Monitoring data from different rivers being not connected to each other in different regions of the Netherlands and Germany proved the occurrence of melamine in surface waters. Concentrations to be found reached up to 19.90 µg/L. Where surface waters are used as a source for raw water in the drinking water production (i.e., via bank filtration or ground water enrichment) melamine can remain in the water following the purification process by generally applied techniques. This hypothesis is proven by findings of melamine in tap water samples, where residual concentrations above 1 µg/L have been found.

4. Human health hazard assessment

Urinary tract toxicity after oral melamine exposure has been shown in experimental animal studies. In addition, numerous observational studies have established a causative role of melamine in the increased occurrence of urinary precipitation and nephrotoxicity in Chinese children in the wake of the deliberate adulteration scandal (Dalal and Goldfarb, 2011; Wen et al., 2016; WHO / FAO, 2009). Following oral exposure, melamine is rapidly excreted mostly unchanged via the urine. Once the urinary concentration of melamine exceeds a certain threshold, precipitation occurs within the urinary tract leading to the formation of crystals and calculi. Melamine-related uroliths have been linked to the formation of rare urinary tract tumours in rodents (with an unusually short latency) and nephrotoxicity in humans. Epidemiological data show that the intake of high amounts of melamine leads to precipitation in the lower urinary tract and to melamine-induced formation of urinary stones in humans (EFSA, 2010; Guan and Deng, 2016; Li et al., 2009; Lu et al., 2011; Yang et al., 2013). Based on these effects, a harmonised classification as Carc. 2 and STOT RE 2 (urinary tract) has been set and it will enter into force as of the 23 November 2023 (EC, 2022).

Although precipitation with its associated adverse effects on the urinary tract is not expected to occur at exposure levels leading to urinary concentrations below the threshold of precipitation, RAC concluded that such a threshold cannot be established based on the available human data as there are too many uncertainties on actual exposure levels: "The precise threshold of exposure in humans leading to precipitation of melamine is not possible to derive based on the available data". RAC also noted that "humans may be more sensitive than rats to the formation of uric-acid calculi". The interaction of the natural occurring uric acid and melamine is thought to cause the precipitation of melamine-uric acid salts and thus the formation of melamine-related crystals/calculi. Due to the lack of the enzyme urate oxidase, which converts uric acid to allantoin, humans, especially neonates, excrete much higher levels of uric acid in the urine as compared to rats (WHO / FAO, 2009). Because of the high levels of uric acid together with an acidic urinary pH, the risk of urolithiasis is especially high for infants (EFSA, 2010). Prematurity, as well as male gender, are known risk factors for the development of melamine-induced urolithiasis consistently identified in exposed Chinese children (Gao et al., 2011; Li et al., 2010; Liu et al., 2010; Lu et al., 2011; Wang et al., 2009; Wang et al., 2011).

A tolerable daily intake (TDI) of 0.2 mg/kg bw/day was established by WHO and EFSA based on animal data (EFSA, 2010; WHO / FAO, 2009). Human data were not considered

sufficiently robust to derive a TDI. Some reports related to the adulteration scandal suggest that the risk of calculi formation may be increased even at doses below the TDI (Chen et al., 2009; Li et al., 2010). RAC, however, notes that there are considerable uncertainties regarding the effects in humans at low-dose melamine exposure.

Adverse effects seen in Chinese children following exposure to high concentrations of melamine and considered relevant for STOT RE classification by RAC included acute renal obstruction failure and acute injuries (e.g. hydronephrosis, haematuria, kidney injuries). The majority of Chinese paediatric patients passed their melamine-related calculi spontaneously or in response to conservative treatment (hydration and urinary alkalisation) (Wen et al., 2016). However, a number of melamine-related calculi had been resistant to conservative management (Jia et al., 2009), especially when they had a large size (> 10 mm) (Sun et al., 2010). Several follow-up studies indicate consistently that melamine-related urolithiasis and renal abnormalities persisted in approximately 8 - 10 % of the patients (Chang et al., 2017; Gao et al., 2011; Shen et al., 2011; Wang et al., 2013; Zou et al., 2013). In some cases, the stone size increased in 8 % of the study participants during a 12 month follow-up (Dai et al., 2012). RAC noted that "Several studies suggested potential persistence of uroliths and kidney abnormalities following children exposure to melamine-tainted formula" and that "Although the available studies have limitations, it is plausible that asymptomatic residual stones may persist in humans". In addition, adverse renal effects observed in monkeys following sub-chronic exposure did not fully resolve during the post-exposure recovery period (Early et al., 2013). Thus, there is a particular concern regarding long-lasting health effects in humans from melamine-related renal injuries at an early age. Children with acute kidney injury (AKI), for instance, may have an elevated risk to develop cardiovascular events and an increased mortality risk (Coca et al., 2009). A history of kidney stones was associated with an increased risk of chronic kidney disease (CKD) in a meta-analysis (Shang et al., 2017). Urolithiasis is associated with an increased risk of urinary tract carcinogenesis (Cheungpasitporn et al., 2015).

In 2007, numerous cases of renal damage, kidney failure, and increased mortality had been reported in dogs and cats exposed to melamine-contaminated animal feed (Brown et al., 2007; WHO / FAO, 2009). Adverse effects observed in these animals were attributed to the presence of crystals in the kidney tubules and comprised renal tubular necrosis and inflammation, crystalluria, and haematuria (Cianciolo et al., 2008; Dobson et al., 2008). In contrast to the administration of pure melamine in experimental animal studies, a mixture of several triazines (especially melamine and cyanuric acid but also ammeline and ammelide) was found in the animal feed (Puschner and Reimschuessel, 2011). The pattern of adverse renal effects seen in pets was consistent with results obtained from studies using a combination of melamine and cyanuric acid in experimental animals (Dalal and Goldfarb, 2011). Melamine-related toxicity is exacerbated in the presence of cyanuric acid (WHO / FAO, 2009). Co-exposure to melamine and cyanuric acid in livestock, fish, pets and laboratory animals shows higher toxicity compared with melamine or cyanuric acid alone. Crystals derived from combined exposure to melamine and cyanuric acid are distinguishable from crystals that form upon exposure to melamine only (Stine et al., 2014). Evidence for crystal formation between melamine and other structural analogues i.e. ammeline and ammelide is limited (Dorne et al., 2013; EFSA, 2010) but cannot be excluded.

With fulfilment of the criteria for classification as STOT RE 2 (urinary tract), melamine fulfils the T-criterion according to Annex XIII of the REACH Regulation.

In a newly conducted extended one-generation reproductive toxicity study (EOGRTS; EU B.56./OECD TG 443), adverse histopathological changes (tubular degeneration/atrophy with related cellular debris in the epididymis) have been observed in the testis of F0 and F1 animals together with abnormal sperm cell morphology (detached head) seen both in F0 and F1 animals. According to the registrants, the observed testicular and sperm effects

of melamine identified in experimental animals are of concern and warrant classification. However, the severity of the testicular findings was generally low and adverse effects on fertility parameters were not observed. Therefore, self-classification in Repr. category 2 (H361f) is considered most appropriate.

5. Environmental hazard assessment

5.1 Aquatic compartment (including sediment)

5.1.1 Fish

5.1.1.1 Short-term toxicity to fish

Several studies on short term toxicity to fish are available on the ECHA dissemination website¹⁶ 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 rainbow trout the LC50 was > 3000 mg/L melamine (nominal) (report dated 1984).

In the feeding study by Pirarat et al. (2012) 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 exists, but 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 (see above).

There are further acute fish studies mentioned on the ECHA dissemination website with exposure via water with reliability 3 or 4 due to missing test details, too short testing duration or other problems. These studies are not assessed here, but the results can be found in the table below.

In conclusion, melamine has a low acute toxicity to fish, at higher concentrations sublethal effects appear.

Table 14: Acute fish tests

Test design	Effects	Comments ¹⁷	Source
<i>O. mykiss</i> (Rainbow trout) Exposure via water, duration 28 d (also examined after 96 h), rainbow trout fingerlings,	LC50 (96 h) > 3000 mg/L (n)	Klimisch 2	ECHA dissemination website, report dated 1984

¹⁶ <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/6/2/2/?documentUUID=bce1af7c-451c-4701-aeef-5dcf9982fc6d>

¹⁷ Unless stated otherwise, the conclusion for reliability is the registrant's overall rating of the study quality

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semi-static, 10 fish per treatment, Conc.: 0, 750, 3000 ppm, no analytics			
<i>Poecilia reticulata</i> (Guppy) Exposure via water, duration 96 h, 4 – 5 week old fish, static, Conc.: 1.14, 2.31, 4.59 g/L (presumably nominal)	LC50 (96 h) >4.59 g/L	Klimisch 4 (missing test details)	ECHA dissemination website, report dated 1982
<i>Leuciscus idus</i> (Ide) Exposure via water, duration 48 h, static, length: 5.78 cm, mass 2.5 g, 3 fish/group, Conc: 100, 500, 1000, 3000, 5000, 10000 mg/L (specified as pretest)	LC50 > 500 mg/L (no information given on effect at higher concentration)	Klimisch 3 (only 3 fish per group, short duration, only few details reported)	ECHA dissemination website, report dated 1979
<i>Oryzias latipes</i> (Japanese medaka) Exposure via water, duration 48 h, semi-static, Conc: no data	LC50 > 1000 mg/L	Klimisch 3 (only few details known, short duration)	ECHA dissemination website, report dated 2010
<i>Poecilia reticulata</i> (Guppy) Exposure via water, duration 96 h	LC50 > 4400 mg/L	Klimisch 4 (only few details known)	ECHA dissemination website, report dated 1978; Secondary source: ECETOC
<i>Leuciscus idus melanotus</i> (Ide) Duration 48 h	LC50 > 500 mg/L	Klimisch 3 (only few details known, short duration)	ECHA dissemination website
<i>Poecilia reticulata</i> (Guppy) Duration 96 h	LC50 > 3000 mg/L	Klimisch 4 (only few details known)	ECHA dissemination website
<i>Clarius batrachus</i> (Walking catfish) Feeding study Body weight: 80 – 140 g, Duration 2 weeks, flow-through, 10 fish per treatment, fish were sampled at days 7 and 14 post feeding (for histopathology), Doses: 5 and 20 g/kg feed, (0.5 and 2 % melamine in feed, nominal), fed at 5 % body weight	No mortality appeared Gross change: - darkening of skin at 20 g/kg feed Histopathology: - Skin: at 5 and 20 g/kg melanophore hyperplasia, at 20 g/kg club cell degeneration and inflammatory cell infiltration - Kidney: at 5 and 20 g/kg degeneration of the renal tubular epithelium with hyaline droplet accumulation or vacuolation of tubular epithelial cells - Liver: at 20 g/kg single cell necrosis	Purity not specified Klimisch 2	(Pirarat et al., 2012)

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	- Gills: at 20 g/kg abnormal histopathology (moderate hyperplasia of the epithelium of the secondary and primary lamellae). Chondrocytes of the cartilaginous support in the primary lamellae had degenerative changes. Dilation and congestion of the capillaries of the gill lamellae were noted. No statistics.		
4 species: <i>Oreochromis</i> spp. (Tilapia), <i>Oncorhynchus mykiss</i> (Rainbow trout), <i>Ictalurus punctatus</i> (Channel catfish), 6 fish of each species above; <i>Salmo salar</i> (Atlantic salmon) only 3 fish, Mature fish Feeding study, fish were fed 3 days and examined at days 1, 3, 6, 10, 14 after administration ceased, doses ranged from 300 to 479 mg/kg melamine	No mortalities occurred no crystals detected in kidneys	Purity not specified Klimisch 3 (not enough fish in controls (1 to 2 per species), 6 fish in treatments, aside from salmon (3 fish)); acc. to OECD 203 seven fish in treatments/control necessary, however information is plausible	(Reimschuessel et al., 2008)
<i>O. mykiss</i> (Rainbow trout) Similar to a Fish Embryo Acute Toxicity Test Duration 18 to 26 days (period until hatch), exact beginning of test not known, at least 106 embryos in total, hence 17 to 18 per conc. or controls. Temperature 15°C Conc: 125, 250, 500, 1000 mg/L (n)	No effect on mortality until hatching. Hatching decreased at 500 and 1000 mg/L to 24 and 19 %, respectively. Controls 31 to 33 % Malformations (seen by dissecting microscope) and histological malformations were seen in a U-shaped curve.	Klimisch 3 (hatching success in controls 31 and 33 %, certainly caused by high temp.: 15°C, instead of recommended 10°C for rainbow trout embryos (see OECD 212)	(Ramusino and Vailati, 1982)

5.1.1.2 Long-term toxicity to fish

In a fish early life stage study available on the ECHA dissemination site¹⁸ 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), (report dated 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 (ECHA dissemination website, report dated 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) (ECHA dissemination website, report dated 1984).

¹⁸ <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/6/2/3/?documentUUID=5afb066a-cdff-4cf0-95ce-25c11cf96707>

As melamine was illegally used to adulterate food for animals (also for fish in aquaculture) due to high nitrogen content of melamine in order to pretend a high "protein content" in the food several feeding studies with fish are available. 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.

Residues of melamine in the fish tissues were examined in two species: red tilapia and Asian sea bass. In Asian sea bass the residue in fillet was 114.3 mg/kg after exposure to 10 g/kg melamine in feed for 12 weeks; in red tilapia the residue in fillet was 65 mg/kg at 5 g/kg feed, 148 mg/kg at 10 g/kg feed and 332 mg/kg at 30 g/kg feed after 8 weeks exposure to melamine (exemplarily values). This might also be of importance for humans. The addition of melamine to food used in aquaculture in the years around 2007 is clearly documented by several authors, e.g. (WHO, 2009a); (Reimschuessel et al., 2010a); (Davis and Tangendjaja, 2015); (Iheanacho et al., 2021). It is not known if melamine could be contained in food used in aquaculture today; information about this is not available. If so, then probably rather outside the EU and therefore perhaps of relevance in imported fish.

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

Table 15: Chronic fish tests

Test design	Effects	Comments 19	Source
<p><i>P.promelas</i> (Fathead minnow) Fish early life-stage (FELS) study (OECD TG 210), with GLP</p> <p>flow through, Test started with eggs < 4 h old</p> <p>Conc.: 0.618, 1.18, 2.30, 5.25 and 10.1 mg/L melamine (measured) Duration 36 d</p>	<p>Survival and growth (length) sign. decreased: NOEC 5.25 mg/L, LOEC 10.1 mg/L</p> <p>Mortalities appeared in the time after swim-up (day 6) until day 36</p>	Klimisch 1	ECHA dissemination website, report dated 2015
<p><i>Jordanella floridae</i> (American flagfish) Long-term toxicity to fish, egg- larval development,</p> <p>no analytical monitoring, semi-static, test started with eggs < 6 h old,</p> <p>Conc.: 100, 180, 320, 560, 1000 mg/L (nom.) Duration: 35 d</p>	<p>Length, weight, hatching, survival, condition: NOEC >= 1000 mg/L (nominal)</p> <p>At 1000 mg/L length and weight were slightly decreased, but not significantly</p>	Klimisch 2	ECHA dissemination website, report dated 1982
<p><i>O. mykiss</i> (Rainbow trout) Fish, juvenile growth test</p> <p>semi-static test Conc: 750, 1500, 3000 mg/L melamine (nom.)</p> <p>Duration 28 d</p>	<p>Survival and growth (weight) sign. decreased: NOEC 1500 mg/L, LOEC 3000 mg/L (nominal)</p> <p>At 1550 mg/L weight was also reduced (no statistics).</p>	Klimisch 2	ECHA dissemination website, report dated 1984
<p><i>Oreochromis niloticus</i> (L.) x <i>O.</i> <i>mossambicus</i> (Peters), (sex- reversed red tilapia) Based on fish, juvenile growth test,</p> <p>Fingerlings, weight: 5.63 g, 25 fish per vessel, three replicates,</p> <p>Feeding study, Doses: 5, 10, 15, 20, 25, 30 g/kg feed (n), 4, 11, 15.6, 20.7, 25, 31.2 g/kg melamine feed (measured)</p> <p>Duration 8 weeks,</p> <p>Melamine residue measured in a) whole fish</p>	<p>Mortality significantly increased at all doses, LOEC 5 g/kg feed</p> <p>Reduced average body weight, LOEC 5 g/kg feed</p> <p>Reduced specific growth rate (SGR²⁰), LOEC 5 g/kg feed</p> <p>Skin discolouration: at 5 g/kg feed (1- 10%), at 10 g/kg (10-25 %), at 15 g/kg (>25 % abnormality)</p> <p>Sluggish swimming behavior at 10 g/kg and higher</p> <p>Anorexia at all doses</p> <p>Fin erosion (dose not specified)</p> <p>Kidney, liver and gills: dose dependent histological changes: - enlargement of renal tubules at 10 g/kg feed</p>	<p>Purity 99.5 %</p> <p>Klimisch 2</p>	(Phromkunthon g et al., 2013)

¹⁹ Unless stated otherwise, the conclusion for reliability is the registrant's overall rating of the study quality

²⁰ Calculation of SGR (% pro day) = $(\ln W_t - \ln W_i)/t \times 100$; (W_t : final mean weight; W_i : initial mean weight; t: feeding days).

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<p>b) fillet c) viscera</p>	<p>- vacuolisation of liver hepatocytes at 5 g/kg feed</p> <p>- effects on gills: epithelial hyperplasia of the primary and secondary lamellae, interlamellar hyperplasia of the epithelium of primary lamellae at 5 g/kg feed; blood clotting and lamellar disorganisation frequently observed at \geq 15 g/kg feed; detachment of respiratory epithelium at \geq 25 g/kg feed</p> <p>(no statistics)</p> <p>Melamine residue in a, b, and c dose-dependently increasing. Exemplarily melamine content after exposure to the doses (5 g/kg; 10 g/kg; 30 g/kg feed):</p> <p>a) 535; 717; 1941 mg/kg dry weight b) 65; 148; 332 mg/kg dry weight c) 442; 1005; 1517 mg/kg dry weight In control melamine not detected</p>		
<p><i>Cromileptes altivelis</i> (Humpback grouper) Juveniles Weight 5.6 g (2 weeks before exposure begin)</p> <p>Dose: 167 mg/kg feed (200 mg melamine + 1 kg pellet), fed 2 times daily ad libitum</p> <p>Two tests: test 1: 20 fish per tank, test 2: 30 fish per tank</p> <p>Duration of both tests: 3 months</p>	<p>At 167 mg/kg feed:</p> <p>80 % mortality after 3 months exposure in both groups, Mortality increased with exposure time, No mortality in controls</p> <p>Histology: swollen kidneys, necrosis of renal tubules, some of them showed crystal like structures</p>	<p>Purity not specified</p> <p>Klimisch 2</p>	<p>(Mahardika et al., 2017)</p>
<p><i>Lates calcarifer</i> Bloch (Asian sea bass)</p> <p>Fingerlings (mean weight 8.62 g),</p> <p>Feeding study,</p> <p>Dose: 10 g/kg feed,</p> <p>Duration 12 weeks</p> <p>Melamine residue in fillet measured</p> <p>Co-exposure melamine and cyanuric acid: Each substance at the doses 2.5, 5, 7.5, 10 g/kg diet</p>	<p>At 10 g/kg feed:</p> <p>- No effect on survival</p> <p>- Sign. reduced specific growth rate</p> <p>- Sign. reduced weight (42 % of control fish)</p> <p>- Histology:</p> <p>Kidney: enlargement of renal tubules, degenerated tubular epithelium, pyknotic nuclei, melano-macrophages no crystals after melamine only diet</p> <p>Liver: distinctive vacuolisation</p> <p>- Hematology:</p> <p>Erythrocytes, leukocytes, hemoglobin, hematocrit sign. decreased</p> <p>- Antioxidant enzymes:</p> <p>GPx sign. decreased in liver, not in kidney; CAT not affected in both</p> <p>- Melamine content in fillet: 114.3 mg/kg</p> <p>- Crystal formation:</p> <p>Co-exposure melamine and cyanuric acid:</p>	<p>Purity 99.5 %</p> <p>Klimisch 2</p>	<p>(Phromkunthong et al., 2015)</p>

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	<p>At all doses irregular golden-brown or needle-like crystals were found within the lumina of the renal tubules</p> <p>No crystals were found after melamine exposure only</p>		
<p><i>Pelteobagrus vachelli</i> (Darkbarbel catfish),</p> <p>Juveniles (initial weight about 14.3 g), Feeding study, Doses: 0.2, 0.5, 1 %, equal to 2, 5, 10 g/kg feed Duration 8 weeks, 3 replicates with 30 fish in each replicate</p>	<p>No effect on mortality</p> <p>Specific growth rate (SGR) sign. decreased at all doses, LOEC 2 g/kg feed</p> <p>Skin: abnormal lightness at 5 and 10 g/kg feed, LOEC 5 g/kg feed, statistically significant decreased content of melanin (pigment in the epidermis) at the same concentrations</p>	<p>Purity 99.5 %</p> <p>Klimisch 2</p>	<p>(Jipeng et al., 2011)</p>
<p><i>Clarias gariepinus</i> (African catfish)</p> <p>Juveniles (weight 15.1 g),</p> <p>Feeding study Dose 3 g/kg feed (at 3 % of fish body weight),</p> <p>Duration 45 d</p>	<p>At 3 g/kg feed:</p> <p>- Neurotoxicity: Acetylcholinesterase activity in brain sign. decreased</p> <p>- Antioxidant enzymes in brain: SOD, GPx, CAT activities sign. decreased (superoxide dismutase, glutathione peroxidase, catalase)</p> <p>MDA level increased (malondialdehyde, biomarker of lipid peroxidation in cells)</p> <p>- Serum biochemistry: ALT and AST enzyme activities sign. elevated (aspartate amino transferase and alanine amino transferase, involved in metabolism of amino acids, indicators of tissue damage in liver and kidney)</p>	<p>Purity 99.8 %</p> <p>Klimisch 2</p>	<p>(Iheanacho et al., 2020)</p>
<p><i>Clarias gariepinus</i> (African catfish)</p> <p>Juveniles (weight 15.1 g),</p> <p>Feeding study at 3 % of body weight Dose 3 g/kg feed,</p> <p>Duration 45 d, followed by depuration period of 30 d</p>	<p>At 3 g/kg feed:</p> <p>- Weight sign. decreased after 45 d</p> <p>- Hematology: Erythrocyte, leukocyte count and hemoglobin sign. decreased</p> <p>PCV (packed cell volume) sign. increased</p> <p>- Antioxidant enzymes: Liver: SOD, GPx activity sign. decreased Gill: SOD, GPx activity no effect No effects on CAT activity in liver and gills Gills and liver: MDA level sign. increased</p> <p>- Effects on weight, hematology and enzymes resolved during depuration period</p>	<p>Purity 99.8 %</p> <p>Klimisch 2</p>	<p>(Iheanacho et al., 2021)</p>
<p><i>O. mykiss</i> (Rainbow trout) Adult females,</p> <p>Body weight about 350 g and 32 cm length,</p> <p>Feeding study, Doses: 250, 500, 1000 mg/kg feed,</p>	<p>Mortality, growth: no effects</p> <p>No formation of crystal complexes</p> <p>Oxidative stress:</p> <ul style="list-style-type: none"> ▪ Glutathione peroxidase activity enhanced at 5 and 10 mg/kg bw in the liver 	<p>Melamine purity 99%</p> <p>Klimisch 3 (not enough animals per treatment, no replicates), however</p>	<p>(Pacini et al., 2013)</p>

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<p>feed at 1% bw of fish, 2.5, 5, and 10 mg/kg bw</p> <p>Additional co-exposure to cyanuric acid at the same doses</p> <p>5 animals per treatment, without replicates</p> <p>Duration 8 weeks</p>	<ul style="list-style-type: none"> ▪ Glutathione S-transferase (GST) activity elevated in the liver at all concentrations ▪ GST activity increased in the kidney at 10 mg/kg bw (decreased at 2.5 and 5 mg/kg bw) ▪ Catalase activity enhanced at 2.5 and 5 mg/kg bw in kidney <p>Melamine-cyanurate complexes after co-exposure to cyanurate and melamine at the middle and high doses</p>	<p>information is plausible</p>	
<p><i>O. mykiss</i>, Adult females, Body weight about 351 g, total length 32 cm;</p> <p>Feeding study,</p> <p>Doses: 250, 500 and 1000 mg/kg feed, Fed at 1 % bw per day, 2.5, 5 and 10 mg/kg bw, Duration 10 weeks, Aim of the test was crystal analysis and biochemistry</p>	<p>Oxidative stress:</p> <ul style="list-style-type: none"> ▪ GST activity increased at 10 mg/kg bw in the kidney (also seen in Pacini et al. 2013) ▪ Catalase activity enhanced at 2.5 and 10 mg/kg bw in kidney 	<p>Klimisch 3 (missing information)</p>	<p>(Pacini et al., 2014)</p>
<p>Catfish (warm water species, 23.9 °C)</p> <p>Rainbow trout (cold water species, 12.2 °C)</p> <p>Only co-exposure to melamine and cyanuric acid</p> <p>Exposure 14 d (dose of each substance: 0.5, 1, 2.5, 5, 10, 20 mg/kg bw)</p> <p>Exposure 28 days (dose of each substance: 0.1 mg/kg bw)</p> <p>Additional sequential co-exposure (not described here)</p>	<p>Co-exposure to melamine and cyanuric acid:</p> <p>No crystals were observed in catfish after co-exposure to melamine and cyanuric acid at 0.1 mg/kg bw after 28 d</p> <p>NOAEL (14 d repeated dose) for both species was 0.5 mg/kg bw co-exposure</p> <p>At 1 mg/kg co-exposure (14 day repeated dose) one third of fish of both species had crystals in the kidney (intensity: single)</p> <p>The intensity of crystals per histological section was dose-dependent, at 2.5 mg/kg few (catfish), moderate to extensive (trout)</p>		<p>(Reimschuessel et al., 2010b)</p>

5.1.2 Aquatic invertebrates

5.1.2.1 Short-term toxicity to aquatic invertebrates

In two acute *Daphnia* studies effects on mobility/behaviour (poor condition) were seen after 48 h exposure: EC50 200 mg/L and EC50 < 180 mg/L (ECHA dissemination website²¹, reports dated 1978 and 1988).

In conclusion, melamine has a low acute toxicity to invertebrates.

Table 16: Acute *Daphnia* tests

Test design	Effects	Comments ²²	Source
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²¹ <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/6/2/4>

²² unless stated otherwise, the conclusion for reliability is the registrant's overall rating of the study quality

<i>Daphnia magna</i> guideline acc. to EU Method C.2 or EPA OPP 72-2, 48 h, static, no analytics Conc.: 56, 100, 180, 320, 560, 1000 mg/L (nominal)	EC50 200 mg/L (mobility, behaviour)	Klimisch 1	ECHA dissemination website, report dated 1988
<i>Daphnia magna</i> proposed standards NEN 6501 and 6502 (editor: Dutch competent authority (RIVM)), 48 h, static, no analytics Conc.: 180, 320, 560, 1000, 1800, 2000 mg/L (nominal)	EC50 < 180 mg/L (behaviour, poor condition)	Klimisch 2	ECHA dissemination website, report dated 1978

5.1.2.2 Long-term toxicity to aquatic invertebrates

In a chronic *Daphnia* study according to OECD TG 211 available on the ECHA dissemination website²³ 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 (ECHA dissemination website, report dated 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, see column 1 in the table below.

Melamine was illegally used to adulterate food for animals (also for shrimp in aquaculture) due to high nitrogen content of melamine and hence in order to pretend a high "protein content" in the food. A study by Nuntapong et al. (2019) with Pacific white shrimp and exposure to melamine over food (dose 10.1 g/kg feed) is available with testing duration of 10 weeks. Survival, growth and specific growth rate were significantly reduced. 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 (see table below). 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.

In conclusion, melamine has a low chronic toxicity to invertebrates, effects appear at higher concentrations.

Table 17: Chronic invertebrate tests

Test design	Effects	Comments ²⁴	Source
<i>Daphnia magna</i> OECD TG 211 Duration: 21 d, semi-static, Conc.: 0.7, 1.4, 2.6, 5.5, 11.0 mg/L (measured)	No sign. mortality, reduced reproduction or behavioural effects up to 11 mg/L NOEC >= 11 mg/L	Klimisch 1	ECHA dissemination website

²³ <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/6/2/5>

²⁴ unless stated otherwise, the conclusion for reliability is the registrant's overall rating of the study quality

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<p><i>Daphnia magna</i> According to proposed standards NEN 6501 and 6502 (editor: Dutch competent authority (RIVM)) Duration 21 d, semi-static, Mortality test: Conc.: 10, 18, 32, 56, 100 mg/L. Reproduction test: Conc.: 5.6, 10, 18, 32, 56 mg/L For the reproduction test egg-bearing females were selected from all exposed animals after 7 days exposure. These were further exposed to examine reproduction. By doing so more sensitive animals were excluded from examination of reproduction. Therefore, the reproduction part has a lower reliability. (However, the other chronic <i>Daphnia</i> test shows a NOEC of ≥ 11 mg/L. As the difference between 18 and 11 mg/L is not high, this test was rated of being applicable for the purpose of the SVHC identification.)</p>	<p>LC100 (21 d) 56 mg/L LC50 (21 d) between 32 and 56 mg/L NOEC (21 d) 18 mg/L based on reproduction and mortality (more sensitive value might be possible by using another test design, see left)</p>	<p>Klimisch 3 (part reproduction test, due to less sensitive test design, see column 1) Klimisch 2 (part mortality test)</p>	<p>ECHA dissemination website, report dated 1978</p>
<p><i>Litopenaeus vannamei</i> (Pacific white shrimp) Feeding study Initial body weight: ca. 2.37 g Duration 10 weeks 18 shrimp per vessel, 5 replicates Dose 10.1 g/kg feed (measured), four times daily, fed to satiation</p>	<p>At 10.1 g/kg feed: Survival, growth and specific growth rate sign. reduced Histology: degenerated and shrunken hepatopancreatic tubules Hemocyte (cell in hemolymph) count and lysozyme activity sign. reduced → impact on immune system Antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase) activities sign. decreased in gill and hepatopancreas Malondialdehyde (MDA, main lipid peroxidation indicator) content in hepatopancreas sign. elevated → oxidative stress and damage</p>	<p>Purity 99.5 % Klimisch 2</p>	<p>(Nuntapong et al., 2019)</p>

5.1.3 Algae and aquatic plants

There are two algae studies available on the ECHA dissemination website²⁵ with the effect value EC50 325 mg/L and NOEC 98 mg/L (nominal) in the more reliable test (report dated 1988) and ErC50 940 mg/L (initial measured) and NOErC: 320 mg/L (nominal) in a less reliable test (report dated 1982).

In conclusion, melamine has a low toxicity to algae.

Table 18: Algae tests

Test design	Effects	Comments ²⁶	Source
<i>Pseudokirchneriella subcapitata</i> According to guideline PRO/FT Algae-AC090-6 (unknown guideline) Duration 96 h 10, 32, 100, 320, 1000 ppm (nom.)	EC50 325 mg/L (based on area under the growth curve) NOEC 98 mg/L	Klimisch 1	ECHA dissemination website, report dated 1988
<i>Scenedesmus pannonicus</i> According to draft Standard Method NEN 6506 (editor: Dutch competent authority (RIVM), 1979) Duration 90.5 h 10, 32, 100, 320, 560, 1000 and 2000 mg/l.	ErC50 940 mg/L (initial measured) NOErC 320 mg/L (nominal)	Klimisch 2	ECHA dissemination website, report dated 1982

5.2 Summary and discussion of the environmental hazard assessment

The substance exerts a low mortality in acute fish tests with exposure via water or feeding as seen in two reliable fish studies: LC50 > 3000 mg/L after 96 h exposure, no mortality up to 20 g/kg melamine in feed after 2 week exposure (ECHA dissemination website, report dated 1984; Pirarat et al. (2012)). The studies were assigned Klimisch 2. In the acute feeding study sublethal effects were seen as darkening of skin and histopathological effects on kidney, liver and gills (Pirarat et al., 2012).

In a chronic fish test (OECD TG 210) with exposure via water the LOEC was 10.1 mg/L (NOEC 5.25 mg/L) for both survival and growth in a Klimisch 1 study (ECHA dissemination website, report dated 2015). In two chronic fish feeding studies mortality appeared at 5 g/kg feed and 167 mg/kg feed (Phromkunthong et al., 2013), (Mahardika et al., 2017). Histological changes were observed in kidney, liver and gills in the study by Phromkunthong, and in the study by Mahardika the kidneys were affected (other organs not examined). The same organs were affected as in the acute feeding study by Pirarat et al. (2012). Furthermore reduced growth and discolouration of skin were seen in two feeding studies ((Phromkunthong et al., 2013), (Jipeng et al., 2011)). In the studies by (Iheanacho et al., 2021) and (Phromkunthong et al., 2015) hematological effects appeared: erythrocytes, leukocytes and hemoglobin were significantly decreased at 3 and 10 g/kg feed. Signs of oxidative stress were seen in three Klimisch 2 studies (Iheanacho et al., 2020; Iheanacho et al., 2021; Phromkunthong et al., 2015) and also in two Klimisch 3 studies (Pacini et al., 2014; Pacini et al., 2013). In a study by Iheanacho et al. (2020) a neurotoxic effect was seen, as acetylcholinesterase activity in brain was significantly decreased at 3 g/kg feed.

²⁵ <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/6/2/6>

²⁶ unless stated otherwise, the conclusion for reliability is the registrant's overall rating of the study quality

Melamine has a low acute toxicity and a moderate chronic toxicity to fish based on the NOEC of 5.25 mg/L in the FELS study.

One acute Daphnia test has an EC50 of 200 mg/L melamine, another acute test has an EC50 of <180 mg/L (lowest tested concentration). One chronic Daphnia test shows no effects up to 11 mg/L, another test has an LC50 between 32 and 56 mg/L, the NOEC for reproduction was given to be 18 mg/L, however the test design was less sensitive (Klimisch 3 for the reproduction part). A feeding study with Pacific white shrimp (Nuntapong et al., 2019) showed effects on survival, growth and growth rate and histological effects in the hepatopancreas at 10 g/kg feed, furthermore the immune system was affected and signs for oxidative stress were seen.

Melamine has a low acute and chronic toxicity to invertebrates, as visible effects are only observed at rather high doses.

The toxicity of melamine to algae is low (NOEC ca. 100 mg/l or higher in two tests).

T-criterion:

The ecotoxicity data do not give evidence for fulfilling the T-criterion as specified in REACH Annex XIII, section 1.1.3a.

However, in accordance with REACH Annex XIII, section 1.1.3.c, melamine fulfils the T-criterion based on its harmonised classification as STOT RE 2 (urinary tract) (see sections 2 and 4).

6. Conclusions on the SVHC Properties

6.1 CMR assessment

Section is not relevant for the identification of melamine as SVHC in accordance with Article 57 (f) REACH.

6.2 PBT and vPvB assessment

Section is not relevant for the identification of melamine as SVHC in accordance with Article 57 (f) REACH.

6.3 Assessment under Article 57(f)

6.3.1 Summary of the data on the intrinsic/hazardous properties

6.3.1.1 Persistency/Degradation

Abiotic degradation of melamine by hydrolysis under environmentally relevant conditions and phototransformation in air as well as water is expected to be negligible.

In a screening test on ready biodegradation no degradation, with regard to oxygen demand occurred until the end of the test period.

In two tests on inherent biodegradation equivalent to OECD TG 302 using different test concentrations, degradation of less than 16% of melamine according to measured DOC removal was observed. According to the ECHA R.11 Guideline (ECHA, 2017) a "lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD TG 302 series may provide sufficient information to confirm that the P-criteria are fulfilled without the need for further simulation testing for the purpose of PBT/vPvB assessment". Therefore, comparing the results from both inherent biodegradation tests against the recommendation of Guidance R.11 would already be sufficient to demonstrate that the substance is persistent in the environment according to the persistency criteria of REACH Annex XIII.

Further studies (Fimberger, 1997; Niemi et al., 1987) which do not follow standard test guidelines, investigated the degradation of melamine in the presence of activated sludge and show that degradation was slow or negligible.

On the basis of a higher tier simulation degradation test under environmentally relevant conditions according to test guideline OECD 309 with surface water, Hofman-Caris and Claßen showed that no degradation of the substance occurs within 60 days under the test conditions applied. Therefore, the DT₅₀ of melamine is concluded to be over 60 days (Hofman-Caris and Claßen, 2020). Based on the degradation kinetics received from the test, the degradation half-life is expected to exceed the 60 day threshold for very persistent substances by several orders of magnitude. The data confirm that the substance qualifies as very persistent in water according to the persistency criteria of REACH Annex XIII.

For degradation in soil, no studies following (or equivalent to) OECD test guidelines are available. Based on a non-standard test on degradation of melamine in Webster silty clay loam soil, it is concluded, that the degradability of melamine is expected to be slow in the soil compartment (Hauck and Stephenson, 1964). In their report ECETOC concluded, that the degradation half-life of melamine in soil is in the range of 2-3 years (ECETOC, 1983).

In summary, it can be concluded that melamine is very persistent in the aquatic environment.

6.3.1.2 Mobility in the environment

Melamine has a calculated Henry's Law constant of 2×10^{-9} Pa * m³ / mol (QSAR HenryWIN from EPI Suite v4.10) by using water solubility and vapour pressure of the substance according to equation R.16-4 (ECHA, 2016). Therefore, the substance is assessed of being of low volatility. It will remain in the water phase.

Information included on the ECHA dissemination website²⁷ report two adsorption coefficients for organic carbon / water: The first is based on a QSAR using the programme KOCWIN v2.00 the log K_{oc} = 1.51 was calculated (ECHA dissemination website, report dated 2009). The second one is based on an equation provided in the ECB Technical Guidance Document of 2000 for calculating the log K_{oc} from the log K_{ow} (partitioning coefficient octanol/water) the calculated log K_{oc} equals 1.13. Additional data from INERIS database and CompTox Chemicals Dashboard show results in comparable ranges (log K_{oc} = 1.81 (measured value, no further information about pH value and temperature) and log K_{oc} = 1.37 (QSAR value), respectively).

As melamine has a pK_{b1} of 7.3, non-ionic and ionic species of the molecule occur under environmental conditions. It has to be considered, that the QSAR calculations normally refer to the non-ionic (undissociated) form of a substance. For the ionic species of melamine, a log K_{oc} of around 3.41 is expected based on a study investigating the experimental and calculated log K_{oc} of a cationic substance (Claßen, 2019). Here the authors show that the experimental determined log K_{oc} value for the ionic species of the model cation was 1.6 log units higher compared to the calculated log K_{oc} for the non-ionic species of the molecule using EPISuite Software (EPIWEB Version 4.1).

Melamine has, therefore, a low tendency to adsorb to organic matter and is expected to remain in the water phase, once the substance is released to the environment.

Further, due to its low log K_{oc} and high water solubility (3.4 g/L) melamine is not likely to be efficiently removed by adsorption to organic materials in sewage treatment plants (WWTP) or in drinking water production. Please see section 3.1.3 for more details on the behaviour of melamine in WWTPs.

The available monitoring data show that melamine has been detected in groundwater, surface water and drinking water in Europe.

In summary, it can be concluded that melamine is very mobile and therefore evidently contaminates the aqueous environment.

6.3.1.3 Decontamination and removal of melamine from water resources

In general, whether or not a substance can be removed from water basically relies on the combination of its persistency and mobility.

Melamine is a very persistent substance. Therefore, the substance will withstand biodegradation-based water purification techniques, e.g., as applied in the wastewater treatment. The high water solubility and low sorption potential of melamine make the substance mobile in water and difficult to remove from water as it only has a low potential to adsorb to materials and prefers to remain in the water phase.

Once melamine is released into the environment, the substance will be preferentially

²⁷ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/5/2>

distributed to the aquatic phase. Consequently, melamine will bypass filters, e.g., in river bank filtration.

These properties make it difficult to remove melamine from drinking water, contaminated groundwater, wastewater and industrial effluents by treatment techniques applied in the majority of sites, e.g. river bank filtration (Alhelou et al., 2019).

6.3.1.4 Potential for long-range transport via air and water

Melamine has an atmospheric half-life in the vapour phase of 16.22 days, thus indicating a potential for long-range transport in air.

The substance has a high water solubility, but a low tendency for volatilisation from the water phase. In combination with the low potential for adsorption and the proven recalcitrant behaviour against degradation as seen in the surface water simulation test, this indicates the substance's potential to be transported over large distances in the water body.

Calculations with the OECD Long-Range Transport Prediction (LRTP) tool support this assumption. According to the prediction - and keeping in mind the to some extent limited prediction validity due to the need for application of environmental degradation half-lives of 10,000 days - the substance is capable to travel about 3500 km. It is therefore capable to reach regions far away from the point of initial release to the environment.

6.3.1.5 Human health effects

Urinary tract toxicity pertinent to oral melamine exposure has been shown both in experimental animal studies and observational studies in humans. Following oral exposure, melamine is rapidly excreted via the urine.

Once the urinary concentration of melamine exceeds a certain threshold, precipitation occurs within the urinary tract leading to the formation of crystals and calculi. Melamine-related uroliths have been linked to the formation of urinary tract tumours in rodents and nephrotoxicity in humans.

Based on these effects, a harmonised classification as Carc. 2 and STOT RE 2 (urinary tract) will enter into force as of the 23 November 2023 (EC, 2022).

Although precipitation with its associated adverse effects on the urinary tract is not expected to occur at exposure levels leading to urinary concentrations below the threshold of precipitation, RAC concluded that such a threshold cannot be established based on the available human data as there are too many uncertainties on actual exposure levels. Observational studies consistently reported a clear difference between ages regarding the risk of developing melamine-induced urolithiasis in Chinese children. Given the high urinary levels of uric acid together with an acidic urinary pH, the risk of urolithiasis attributed to melamine is especially high for infants (EFSA, 2010), increasing the concern for more susceptible populations. Follow-up studies show that kidney abnormalities and urolithiasis may persist in a small percentage of exposed Chinese children, indicating a potential concern for long-lasting human health effect.

With fulfilment of the criteria for classification as STOT RE 2 (urinary tract), melamine fulfils the T-criterion for PBT according to Annex XIII of the REACH Regulation.

According to the registrants, the observed testicular and sperm effects of melamine identified in experimental animals are of concern and warrant self-classification. However, the severity of the testicular findings was generally low and adverse effects on fertility parameters were not observed. Therefore self-classification in Repr. category 2 (H361f) is considered most appropriate.

6.3.2 Concerns arising from the substance properties

6.3.2.1 Concern for an irreversible and increasing presence in the environment

As elaborated below the properties of melamine of persistency, mobility and potential for being transported in the water phase over long distances lead to an irreversible presence in the environment together with a widespread contamination of the aquatic environment.

Due to the lack of abiotic and biotic degradation of melamine, it is expected that there is no significant removal of melamine by biological processes in conventional sewage treatment plants dealing with mixed sewage. Section 3.1.3 summarises studies providing scientific and experimental evidence to support the concern that melamine is hard to be removed from wastewater in sewage treatment plants. Studies done by (Alhelou et al., 2019), (Seitz and Winzenbacher, 2017), (Henninge et al., 2020) reported consistently, that the removal of melamine during wastewater treatment is limited.

Thus, the overall amount of melamine emitted from production and use is, if at all, only marginally reduced in sewage treatment plants. Once released, melamine remains in the environment due to its persistency.

Melamine is already present in the aquatic environment. Section 3.2.4 summarises studies, in which melamine was detected in surface waters. For all rivers investigated in the studies above (e.g. Rhine, Meuse, Mulde, Danube, Nau), melamine concentrations above the limit value derived at the European River Memorandum (ERM) of 0.1 µg/L were found (ERM, 2020).

Only limited information is available for the occurrence of melamine in groundwater. However, melamine was found in groundwater in Germany and Switzerland (Seitz and Winzenbacher, 2017), (Kiefer et al., 2021).

Due to the global water cycle and the fact that the aqueous compartments are all well connected, the high persistency and the high mobility of melamine lead to long distance transport processes in the environment. The properties of melamine are likely to cause a transport across water bodies to pristine oceans and groundwaters, raising the concern that any effects caused by the substance might also occur at remote locations from the origin of release.

6.3.2.2 Decontamination of melamine from the aquatic environment and drinking water resources

Once, melamine is released into the aquatic compartment and widely distributed in the environment, it is difficult to be removed from there. As described in section 3.1.1, abiotic degradation processes are not relevant pathways for the removal of melamine from the aquatic environment. As described in section 3.1.2 Biodegradation biodegradation in the aquatic environment is expected to be slow or negligible.

As specified in the European River Water Memorandum (ERM, 2020), "it is a clear aim to achieve a water in the these water bodies that permits drinking water supply using natural treatment methods only. [...] Natural treatment methods minimise the otherwise required technical impact on the water".

Melamine is not expected to be easily removed with natural (or even conventional) water purification techniques (please see section 3.4.3 Field data).

One report is available which estimates the behaviour of melamine in drinking water purification steps. Alhelou et al. (2019) investigated a bank filtration site at the river Mulde. For melamine, no concentration decrease was visible for the bank filtration

passage.

Therefore, there is substantial evidence that melamine may not be removed from the water cycle, once a contamination of aquatic resources occurs. The same applies for the drinking water purification, as melamine will bypass commonly used drinking water purification techniques and has been found in drinking water (Kolkman et al., 2021), (Zhu and Kannan, 2020).

6.3.2.3 Human health effects

Melamine is classified as STOT RE 2, H373 (urinary tract), and Carc. 2, H351, and therefore its presence in drinking water may become a concern if critical dose levels are exceeded. A substance classified as STOT RE 2 fulfils the T criterion for PBT substances according to REACH Annex XIII.

Aside from effects caused by exposure to melamine only, combined effects due to co-exposure with other chemicals, e.g. cyanuric acid, have been reported in rats, pets and livestock (WHO / FAO, 2009) Dorne et al., 2013). Effects occur at lower melamine concentrations following co-exposure to melamine and cyanuric acid. Potential combined effects as a consequence of co-exposure of melamine and cyanuric acid prompted the Dutch competent authority (RIVM) to lower their derived limit values for drinking water (Smit, 2018; Smit, 2019).

According to the registrants, the observed testicular and sperm effects of melamine identified in experimental animals are of concern and warrant self-classification. However, the severity of the testicular findings was generally low and adverse effects on fertility parameters were not observed. Therefore self-classification in Repr. category 2 (H361f) is considered most appropriate.

6.3.2.4 Human exposure

The most significant sources of human exposure to melamine are food contact materials and, with several uncertainties, articles containing melamine as flame retardant.. Apart from cases of criminal food alteration, the contribution of nutrition and drinking water to human exposure to melamine is several orders of magnitude lower than the current health-based guidance value (EFSA 2010).

Melamine has been repeatedly detected in human urine samples from the general population in the USA and in East Asia (see (Health Canada, 2020), for an overview of these biomonitoring studies).

Melamine can also be ingested by infants through breast milk (Zhu and Kannan, 2019; Zhu and Kannan, 2020). Lactational and/or placental transfer was shown by (Chan et al., 2011; Cruywagen et al., 2009; Kim et al., 2011) following exposure to melamine in animal studies.

Water is used for drinking and cooking each day and it is the basis of all food and meals throughout the human life. Water taken from environmental sources is not only used for drinking water production but also for applications like irrigation in agriculture or cattle watering tanks. According to the German Association for Nutrition (DGE), the intake of water as such for adolescents and adults is approximately 1500 mL per day (DGE, 2020), not envisaging the incidental co-intake via nutrition. EFSA has established the tolerable daily intake (TDI) for melamine at 0.2 mg/kg bw/day in 2010 (EFSA, 2010). At the current stage the TDI is not exceeded via nutrition including beverages and tap water, and there are currently no risks for human health via this exposure pathway.

Due to the persistence and mobility of melamine, it is able to reach the resources of drinking water. Continued emissions and the limits of retrievability from the aqueous phase

may result in increasing concentrations in raw water in the future and humans may be exposed to increasing concentrations in drinking water, too.

6.3.2.5 Environmental effects

Due to the properties of (persistence, mobility, potential for being transported in the water phase over long distances and carcinogenicity) it is not possible to derive a safe concentration limit for the environment by the means of standardised acute and chronic ecotoxicological tests and the assessment criteria investigated within.

A concern observed for environmental organisms are sublethal effects in fish. Although available acute aquatic studies in fish do not show effects on mortality, sub-lethal effects were seen. In chronic fish studies mortality appeared and growth was decreased (effect value in one study: NOEC 5.25 mg/L). Sub-lethal effects appeared in the long-term fish studies on the same organs as in the acute study: Kidney, liver and gills were affected with dose-dependent histological effects. Furthermore, in several fish studies there were effects on skin coloration and on blood cells, additionally the antioxidant system was impaired. In aquatic invertebrates, the following effects were observed as well. In a chronic Daphnia test, the NOEC for reproduction was 18 mg/L. In a feeding study with Pacific white shrimp, effects on survival, growth and histological effects on the hepatopancreas, as well as effects on the antioxidant system were observed at 10 g/kg feed. Therefore, the substance properties raise the concern that these effects or yet unknown effects could appear in the environment and lead to population-relevant effects, due to long-term exposure over the whole life and over several generations, keeping in mind continuous exposure via water and potentially increasing concentrations.

6.3.2.6 Societal concern

Article 7.3 of the Water Framework Directive (2000/60/EC) stipulates that "Member States shall ensure the necessary protection for the bodies of water identified with the aim of avoiding deterioration in their quality of water to reduce the level of purification treatment required in the production of drinking water."

Due to its mobility and persistence, melamine is found in surface water, groundwater and processed drinking water.

Decontamination may only be achieved by advanced water purification techniques, if at all and at high costs that have to be paid predominantly by the society, not by the body that is responsible for the initial emission of melamine into the environment.

Furthermore, melamine has been classified for target organ toxicity after repeated exposure (STOT RE 2 (urinary tract)) and is likely carcinogenic to humans (Carc. 2). Exposure to humans might occur via consumption and use of contaminated drinking water. In case that humans may be harmed due to this potential effects, costs for medical treatment will be the result and those will be handed over predominantly to the society.

Consequently, there is societal concern regarding increasing concentrations of melamine in sources of drinking water, which requires action based on precautionary considerations.

6.3.2.7 Increasing presence in the aquatic environment results in increasing exposure of humans and environmental organisms

Melamine is already present in surface waters. As described in detail in section 3.3, the characteristic travel distance (CTD) of melamine calculated with the OECD LRTP tool is 3530 km with an estimated overall environmental persistence (Pov) of 2181 days – when applying 'pragmatic worst-case' inputs, not maxing out the applicability domain of the model. It is clear from this estimate that the substance is capable to reach regions far away from the point of initial release by aqueous distribution, for example via surface waters.

The use of surface water as a source for drinking water is common practice in Europe, regardless of whether the water taken is more or less directly processed or in case it is used for groundwater enrichment in an intermediate step. Due to the substance's intrinsic lack of degradability and lack of adsorption potential, melamine is capable to permeate bank filtration and soil horizons. Details are described in sections 3.1 and 3.2.1 Adsorption/desorption respectively.

If release of melamine to the environment continues, the concentration of melamine in the aquatic resources may increase over time due to high persistency. This will result in an increase of the concentrations in drinking water, too.

The time span, until specific concentrations of melamine are reached in "natural" water, is not predictable. It depends on future emissions of the substance to the environment and the specific conditions of the single water body. Consequently, exposure of environmental organisms to melamine via "natural" waters

Under real-life conditions organisms and humans are not solely exposed to melamine, but also to other melamine-based substance and other chemicals. This makes it even harder to predict "safe" concentrations until which emissions seem to be tolerable and unacceptable effects may not occur.

Data shows that after release to the environment, melamine reaches the drinking water, too. Consequently, human exposure to melamine via drinking water is expected to increase over time.

Humans could be exposed to melamine, which is of increasing concern, as melamine concentrations into the environment are expected to increase over time due to very high persistency, and the substance is classified as STOT RE 2 (urinary tract) and Carc. 2. For these reasons, the release of melamine to the environment should be prevented.

6.3.2.8 Concern related to co-exposure and combined effects and inability to derive a safe concentration

Melamine and other melamine analogues occurring in the environment can act jointly, so that exposures at comparatively low concentrations may lead to health and environmental effects.

Melamine is present as a trace contaminant in nitrogen supplements used in animal feeds (e.g. urea), and can be found as a metabolite and degradation product of the active substance cyromazine, a biocidal active substance approved in Europe. Several melamine salts and derivatives are also registered under REACH for various uses. Depending on the purification process, melamine may contain a number of structurally related by-products, including cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine) potentially leading to melamine-cyanuric acid complex formation in renal tubules and acute kidney failure in exposed humans. Dichloroisocyanurates used in disinfection of water (e.g. swimming pools) and food contact materials can also be hydrolysed into cyanuric acid (EFSA, 2010; Dorne et al., 2013; WHO, 2004). In this context, the occurrence of melamine, its structural analogues or metabolites from authorised uses can all contribute to background levels.

Regarding co-exposure to melamine and similar substances, (Zhu and Kannan, 2020) showed the occurrence of both melamine and cyanuric acid in tap water in the USA, and both substances were detected in the urine of children from the USA (Sathyanarayana et al., 2019). Sodium dichloroisocyanurate, the salt of cyanuric acid can inter alia be used for drinking water disinfection (WHO, 2009a); (WHO, 2009b), primarily for emergency situations. Both cyanuric acid (EC 203-618-0) and sodium dichloroisocyanurate (EC 220-767-7) are registered under REACH at tonnages above Annex X (1000 T/y) and have uses

indicating that they are likely to be released to the environment²⁸. Cyanuric acid was detected in drinking water in Europe in one drinking water facility (Kolkman et al., 2021) and the presence of cyanuric acid in drinking water was also confirmed by RIVM (Smit, 2018; Smit, 2019). Sodium dichloroisocyanurate is used as a biocidal active substance for the treatment of swimming pool water, which results in an additional exposure route to humans. (WHO, 2009a; WHO, 2009b).

The mode of action of melamine involves the dose-dependent formation of crystals with endogenous uric acid or, during co-exposure to cyanuric acid. These may be known substances with similar MoA but also chemicals with different MoA that act additively or even synergistically.

Because of the occurrence of melamine in different environmental compartments, co-exposure and combined effects with other substances cannot be excluded.

EFSA states that "the TDI for melamine is not appropriate if there is significant concomitant exposure to cyanuric acid, ammeline or ammeline. Such concomitant exposure could result in considerable health impact and the currently available data are inadequate to establish a TDI for this scenario." (EFSA, 2010). Also the WHO states, that the TDI is specific for exposure to melamine alone (WHO, 2009b).

Once melamine is in the environment, it is hard to remove due to high persistency and the lack of natural removal processes. Contaminated areas might be sources of continued releases into the environment a long time after the phase-out of melamine and the high mobility of melamine leads to wide distribution in the water body. The substance properties raise the concern of yet unknown effects on the environment that were not observed in the standard toxicity tests or may only develop after life-long exposure.

Indeed, the concern for effects that may emerge only after lifetime exposure is part of the concern for PBT/vPvB substances. ECHA Guidance R11, page 11 states "vPvB substances are characterised by a particular high persistence in combination with a high tendency to bioaccumulate, which may, based on experience from the past with such substances, lead to toxic effects and have an impact in a manner which is difficult to predict and prove by testing, regardless of whether there are specific effects already known or not." Further the Guidance states: "such accumulation is in practice difficult to reverse as cessation of emission will not necessarily result in a reduction in substance concentration".

The same concern applies to persistent and mobile substances such as melamine. The difference between PBT/vPvB and persistent and mobile substances is their route of exposure. Persistent and mobile substances can enrich in the water cycle, giving special emphasis to the (semi-)closed drinking water cycles exploiting recycled waste waters. Further, both groups of substances are capable to migrate to pristine remote areas, either via food chain or water cycle, respectively (Hale et al., 2020). Therefore, in case such substances do cause effects to humans or the environment, those effects might occur far from the point of release.

Due to its high persistence, melamine will remain in the environment for a long time and due to its high water solubility and low adsorption potential, it will remain widely distributed in the water compartment and be bioavailable for uptake by wildlife or humans.

Furthermore, environmental concentrations will inevitably increase with continued release. However, there are still no test systems available that are capable of detecting effects which may appear in long-living wildlife only after lifelong (i.e., potentially decades of) exposure.

In addition, there is a potential for combined effects resulting from concomitant exposure

²⁸ <https://echa.europa.eu/registration-dossier/-/registered-dossier/15028/3/1/6>;
<https://echa.europa.eu/registration-dossier/-/registered-dossier/14822/3/1/6>

to other substances. Co-exposure to structural analogues such as cyanuric acid, for instance, has been shown to increase the toxicity of melamine in rats, pets and livestock (Dorne et al., 2013; EFSA, 2010; WHO, 2009b)

Both melamine and cyanuric acid were detected in drinking water (Zhu and Kannan, 2020) in the USA, and both substances were detected in the urine of children (from USA) in studies conducted between 2013 and 2017 (Sathyanarayana et al., 2019). The salt of cyanuric acid, sodium dichloroisocyanurate can inter alia be used for drinking water disinfection, primarily in emergency situations (WHO, 2009a) and cyanuric acid was detected in European drinking water of one drinking water facility (Kolkman et al., 2021). RIVM (Smit, 2018) states that according to the Dutch Watercycle Research Institute, the presence of structural analogues of melamine, that is e.g. cyanuric acid, is confirmed with monitoring data. This means that there is a concern of possible mixture effects regarding the formation of crystals and calculi in the urinary tract due to the consumption of drinking water that contains both, melamine and cyanuric acid. To account for such mixture toxicity, RIVM recommended to set the value for melamine lower if there is a co-exposure to cyanuric acid than without such simultaneous exposure in 2018 (Smit, 2018; Smit, 2019).

Regarding mixture effects in fish, the formation of crystals was observed if co-exposure of melamine and cyanuric acid occurred (Phromkunthong et al., 2015), (Reimschuessel et al., 2010b), (Pacini et al., 2013).

In addition, due to the high persistence and low adsorption potential (which prevents removal of the substance over time by sorption to particles and sedimentation) the aquatic concentrations will rise with continued emissions and may eventually reach exposure levels sufficient to trigger the already identified severe effects but potentially also further adverse effects which are currently not known. Hence, high persistence in combination with low adsorption potential and high water solubility of melamine is in terms of the associated concerns for human health and the environment comparable with vPvB properties of a substance in sense that there are the same difficulties to cease exposure and remove the substances from the environment. Further, there are the same difficulties to quantify the development of exposure in the long-term and the related risks with sufficient certainty, as for PBT/vPvB substances.

6.3.3 Conclusion on hazard assessment and Equivalent level of Concern assessment

Melamine is identified 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.

Intrinsic properties

Degradation:

Abiotic degradation of melamine by hydrolysis and phototransformation in air and water is regarded as negligible.

Based on modelling data, melamine is predicted to be not readily biodegradable and hence potentially persistent. A reliable OECD TG 301C study shows the melamine is not readily biodegradable (0% degradation after 14 days). Biotic degradation of melamine was investigated in a surface water over 60 days according to OECD TG 309, showing no degradation of the substance. Therefore, the degradation half-life of melamine is longer than 60 days. Considering the data on abiotic and biotic degradation, melamine fulfils the P and vP criteria (half-life in water >60 days) of REACH Annex XIII.

Volatility, water solubility, adsorption:

The substance has an experimentally derived log K_{OC} of 1.81 indicating a low potential for adsorption on organic matter and clay minerals. As melamine has a pK_b1 of 7.3, non-ionic and ionic species of the molecule occur under environmentally relevant conditions. It has to be considered, that the QSAR calculations normally refer to the non-ionic (undissociated) form of a substance. For the cationic species of melamine, a log K_{OC} of around 3.41 is assumed based on a study investigating the experimental and calculated log K_{OC} of a model cationic substance. Thus, also for the ionic melamine species, adsorption on organic matter and clay minerals is still expected to be low.

The substance intrinsic behaviour of low volatility from water (calculated Henry's law constant $2.0e-08 \text{ Pa}\cdot\text{m}^3/\text{mol}$) together with its low potential to adsorb to organic matter result in a high mobility in water. Additionally, the intrinsic substance properties indicate that the substance will partition primarily to the water compartment and will undergo environmental distribution via aqueous media, easily reaching groundwaters.

High water solubility (3.48 g/L at 20°C and pH 7.7 using EU Method A.6) and low adsorption potential of melamine makes it difficult to remove the substance from water by commonly applied sewage treatment and water purification techniques as it only has a low potential to adsorb to materials and prefers to remain in the water phase.

Because of melamine's high water solubility, low volatility from water and low potential for adsorption, water will be the dominant transport medium in the environment once the substance is released. In combination with its long environmental half-life, there is a potential for widespread contamination of the water environment.

The OECD tool for Long-Range Transport Potential (LRTP) predicts a characteristic travel distance (CTD) of 3530 km together with an overall environmental persistence (P_{ov}) of 2181 days for melamine. This indicates that melamine is capable of reaching regions far away from the point of initial emission.

Toxicity:

Urinary tract toxicity after oral melamine exposure has been shown both in experimental animal studies and observational studies in humans. Following oral exposure, melamine is rapidly excreted mostly unchanged via the urine. Once the urinary concentration of melamine exceeds a certain threshold, precipitation occurs within the urinary tract leading to the formation of crystals and calculi. Melamine-related uroliths have been linked to the formation of rare urinary tract tumours in rodents (with an unusually short latency) and nephrotoxicity in humans. Epidemiological data show that the intake of high amounts of melamine leads to precipitation in the lower urinary tract and to melamine-induced formation of urinary stones in humans.

Based on these effects, the Committee for Risk Assessment (RAC) has concluded that melamine fulfils the criteria for classification as STOT RE 2 (urinary tract) and Carc. 2. The harmonised classification has been included in the 18th ATP to CLP²⁹. This classification is of relevance for the assessment of melamine as a substance of very high concern according to Article 57 (f), i.e., applying the T-criterion of REACH Annex XIII; Section 1.1.3 c).

Melamine fulfils the T-criteria of REACH Annex XIII Section 1.1.3 c) through its harmonised classification as STOT RE 2 (urinary tract) as of 23 November 2023. Melamine therefore

²⁹ Commission Delegated Regulation (EU) 2022/692 of 16 February 2022 amending, for the purposes of its adaptation to technical and scientific progress, Part 3 of Annex VI to Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (the 18th ATP to CLP). Pursuant to the second paragraph of Article 2 of this Regulation, this new harmonised classification applies from 23 November 2023. However, pursuant to the third paragraph of that provision substances and mixtures may already be classified, labelled and packaged in accordance with this classification.

has the potential to produce the same adverse toxic effects as a PBT substance.

According to the registrants, the observed testicular and sperm effects of melamine identified in experimental animals are of concern and warrant self-classification in Repr. category 2 (H361f).

Concerns arising from the substance properties

The concern raised by melamine is triggered by individual properties as well as by combination of its properties.

Concern for an irreversible and increasing presence in the environment

The combination of the substance intrinsic properties persistency, mobility and potential for being transported in the water phase over long distances lead to an irreversible presence in the environment, together with a widespread contamination of the aquatic environment.

Due to its low tendency for adsorption, the substance will not attach to suspended or organic matter in the environment to any significant degree.

Due to the lack of abiotic and biotic degradation of melamine, it is expected that there is no significant removal of melamine by biological processes in conventional sewage treatment plants dealing with mixed sewage. Thus, the overall amount of melamine emitted from production and use is, if at all, only marginally reduced in sewage treatment plants. Once released, melamine remains in the environment due to its persistency.

Monitoring already confirmed occurrence of melamine in various rivers (e.g. Rhine, Meuse, Mulde, Danube, Nau), where sampling points that do not influence each. Concentrations found exceeded the value of 0.1 µg/L. Even while not being subject to the list of substances for regular groundwater monitoring, recent projects were able to identify and quantify melamine in groundwater samples in Germany and Switzerland, too.

Due to the global water cycle and the fact that the aqueous compartments are all well connected, the high persistency and the high mobility of melamine lead to long distance transport processes in the environment. The properties of melamine are likely to cause a transport across water bodies to pristine oceans and groundwaters, raising the concern that any effects caused by the substance might also occur at remote locations from the origin of release.

The substance stays in the environment even if releases of the substance have already ceased, as can be concluded from the substance properties and the recurring findings in groundwater samples.

Decontamination from the aquatic environment and from drinking water resources

Sources of emission in the vicinity are seldom apparent. Once, melamine is released into the aquatic compartment and widely distributed in the environment, it is difficult to be removed from there. This results from the recalcitrant degradation potential of melamine against abiotic and biological processes, leading to very slow or negligible removal from the water phase.

Due to its intrinsic properties melamine will not be easily removed with natural processes or conventional water purification techniques. This includes procedures such as river bank filtration or soil infiltration for groundwater enrichment, which are commonly applied on European level. Therefore, there is substantial evidence that melamine may not be removed from the water cycle, once a contamination of aquatic resources occurs. The same applies for the drinking water purification, as melamine will bypass these commonly used drinking water purification techniques and has been already found in drinking water.

Human Health effects:

Melamine is classified as STOT RE 2, H373 (urinary tract), and Carc. 2, H351, and therefore its presence in drinking water may become a concern if critical dose levels are exceeded. A substance classified as STOT RE 2 fulfils the T criterion for PBT substances according to REACH Annex XIII.

Aside from effects caused by exposure to melamine only, combined effects due to co-exposure with other chemicals, e.g. cyanuric acid, have been reported in rats, pets and livestock (WHO / FAO, 2009) Dorne et al., 2013). Effects occur at lower melamine concentrations following co-exposure to melamine and cyanuric acid. Potential combined effects as a consequence of co-exposure of melamine and cyanuric acid prompted the Dutch competent authority (RIVM) to lower their derived limit values for drinking water (Smit, 2018; Smit, 2019).

According to the registrants, the observed testicular and sperm effects of melamine identified in experimental animals are of concern and warrant self-classification. However, the severity of the testicular findings was generally low and adverse effects on fertility parameters were not observed. Therefore self-classification in Repr. category 2 (H361f) is considered most appropriate.

Human exposure:

The most significant sources of human exposure to melamine are food contact materials and, with several uncertainties, articles containing melamine as flame retardant. Apart from cases of criminal food alteration, the contribution of nutrition and drinking water to human exposure to melamine is several orders of magnitude lower than the current health-based guidance value (EFSA 2010).

Melamine has been repeatedly detected in human urine samples from the general population in the USA and in East Asia (see Health Canada (2020), for an overview of these biomonitoring studies).

Melamine can also be ingested by infants through breast milk (Zhu and Kannan, 2019; Zhu and Kannan, 2020). Lactational and/or placental transfer was shown by (Chan et al., 2011; Cruywagen et al., 2009; Kim et al., 2011) following exposure to melamine in animal studies.

Water is used for drinking and cooking each day and it is the basis of all food and meals throughout the human life. Water taken from environmental sources is not only used for drinking water production but also for applications like irrigation in agriculture or cattle watering tanks. According to the German Association for Nutrition (DGE), the intake of water as such for adolescents and adults is approximately 1500 mL per day (DGE, 2020), not envisaging the incidental co-intake via nutrition. EFSA has established the tolerable daily intake (TDI) for melamine at 0.2 mg/kg bw/day in 2010 (EFSA, 2010). At the current stage the TDI is not exceeded via nutrition including beverages and tap water, and there are currently no risks for human health via this exposure pathway.

Due to the persistence and mobility of melamine, it is able to reach the resources of drinking water. Continued emissions and the limits of retrievability from the aqueous phase may result in increasing concentrations in raw water in the future and humans may be exposed to increasing concentrations in drinking water, too.

Environmental effects:

Due to the properties of melamine (persistency, mobility, potential for being transported in the water phase over long distances and carcinogenicity) it is not possible to derive a

safe concentration limit for the environment by the means of standardised acute and chronic ecotoxicological tests and the assessment criteria investigated within.

A concern observed for environmental organisms are sublethal effects in fish. Although available acute aquatic studies in fish do not show effects on mortality, sub-lethal effects were seen. In chronic fish studies mortality appeared and growth decreased (effect value in one study: NOEC 5.25 mg/L). Sub-lethal effects appeared in the long-term fish studies on the same organs as in the acute study: Kidney, liver and gills were affected with dose-dependent histological effects. Furthermore, in several fish studies there were effects on skin coloration and on blood cells, additionally the antioxidant system was impaired. In aquatic invertebrates, the following effects were observed as well. In a chronic Daphnia test, the NOEC for reproduction was 18 mg/L. In a feeding study with Pacific white shrimp, effects on survival, growth and histological effects on the hepatopancreas, as well as effects on the antioxidant system were observed at 10 g/kg feed. Therefore, the substance properties raise the concern that these effects or yet unknown effects could appear in the environment and lead to population-relevant effects, due to long-term exposure over the whole life and over several generations, keeping in mind continuous exposure via water and potentially increasing concentrations.

Societal concern:

Efforts to purify drinking water from surface water or groundwater bodies should be as low as reasonably possible. Therefore, Member States shall introduce measures to protect the water bodies with the aim of avoiding deterioration in their quality (see Article 7.3 of the Water Framework Directive (2000/60/EC)).

The combination of melamine's intrinsic properties of persistence and mobility results in a little effectivity of common and widely applied drinking water purification techniques, such as river bank filtration or soil infiltration for groundwater enrichment. Decontamination may only be achieved by advanced water purification techniques, if at all and at high costs that have to be paid predominantly by the society, not by the body that is responsible for the initial emission of melamine into the environment.

Exposure to humans might occur via consumption and use of contaminated drinking water. Furthermore, melamine received a harmonised classification for target organ toxicity after repeated exposure (STOT RE 2 (urinary tract)) and is likely carcinogenic to humans (Carc. 2). In case that humans may be harmed due to this potential effects, costs for medical treatment will be the result and those will be handed over predominantly to the society.

Consequently, there is societal concern regarding increasing concentrations of melamine in sources of drinking water, which requires action based on precautionary considerations.

Increasing presence in the aquatic environment results in increasing exposure of humans and environmental organisms:

Melamine is already present in surface waters. Computational models predict that the substance is able to remain in the environment for several years or maybe even decades in the environment and, once released, is able to reach remote regions because it can be distributed in the aquatic environment over several thousand kilometres. This means, findings and undesirable effects may occur far away from the point of initial release.

Due to its mobility melamine can widely distribute in the water bodies, making it difficult to control the arising concentrations and conclude on appropriate, effective measures to remove the substance from environmental aquatic media.

Depending on the level of continuation of emissions into the environment and resulting from persistency and mobility of melamine, overall concentration in the environment is expected to increase over time. This will result in an increase of the concentrations in drinking water, too.

Consequently, exposure of environmental organisms to melamine via “natural” waters and exposure of humans via drinking water is expected to increase over time due to persistency and mobility. For these reasons and because the substance has a harmonised classification as STOT RE 2 (urinary tract) and Carc. 2, the release of melamine to the environment should be prevented.

Concern related to co-exposure and combined effects and inability to derive a safe concentration:

Melamine and other melamine analogues occur in the different environmental compartments, therefore co-exposure cannot be excluded. These substances can act jointly, so that exposures at comparatively low concentrations may lead to health and environmental effects. In addition, co-exposure and combined effects with other substances are likely. Therefore, appearance of undesirable effects due to additive or even synergistic Mode of Action cannot be excluded and no safe concentration can be derived.

Overall evaluation of the concerns:

The persistency, mobility and toxicity (specific target organ toxicity after repeated exposure and carcinogenicity) and in addition the irreversibility of the contamination of the aquatic compartment compromise the quality of drinking water resources and give rise to the concern of increasing exposure to wildlife and man via environment due to contaminated water.

Consequently, there is societal concern regarding increasing concentrations of melamine in sources of drinking water, which requires action based on precautionary considerations. The environment provides natural drinking water sources, whose integrity needs to be ensured for future generations.

Equivalent level of concern

The level of concern is considered very high in particular due to the combination of the following concern elements:

- Irreversible and increasing presence in the environment, due to limited potential for removal of melamine from the aquatic environment and drinking water resources, which are leading to a potential for:
 - Human health effects, potentially long-lasting and more likely in susceptible populations such as infants, which may occur in the future due to increasing exposure over time
 - Societal concern through its ubiquitous presence in surface water, groundwater and processing of raw water
- Increasing presence in the aquatic environment results in increasing exposure of humans and environmental organisms
- Concern about other potential effects (e.g., combined effects from concomitant exposure to similar substances)

Conclusion

The combination of melamine’s substance properties causes high concern to the environment and human health (man via environment). The combined intrinsic properties which demonstrate scientific evidence of probable serious effects to human health and the environment and which give rise to an equivalent level of concern are the following: very high persistence, high mobility in water, potential for being transported in the water phase over long distances, difficulty of remediation and water purification. There is a potential for serious effects for human health and the environment that may occur with increasing concentrations in the environment. Thus, melamine may cause damage to the urinary

tract system through prolonged or repeated exposure, is suspected of causing cancer and yet unknown environmental effects. Together, these elements lead to a high potential for irreversible effects.

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Part II

7. Registration and C&L notification status

7.1 Registration status

Table 19 Registration status

From the ECHA dissemination site ³⁰	
Registrations	<input checked="" type="checkbox"/> Full registration(s) (Art. 10) <input checked="" type="checkbox"/> Intermediate registration(s) (Art. 17 and/or 18)

7.2 CLP notification status

Table 20: CLP notifications

	CLP Notifications ³¹
Number of aggregated notifications	21
Total number of notifiers	866

8. Total tonnage of the substance

Table 21: Tonnage status

Total tonnage band for the registered substance (excluding the volume registered under Art 17 or Art 18) ³²	100,000 – 1,000,000 t/pa
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9. Information on uses of the substance

Melamine is synthesised by a trimerisation reaction at high temperatures starting from urea. Both a high pressure and a low pressure process can be used, whereby the low-pressure process requires the use of catalysts.

³⁰ <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978> (accessed 26 July 2022)

³¹ C&L Inventory database, <http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database> (accessed 02. March 2022)

³² <https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/1/2> (accessed 02. March 2022)

Melamine is a starting material for various polymerisation reactions. The main field of application is certainly the production of formaldehyde based resins. These resins are used by the woodworking industry to produce different types of wood based panels (including composite pallet blocks) and melamine-impregnated papers and foils for surface-coating panels for a wide variety of applications including laminate flooring. In addition, these resins are used as amino crosslinkers in coatings, for the production of foams and for the production of consumer goods such as table ware.

A small fraction of the total melamine tonnage is used for fire safety applications. Mainly melamine is used as an intermediate chemical for the production of other melamine derivatives which are used as flame retardants and in intumescent (fire protective) coatings, such as melamine phosphate, melamine polyphosphate, melamine cyanurate, melamine-poly(zinc phosphate) or melamine borate. Most applications of melamine compounds for fire safety are in intumescent coatings of building structures, rigid foams used in technical applications or in technical plastics in industrial applications. In even smaller quantities, melamine itself is also used as such as a flame retardant in certain specific applications, in particular to reduce flammability of foams, especially polyurethane foams (e.g., in furniture, transport seating, ...) and also of rubbers, thermosets, and also in intumescent coatings.

The intumescent coatings, stated above, require somehow special attention, as they are the only use registered for the professional workers. Intumescent coatings are used to protect different materials from fire and heat. Melamine (and its derivatives) is used here exclusively as a blowing agent.

Table 22: Overview of uses according to ECHA dissemination site (non-exhaustive list)³³

	Use(s)	Registered use <i>(If not, specify the source of the information)</i>	Use likely to be in the scope of Authorisation
Uses as isolated intermediate (registered under Art 17 or Art 18)		Yes	No
Formulation or repacking	<p>Generic formulation processes; production of melamine resin, formica and melamine foam, manufacture of coatings and inks</p> <p>Formulations for leather manufacturing processes</p> <p>Formulation for production of adhesives, laquers, pigment intermediates</p> <p>In those applications the substance is used as such, or substance supplied to that use in form of mixture.</p> <p>Product categories (uses provided via ECHA dissemination site contain all or a variable subset of subsequent product categories, therefore no specific differentiation was made)</p> <p>PC 1: PC 9a: PC 9c:</p>	Yes	Yes

³³ For the sake of clarity, comparable uses that had a different pattern of use descriptors applied, but seemed to described similar uses according to the title of the intended use have been combined.

ANNEX XV – IDENTIFICATION OF MELAMINE AS SVHC

	Use(s)	Registered use <i>(If not, specify the source of the information)</i>	Use likely to be in the scope of Authorisation
	PC 15: PC 19: PC 20: PC 21: PC 23: PC 26: PC 32: PC 34: Sector(s) of end use n.a. subsequent service life relevant for use(s) n.a.		
Uses at industrial sites	Use as intermediate for the production of other substances, e.g. melamine salts; intermediate for coatings, inks and polymers substance is used as such, or substance is supplied to that use in form of mixture; Product categories PC 1: PC 9c: PC 19: PC 26: PC 32: Polymer preparations and compounds PC 34: PC 35: PC 39: Cosmetics, personal care products Sector of end use SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: a subsequent service life may or may not be relevant for this use	Yes	No
	Use of resins with unreacted residual melamine; leading to inclusion into/onto article	Yes	unclear
	Use of resins with unreacted residual melamine substance is used as such, or substance is supplied to that use in form of mixture; a subsequent service life may or may not be relevant for this use	Yes	Yes
	Handling, production of melamine resin, formica and melamine foam, manufacture of coatings and inks, professional and consumer use of resin substance is used as such, or substance is supplied to that use in form of mixture; Product categories PC 1: PC 9a: PC 9c: PC 15: PC 19: PC 20: PC 23: PC 26: PC 32: Polymer preparations and compounds PC 34:	Yes	YES

ANNEX XV – IDENTIFICATION OF MELAMINE AS SVHC

	Use(s)	Registered use <i>(If not, specify the source of the information)</i>	Use likely to be in the scope of Authorisation
	<p>Sector of end use SU 5: SU 6a: SU 6b: SU 7: SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: SU 10: SU 12: SU 13: SU 17: SU 18: SU 19:</p> <p>a subsequent service life may or may not be relevant for this use</p>		
	<p>Uses at industrial sites as additive in intumescent coatings</p> <p>substance is used as such, or substance is supplied to that use in form of mixture;</p> <p>Product categories PC 9a: PC 32: Polymer preparations and compounds</p> <p>Sector of end use SU 8: SU 12: SU 17: SU 19:</p> <p>a subsequent service life may or may not be relevant for this use</p>	Yes	Yes
	<p>Uses at industrial sites as intermediate for intumescent coatings</p> <p>substance is used as such;</p> <p>Product categories PC 9a: PC 32: Polymer preparations and compounds</p> <p>Sector of end use SU 8: SU 19:</p> <p>a subsequent service life is relevant for this use</p>	YES	NO
	<p>use at industrial site as additive in foams and coatings</p> <p>substance is used as such, or substance is supplied to that use in form of mixture;</p> <p>Product categories PC 9a: PC 21: PC 32: Polymer preparations and compounds</p>	YES	YES

ANNEX XV – IDENTIFICATION OF MELAMINE AS SVHC

	Use(s)	Registered use <i>(If not, specify the source of the information)</i>	Use likely to be in the scope of Authorisation
	Sector of end use SU 2b: SU 8: SU 11: SU 12: SU 18: a subsequent service life may or may not be relevant for this use		
	use as intermediate for resins (reacted melamine) substance is used as such, or substance is supplied to that use in form of mixture; Product categories PC 1: PC 9a: PC 9b: PC 9c: PC 18: PC 21: PC 23: PC 32: Polymer preparations and compounds PC 35: PC 39: PC 41: Sector of end use SU 1: SU 2a: SU 2b: SU 5: SU 6a: SU 6b: SU 8: SU 9: SU 11: SU 12: SU 13: SU 18: SU 19: SU 24: a subsequent service life may or may not be relevant for this use	YES	NO
	Textile applications – coating, calendaring, dipping and pouring substance is used as such, or substance is supplied to that use in form of mixture; Product categories	YES	YES

ANNEX XV – IDENTIFICATION OF MELAMINE AS SVHC

	Use(s)	Registered use <i>(If not, specify the source of the information)</i>	Use likely to be in the scope of Authorisation
	PC 20: PC 34: Sector of end use SU 5: a subsequent service life is relevant for this use		
	Use in leather manufacturing process substance is used as such; Product categories PC 23: Sector of end use SU 5: a subsequent service life is not relevant for this use	YES	YES
	use as laboratory reagent substance is used as such, or substance is supplied to that use in form of mixture; Product categories PC 21: Laboratory chemicals Sector of end use SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) a subsequent service life may or may not be relevant for this use	YES	NO
	Use as monomer in polymer production, e.g. in a polymer backbone substance is used as such, or substance is supplied to that use in form of mixture; Product categories PC 32: Polymer preparations and compounds Sector of end use SU 3: SU 8: SU 9: SU 12: a subsequent service life is relevant for this use	YES	NO
	Manufacture of coating and inks substance is used as such, or substance is supplied to that use in form of mixture; Product categories PC 1: PC 9a: Sector of end use SU 10:	YES	YES

ANNEX XV – IDENTIFICATION OF MELAMINE AS SVHC

	Use(s)	Registered use <i>(If not, specify the source of the information)</i>	Use likely to be in the scope of Authorisation
	a subsequent service life is relevant for this use		
	Manipulation of substances bound in materials substance is used as such, or substance is supplied to that use in form of mixture; Product categories PC 32: Sector of end use SU 5: a subsequent service life is not relevant for this use	YES	YES
	mixing substance is used as such; Product categories PC 1: PC 32: PC 34: Sector of end use SU 5: a subsequent service life is not relevant for this use	YES	YES
	adhesives, resins, lacquers, pigment intermediates no information available whether the substance is applied as such for this uses, or whether the substance is supplied to that use in form of a mixture; Product categories PC 1: PC 9a: PC 9b: Sector of end use SU 6a: SU 6b: SU 18: SU 19: a subsequent service life is not relevant for this use	YES	Yes
	Extrusion substance is not used as such, or substance is supplied to that use in form of mixture; Product categories PC 31: PC 34: Sector of end use n.a.	YES	unclear

ANNEX XV – IDENTIFICATION OF MELAMINE AS SVHC

	Use(s)	Registered use <i>(If not, specify the source of the information)</i>	Use <u>likely</u> to be in the scope of Authorisation
	a subsequent service life is not relevant for this use		
	Recycling substance is used as such, or substance is supplied to that use in form of mixture; Product categories n.a. Sector of end use SU 23: a subsequent service life may or may not relevant for this use	YES	Yes
Uses by professional workers	use as laboratory reagent Product categories PC 21: Laboratory chemicals PC 0: Other: Not Applicable Sector of end use SU 8: Manufacture of bulk, large scale chemicals (including petroleum products)	Yes	No
	use as additive in intumescent coatings for articles, whereas application can occur indoors and outdoors Product categories: PC 9a: Coatings and paints, thinners, paint removes PC 21: Laboratory chemicals PC 32: Polymer preparations and compounds Sector of end use SU 12: Manufacture of plastics products, including compounding and conversion SU 19: Building and construction work	Yes	Yes
Consumer uses	There are currently no intended uses of melamine as such by consumers reported on the ECHA dissemination site.	NO	NO
Article service life	There are variety of uses by workers, professionals and consumers reported for articles, that contain melamine. These uses seem to be subsequent to some of the aggregated industrial and professional use scenarios already presented in the rows above. However, the generic use description does not allow to make direct references. The service life of articles containing melamine presumably seem to cover articles made of polyurethane foams, where melamine is enclosed in the polymer structure of the PU and is acting as a flame retardant. Other articles are those made of wood, metal, plastic, leather or textiles where melamine as such of as constituent in mixtures was applied for intumescent purposes or to achieve specific decorative and haptic properties of the treated surface (e.g. decorative coatings with melamine for increased level of scratch proofness).	YES	unclear

	Use(s)	Registered use (If not, specify the source of the information)	Use likely to be in the scope of Authorisation
	The most important service life of articles that are supposed to contain melamine as well is the service life of articles made from formica. Here melamine is the substitute needed to decrease the residual concentration of free formaldehyde in the resins used for manufacture of adhesives used in formica production.		

Information from BUA report 105 (1992), (GDCh, 1992):

Dominant use of melamine is the manufacture of resins, which are manufactured by reactions with alcohols, aldehydes and amines.

Formaldehyde adds readily to the amino-groups of melamine, generating hydroxymethylmelamines. These crosslink (harden) readily, with elimination of water. Temperatures need to be 90 °C or higher in weakly alkaline pH regions, in highly acidic ones hardening occurs already in the 20 °C to 40 °C range. The degree of crosslinking of the resin correlates with the duration of heating. More highly condensed resins are insoluble in water.

Further processing of the aminoplastics obtained in this way mainly involves addition of fillers. Melamine resin renders cellulose, starch and other compounds bearing hydroxyl groups waterproof.

Relevant application areas are as follows:

- Surface coatings and paints: etherification of melamine and alcohols in conjunction with
 - a) plasticising resins yields stoving finishes for automotive applications that have high alkali resistance,
 - b) epoxide or epoxide ester resins affords wood finishes for floor seals.
- Laminates: in furniture industry, paper or fabric webs are impregnated with melamine resin to generate impact-resistant and scratch-resistant coatings for particle board and plywood.
- Glues and binders: melamine resins are used as binders for top-quality, water-proof particle board and for reinforcing wood glues.
- Paper finishing: addition of melamine resin increases the wet strength and wet-abrasion resistance of paper
- Compression-moulding materials: melamine resins are processed with cellulose or cotton linters to produce thermosetting, impact-resistant plastics for the manufacture of household appliances or kitchen utensils
- Textile and leather finishing: cellulose-containing fabric is impregnated with melamine resins in order to enhance dimensional stability, abrasion resistance and wet strength. To some extent, the flammability of fibres is also decreased. Melamine resins are moreover used to increase durability and abrasion resistance in the tanning of leather.
- Other: literature reports the possible use of melamine resins as filler in the rubber industry and as matrix for ion-exchanger resins.

The authors also stated that new applications of pure melamine were developed in the area of flame retardants for polymers, especially polyurethane foams during the time the BUA report was generated. In the meantime, the application of pure melamine for the purpose of flame retardants seem to have become a relevant application.

10. Information on structure of the supply chain

According to the publicly available data on ECHA's dissemination site, there are currently 51 active full registrations (Article 10)³⁴.

In addition, there is currently one active registration for manufacture and use of melamine as isolated intermediate under strictly controlled conditions (articles 17/18)³⁵.

The number of registrants and the variety of uses displayed in **Table 22** indicate that the supply chain is widely distributed. This and the submitted use information describing universal application, not only in an overview but also for single exposure scenarios in the registrants' individual CSRs makes it difficult to trace all supply chains with a sufficient level of certainty.

Some supply chains are very straight and can be followed easily. For example, melamine as such is used for the production of melamine resins, which are used in the follow-up for the manufacture of melamine resin foams. The maybe most common use of those foams are the so called "magic erasers" that are used as articles by households and commercial professionals for cleaning stains from hard surfaces.

The more complex the object for the end user is, the more complex the supply chain gets. The increased number of different sources of melamine contribute to the overall relevance of an aggregated distribution. In this example, formulations of melamine resins with free melamine are used for production of laminated papers or Formica at industrial level, which are then further applied by another industry competitor for manufacture of furniture. For decorative purposes and achieve scratch resistant surfaces the furniture may receive an additional coat of a melamine resin based lacquer. Both result in a contribution to the article service life of this piece of furniture. Depending on the intended area of application of the furniture, the customer requests the manufacturer that the furniture has to withstand fires for a certain time span. Therefore, the manufacturer may apply an additional intumescent coating with increased levels of free melamine (or its salts) before the decorative coat.

References for Part II

GDCh (1992): GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance: Melamine, BUA Report 105 date: June 1992

³⁴ <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/1/2>; accessed: 09.03.2022

³⁵ <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/6244/1/2>; accessed: 09.03.2022