Substance Name: Melamine

EC Number: 203-615-4

CAS Number: 108-78-1

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF

MELAMINE

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS EQUIVALENT LEVEL OF CONCERN HAVING PROBABLE SERIOUS EFFECTS TO HUMAN HEALTH (ARTICLE 57(F) - HUMAN HEALTH), EQUIVALENT LEVEL OF CONCERN HAVING PROBABLE SERIOUS EFFECTS TO THE ENVIRONMENT (ARTICLE 57(F) - ENVIRONMENT) PROPERTIES

Adopted on 15 December 2022

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ABBREVIATIONS

B	bioaccumulative
vB	very bioaccumulative
BCF	bioconcentration factor
cmax	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
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
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

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

- The substance is identified 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 experimental studies and Quantitative structure-activity relationship (QSAR) predictions.

The overall weight of evidence shows that degradability of melamine under environmental conditions is low: Based on QSAR 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 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. Additional studies with cultures of single species of bacteria or in wastewater treatment plants treating industrial effluent indicate that melamine might be degradable under specific conditions. However these conditions are not representative of either municipal sewage treatment plants or environmental conditions and therefore are not applicable for persistency assessment under REACH.

Considering the data on abiotic and biotic degradation, the half-life of melamine in water is >60 days.

Volatility, water solubility, adsorption, distribution in the environment:

Melamine has an experimentally derived log normalised organic carbon to water partition coefficient (K_{OC}) of 1.81 (pH value in experiment not disclosed) indicating a low potential for adsorption on organic matter and clay minerals in the environment. The QSAR estimate for the non-ionic molecule results in a log K_{OC} of 1.51 (KOCwin from EPIsuite was applied). As melamine has a base dissociation constant (pK_{b1}) of 7.3, non-negligible quantities of non-ionic and ionic forms of the molecule occur under environmentally relevant conditions.

For the cationic forms of melamine, a higher log $K_{OC}$is assumed. Thus, for the ionic melamine forms, adsorption on soil constituents such as organic matter or clay minerals is expected to be higher when compared to the non-ionic form.

As the data indicates that under environmentally relevant conditions ionic and non-ionic molecules can be found in non-negligible quantities at the same time, the log K_{OC} of 1.81 of the non-ionic form is used in the assessment of environmental fate and behaviour of melamine.

The substance's intrinsic behaviour of low volatility from water (calculated Henry's law constant 2.0e-08 Pa*m^3/mol) together with its low potential to adsorb to organic matter result in a high mobility in water. Additionally, the physical-chemical 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 make it difficult to remove melamine from water by commonly applied sewage treatment and water purification techniques as it only has a low potential to adsorb to materials and tends to remain in the water phase.

Because of melamine's intrinsic property of 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 emerges in the environment. In combination with another intrinsic property, 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.

Distribution of melamine in the environment is influenced by the intrinsic properties of the substance, in addition to the properties and conditions of the environment. Therefore, the source of melamine emissions to the environment is not of relevance for the purpose of SVHC identification.

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. This is due to the chemical properties of melamine resulting in reaction within the urinary tract. Although the exact mechanisms have not been fully elucidated, it is thought that the intrinsic structural properties of melamine allow for hydrogen bonding with urinary uric acid to form a crystalline lattice structure (melamine–uric acid complexes). 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, there is scientific evidence of probable serious human health effects. Committee for Risk Assessment (RAC) has confirmed this by concluding 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 CLP[1]. .

The observed testicular and sperm effects of melamine in an extended one-generation reproductive toxicity study conducted in rats are additional probable effects of melamine.

These effects are serious for human health because they are significant and irreversible effects. This is confirmed by the fact that they fulfil the criteria for classification (STOT RE, carcinogenicity). According to the RAC opinion, classification as STOT RE is required based on the potential to cause acute and chronic renal diseases in humans. RAC also notes that the mode of action of carcinogenesis established in experimental animals cannot be disregarded as potentially relevant in humans. Thus, melamine fulfils the criteria for classification as a suspected human carcinogen. An additional harmonised classification may be warranted based on the observed effects on reproductive toxicity. . These effects are also significant when combined with environmental fate properties leading to irreversible and increasing presence in the environment for the reasons described below.

Ecotoxicity:

The observed testicular and sperm effects of melamine identified in experimental animals (rats) are considered relevant for identification of probable serious effects on the environment. They can impact reproduction and can have an effect on the population level. Rats are rodents and rodents represent approximately 42% of all mammalian species. Rodents play an important role in the environment, for example in the dispersal of seed and spores, pollination, energy and nutrient cycling, modification of plant succession and species composition. Rodents are a food source for many predators.

There is also scientific evidence of probable serious effects on fish (mortality and growth) and on aquatic invertebrates (mobility, poor condition). As supporting information, effects were observed on terrestrial birds (decreased egg shell strength). These effects are serious because they also can have an effect on population level.

These effects are also significant when combined with environmental fate properties leading to irreversible and increasing presence in the environment for the reasons described below.

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 a potential to cause an irreversible presence in the aquatic environment, together with a widespread contamination of the aquatic environment.

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

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[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.

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 municipal sewage treatment plants dealing with mixed sewage that predominantly originates from households. Thus, the overall amount of melamine emitted from production and use is, if at all, only marginally reduced by the treatment processes in such municipal sewage treatment plants.

It is acknowledged that sewage treatment plants that are specifically designed to treat sewage predominantly originating from industrial sites may achieve a higher removal efficiency. Such industrial sewage treatment plants are specifically designed to reflect the local situation and site specific legal requirements. Although such industrial sewage treatment plants may be relevant for melamine, no information is available about removal efficiency in such plants that would allow general conclusions about the removal efficiency to refine the release estimates nor their availability across the European Union. It is therefore assumed that manufacture and industrial applications of melamine (or its precursors) may contribute to distribution of melamine in the environment and it would have a significant cost, supporting the identified concern, to apply efficient methods even for industrial sewage treatment plants in the European Union.

Once released, melamine may remain in the environment for a long time due to its persistency.

Supporting monitoring data already confirmed occurrence of melamine in various rivers (e.g. Rhine, Meuse, Mulde, Danube), where sampling points do not influence each other because as they are located in different river catchment areas. Concentrations found exceeded the value of 0.1 µg/L. In addition, recent projects were able to identify and quantify melamine in groundwater samples in Germany and Switzerland.

In the NORMAN Empodat data base melamine was detected above limit above equantification (LOQ) in 958 cases and below LOQ in 267 cases in 10 countries, indicating a widespread distribution in the environment.

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 intrinsic properties of melamine are likely to cause transport across water bodies to pristine oceans and groundwaters, raising the concern that the substance may also occur at remote locations from the origin of release.

Melamine stays in the environment even if emissions have already ceased, as can be concluded from the melamine's intrinsic properties and supported by the recurring findings in groundwater samples.

Due to the high persistency, the amount of the melamine present in the environment is expected to increase over time if continuous emission occurs. In addition, local concentrations may increase temporarily or permanently due to aridity periods e.g. as result of climate change.

Removal from the aquatic environment and from drinking water resources

Once melamine emerges in the aquatic compartment (as direct consequence of its use pattern or as result of the degradation of precursor substances) and is widely distributed in the environment, it is difficult to remove. This results from the recalcitrance 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 drinking 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.

Increasing exposure of humans and environmental organisms due to increasing presence in the aquatic environment and wide distribution.

Melamine is already present in surface waters. Computational models predict that, once occurring in the environment, melamine is able to remain in the environment for several years or maybe even decades and is able to reach remote regions. This is due to the melamine's intrinsic property to disperse in the aquatic environment over several thousand kilometres. This means that detection of melamine and undesirable effects may occur far away from the point of the 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 can be expected to increase over time. This can 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 melamine has probable serious effects, the occurrence of melamine in the environment should be prevented.

Human Health effects

Melamine has probable serious effects (urinary tract and carcinogenic effects), and therefore its presence in drinking water is a concern as it may cause effects if critical dose levels are exceeded.

Additionally, in an extended one-generation reproductive toxicity study conducted in rats, 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. Furthermore, an assessment on endocrine disrupting properties for melamine is ongoing.

Human exposure

As supporting information, ECHA notes that melamine has been repeatedly detected in human urine samples from the general population in the USA and in East Asia.

Melamine can also be ingested by infants through breast milk. Lactational and/or placental transfer was shown following exposure to melamine in animal studies.

Due to its persistence and mobility, melamine is able to reach the sources 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, and potential for being transported in the water phase over long distances) it is not possible to assess its (local) environmental concentration with sufficient certainty and to consider effect concentration limits for the environment by the means of standardised acute and chronic ecotoxicological tests and the assessment criteria investigated within (in short: no safe concentration limits can be derived).

One aspect that adds to the concern that the effects in the environment might currently be underestimated are sublethal effects observed in several species. 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.

In an extended one-generation reproductive toxicity study conducted in rats, 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. Furthermore, an assessment on endocrine disrupting properties for melamine is ongoing. The observed effects on rats are considered relevant for identification of probable serious effects on the environment.

In a study with birds, effects on egg shell strength and egg shell weight were seen, that both decreased significantly after melamine exposure. These effects are important as they might be relevant for populations. In exposed hens Melamine as well as cyanuric acid were detected in the liver and kidneys. The study authors suggested that Melamine was biotransformed to cyanuric acid. This study on birds is used as supporting information in the assessment of environmental effects of melamine.

Hence the substance properties raise the concern that effects as described above or yet unknown effects could appear in the environment and lead to irreversible 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 little effectivity of common and widely applied drinking water purification techniques, such as river bank filtration or soil infiltration for groundwater enrichment. Removal 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.

This societal concern is further confirmed by reports that extraction of water from the Rhine and Meuse rivers was stopped due to the presence of melamine.

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. For example*,* combined effects due to co-exposure with other chemicals, e.g. cyanuric acid, have been reported in rats, pets and livestock. Effects occur at lower melamine concentrations following co-exposure to melamine and cyanuric acid, which is linked to the formation of highly nephrotoxic melamine–cyanuric acid crystals/stones. 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.

Therefore, appearance of undesirable effects due to additive or even synergistic mode of action cannot be excluded which adds to the concern that no safe concentration can be derived.

Overall evaluation of the concerns and summary with regard to the equivalent level of concern

The persistency, mobility and toxicity (specific target organ toxicity after repeated exposure and carcinogenicity) and the irreversibility of the contamination of the aquatic compartment compromise the quality of drinking water resources and give rise to the concern of increasing and wide spread 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.

Based on these concerns the level of concern is considered very high due to the combination of the above-mentioned concerns which can be clustered into the following concern elements:

- Irreversible, wide spread and increasing presence in the environment (limited potential for removal of melamine from the aquatic environment and drinking water resources, persistency in the environment, potential to be transported to remote areas and continuous emission due to low degradation in sewage treatment plants),
- Increasing exposure of humans and environmental organisms as a result of the irreversible, wide spread an increasing presence in the environment (increasing contamination of drinking water resources, long-term exposure, exposure of remote areas),
- Concern about the effects observed and other potential effects (e.g. combined effects from concomitant exposure to similar substances)
- The combination of these concern elements results in a potential for:

  - Environmental and 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 and groundwater which are used as a source of drinking water

Conclusion

The combination of melamine’s substance properties causes very 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 and toxicity. The combination of persistence and mobility of melamine lead to the difficulty of remediation from the environment and water purification. There is scientific evidence of probable serious effects for human health and the environment that may occur with increasing concentrations in the environment. These probable serious effects to human health are urinary tract toxicity, carcinogenicity and reproductive toxicity. The probable serious effects to the environment are sub-lethal effects on fish and aquatic invertebrates and reproductive toxicity in rats and other mammals.

Registration dossiers submitted for the substance: Yes

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:	$C_3H_6N_6$
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.	
(Base) 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. 2[2].

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).

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[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 at the ECHA dissemination site, 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).

For the purpose of gathering information on hydrolytic degradation for the SVHC dossier, QSAR models were also used.

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

Another trial for a QSAR estimation was undertaken with the Vega Hub models[3]. 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.

In a study by Gong et al. (2016) the pH and temperature dependency of hydrolysis were investigated in two tests. The authors found, that the highest hydrolysis conversion rates at a test temperature of 200 °C and for a reaction time of 2 hours have been found at extreme pH values of pH 1.00 and pH 13.26. In contrast, the lowest conversion rates for this test temperature were found at pH ranges that are of more relevance for the environment (pH 5 to pH 9.26). The conversion rates found are presented in **Table 4**. The second test in this study investigated the temperature dependency of hydrolysis of melamine. In this test, the conversion rates were determined for the temperature range of 150 °C to 200 °C for a single pH value (pH 13.26) and a reaction time of 2 hours. The results are presented in **Table 5**. From the data it is obvious that there is a direct dependency between temperature and hydrolysis rate – with increase of the temperature the hydrolysis rate increases, too. (Gong et al., 2016)

Table 4: conversion rates for hydrolysis of melamine at different pH values

pH	1.00	3.00	5.00	7.00	9.26	11.26	13.26

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[3] QSARs available at https://www.vegahub.eu/
Date of access 19.08.2022

Conversion rate (percent)	95.14	30.11	28.43	13.50	32.39	41.11	99.76

Experimental conditions: Temperature = 200 °C; Reaction time = 2 h; Volume of the reaction liquid = 150 mL; Initial melamine concentration in the reaction liquid = 666.7 mg/L;

Table 5: conversion rates for hydrolysis of melamine in NaOH solution at different temperatures

Temperature (°C)	150.0	160.0	170.0	185.0	200.0
Conversion rate (percent)	35.42	41.46	67.78	87.14	99.76

Experimental conditions: Initial melamine concentration in the reaction liquid = 666.7 mg/L; Reaction time = 2 h; Volume of the reaction liquid = 150 mL; pH of reaction liquid = 13.26

Even with no data available for hydrolysis of melamine from standard tests systems, it it can be concluded from the data of Gong et al., that abiotic degradation via hydrolysis is low at environmental relevant pH ranges (range of about pH 5.5 to pH 9). Taking into account that the hydrolysis rate will decrease with decreasing temperature it becomes quite obvious that the hydrolysis rates of melamine at the European average temperature of 12°C will be by orders of magnitude lower compared to results found at a test temperature of 200 °C.

In summary, the available information for the hydrolytic degradation behavior of melamine provides scientific evidence, that this pathway of abiotic degradation is negligible under environmentally relevant conditions.

3.1.1.2 Oxidation

Melamine does not contain oxygen or halogen atoms. Therefore, it is unlikely that melamine has oxidising 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 site[4] 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 melamine 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/cm^3, 24 h daylight. Assuming a 12 h daylight and an OH-radical concentration of 1.5×10^6 OH/cm^3, the half-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

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[4] https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/2/2; date of access 18.08.2022

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 site[5] for melamine.

The technical guidance document R.11 (ECHA, 2017b) 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."*

Major releases during the life cycle of melamine resulting from manufacture and use of the substance (or its precursors), which are falling under the considerations of the REACH legislation 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.

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.

3.1.1.3.3 Phototransformation in soil

No experimental or calculated data on phototransformation in soil is available on the ECHA dissemination website[6] 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 melamine 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.

3.1.2 Biodegradation

Several standard and non-standard studies on biodegradability are available and described in the following subchapters.

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[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

Some non-standard studies with isolated bacteria culture (e.g. *Rhodococcus sp.*, *Pseudomonas sp.*, *Klebsiella terragena*, *Arthrobacter sp.* and *Nocardioides sp. ATD6*) indicate some potential for biodegradation of melamine (Cook et al., 1985; Dodge et al., 2012; Hatakeyama and Takagi, 2016; Jutzi et al., 1982; Shelton et al., 1997; Takagi et al., 2012; Tolleson et al., 2009). However, as isolated microorganism strains were used, the studies are only applicable to show that melamine is biodegradable under specific conditions. These studies cannot be used on their own for P/vP-assessment (ECHA, 2017b) and are not further assessed in this document.

Furthermore, studies showing removal of a substance in wastewater treatment plants (WWTP) can also not be used on their own for P/vP assessment (ECHA, 2017a). The half-lives determined from those tests are not suitable for comparison with the REACH Annex XIII criteria for persistence. There are several reasons for this: e.g. degradation in a technical sphere such as a WWTP does not represent biodegradation in the environment and it is not possible to differentiate the elimination processes that contribute to the overall removal observed in a WWTP.

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 (2017b).

3.1.2.1.2 Screening tests

Several standard and non-standard screening studies on biodegradability are available on the ECHA dissemination website for melamine[7]:

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 melamine 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

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[7] https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/3/2
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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 this 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. In other words, the low degradation observed in the OECD degradation tests has not been caused by toxicity of melamine to the inoculum.

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 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 melamine 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 (O_2 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, 2017a 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 website[8].

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

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[8] https://echa.europa.eu/registration-dossier/-/registered-dossier/15978

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 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 ± 0.0 µg/L at day 45 and a maximum value of 5.35 ± 0.7 µg/L at day 15 (Figure 1). At the end of the study (day 60), 5.25 ± 0.7 µg/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.

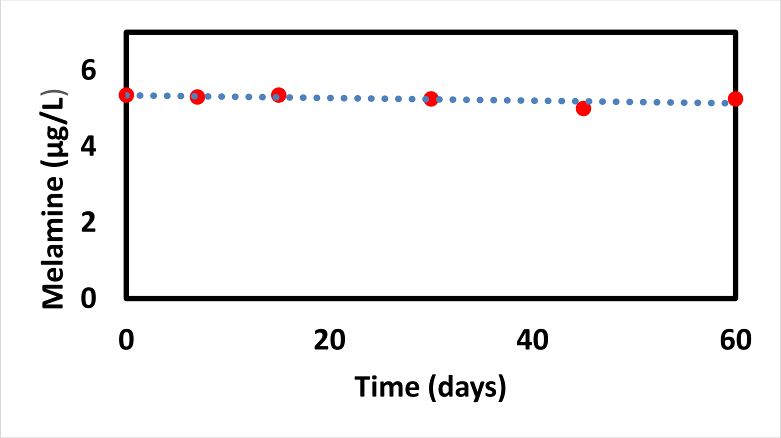

Figure 1: Concentration of melamine (µg/L) in a surface water test system in course of time (days). Data were shown as mean value of two individual bottles for each sampling point. Please note: Error bars indicating standard deviation are included in this figure. However, as the standard deviation resulted in values up to 0.071 µg/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 (k1) followed by a slow phase (k2).

Calculated half-lives (DT_{50}) of melamine are shown in Table 6. The SFO model is the best fit model (lowest Chi2 error value which is below 15 %). Results of the t-test reveal that the parameter k of the SFO model, as well as the k1 and k2 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 6: 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 website[9].

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.

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[9] https://echa.europa.eu/registration-dossier/-/registered-dossier/15978
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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 (NO_3^-). The extent of nitrate formation from melamine in soil was low in the study. Only 0.7 % of organic N was found as NO_3^- 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, even if elevated temperatures (32 °C) were applied.

3.1.2.3 Summary and discussion on biodegradation

Based on BIOWIN 2, 3 and 6 QSAR predictions (BIOWIN v4.10 of the EPISuite tool (US EPA, 2002-2012)), melamine screens as potentially P/vP according to REACH guidance ECHA (2017b).

Low degradation in water is shown in the standard screening test on ready biodegradation, the two inherent tests available and other studies on biodegradation (Fimberger, 1997; Niemi et al., 1987). The OECD TG 309 surface water simulation test over 60 days showed no degradation of melamine indicating that the degradation half-life of melamine is longer than 60 days. 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

This section is about analytical monitoring data for melamine monitored in effluents from waste water treatment plants. For data on distribution modelling in municipal waste water treatment plants, that is solely based on the intrinsic properties of the substance and an assessment of techniques for removal of melamine in water, please see section 3.2.3 (Distribution modelling).

The subsequent publications document the presence of melamine in the effluents of sewage treatment plants.

Alhelou et al. (2019) investigated the concentration of melamine in four WWTPs by applying a combined Ultra High Performance Liquid Chromatography with photodiode array detector (PDA) system was interfaced to quadrupole time-of-flight mass spectrometer G2-XS QTof equipped with an electrospray ionization interface (ESI), 'UPLC-PDA-QTOF-MS/MS'. Limit of detection (LOD 3.3 x S/N) and the limit of quantification (LOQ; 10 x S/N) were within a range of 0.78 and 3.93 µmol/L for melamine. 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 melamine concentrations in the influent and effluent of four WWTPs. They applied high performance liquid chromatography coupled via electrospray ionisation with tandem mass spectrometry (LC-MS/MS). The authors state that the LOQ was specifically determined for every substance, however a higher generic reporting limit of ≥0.025 µg/L for melamine was established by the authors. 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). Extraction of MA insample was based upon solid phase extraction (SPE) by poly-Sery MCX column. The column was eluated with 6 mL 5% ammonium methanol and concentrated before detection. The HPLC system consisted of a C18 column, the resulting LOD was 0.05 µg/L. 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.

On behalf of the Norwegian Environment Agency Henninge et al. (2020) were able to identify melamine in 16 out of 16 effluent samples of wastewater treatment plants collected in Norway. The authors used four different LC techniques for the water samples depending on the substances looking for: LC-APPI / HRMS, DIA screen, and LC-ESI / MS / MS with both C18 and hypercarb columns. However, no information is included in the publication which specific techniques were applied in the analysis for melamine, nor the LOQ/LOD values. The average concentration ranged between 0.7 – 2.9 µg/L with a maximum concentration of 11.00 µg/L observed. Further the authors observed "no indication of reduction over the treatment steps of the plant." This was because corresponding samples from the inlets and outlets at the individual WWTP were taken at the same days and the effluent concentrations were even slightly exceeding the influent concentrations. The Norwegian screening programme in 2019 also measured melamine in house dust. The substance was found in 64 out of 65 indoor dust samples (average concentration 195 µg/kg, median concentration 76.5 µg/kg) from private homes (highest concentration measured 1900 µg/kg) and commercial buildings (highest concentrate measured 2800 µg/kg). These data are in line with findings from Zhu and Kannan (Environ. Sci. Technol. 2018, 52, 12801−12808) who determined melamine in indoor dust collected in 12 countries. This data is included as supporting information exemplary for occurrence of melamine in a non-aquatic compartment (which may however lead to discharge to aqueous compartments e.g. via cleaning).

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 melamine 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, 2017b) already allow to demonstrate that melamine 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 DT_{50} of melamine is concluded to be over 60 days. This result confirms that melamine 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

For assessing the adsorption behaviour of a substance the log Koc is used because it provides a measure of adsorption potential which can be compared with other substances and is not dependent on the properties of the specific matrix to which it is adsorbing

It is possible to have an estimate for the log Koc from the log Kow by applying the respective equations. This approach is often applied for non-ionic molecules.

Grathwohl argues, that the specific organic matter content in soils and sediments influences the adsorption behaviour of non-ionic substances in the specific soil/sediment (Grathwohl, 1990). For ionised substances the adsorption is affected by parameters such as the content of sand, loam and clay minerals, too. Soils with high content of clay and organic matter in general have a much higher adsorption capacity compared to soils with a high sand fraction. Depending on the "substance load" applied to the specific soil matrix, the available adsorption traps are occupied and the soil specific adsorption capacity is consumed. In case there is no retention capacity left, also adsorptive substances are capable to travel through soil layers. This does not only include vertical movement, i.e. as result of precipitation. This behaviour can also be seen for horizontal movement, i.e. due to hydraulic pressure as it can be found in bank filtration systems, where the underground passage of surface waters applied for drinking water production.

Adsorption to specific media (soil, sediment, sewage sludge) can be described via specific adsorption coefficients (Kd). However for having an overall picture for the adsorption behaviour of one substance in different soils, sediments and sewage sludge there seems to be a need for various, specific adsorption coefficients (Kd) to conclude on the adsorption behaviour from a general perspective. The same holds true for lysimeter studies.

In contrast to this laborious approach, the application of the log Koc allows assessment and comparison of adsorption behaviour of different chemicals across the world, regardless which type of the soils/sediments/sewage sludge compositions can be found locally and thus represents both conditions with soil/sediment/sewage sludge with low and high adsorption potential

Information included on the ECHA dissemination website[10] 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 database[11] 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 Dashbord[12] 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 form of a substance.

As melamine has a pK_{b1} of 7.3, non-ionic and ionic forms of the molecule occur under environmentally relevant conditions. In a study investigating the experimental and calculated log K_{OC} of a cationic substance, the author shows, that the experimental determined log K_{OC} value for the ionic forms of the model cation was 1.6 log units higher compared to the calculated log K_{OC} for the non-ionic forms of the molecule using EPISuite Software (EPIWEB Version 4.1) (Claßen, 2019). Assuming that the same difference in log Koc units applies to the non-ionic and cationic forms of melamine, a log K_{OC} of around 3.11 (based on KOCWIN v2.00) - 3.41 (based on experimental data) is expected based on that study.

In the EFSA peer review conclusion for the pesticide risk assessment for the active substance cyromazine published in 2008, EFSA stated: "Reliable adsorption characteristics of melamine are available for 4 soils. This metabolite is medium to high mobile in soil with Kfoc = 54-423 mL/g (1/n = 0.7414-0.83). The meeting of the experts agreed that a clear pH dependency of the adsorption of the parent cannot be concluded, whilst a good correlation between the pH and the adsorption coefficient can be observed for melamine." (EFSA, 2008). Unfortunately, no information for the pH values in the soils were provided in EFSA conclusion document. The Kfoc provided by EFSA translate into logarithmic values of log Kfoc = 1.732-2.626 for describing a soil specific adsorption behaviour of melamine.

Wang et al. studied the adsorption of melamine on five typical Chinese agricultural soils having a pH of between 5.56 and 7.45. They found that the sorption capacity of those soils

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[10] https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/5/2

[11] https://substances.ineris.fr/fr/substance/1176
date of access 18.08.2022

[12] https://comptox.epa.gov/dashboard/chemical/env-fate-transport/DTXSID6020802
date of access 18.08.2022

for melamine is in the range of log Kfoc 1.63 – 2.55 (Wang et al., 2014). The highest sorption was found in loam with a high organic matter content (45.89 g/kg). The lowest sorption was found in the sandy loam soil, showing a low organic matter content (9.93 g/kg). They also concluded, that cationic exchange capacity and pH value of the specific soil has an influence on the adsorption behaviour of melamine.

In summary, the available data consistently shows an adsorption coefficient log K_{OC} below 2 for the non-ionic forms of melamine whereas log K_{OC} below 3.5 was estimated for the ionic forms. The information substantiates that melamine has a low tendency to adsorb to organic matter or sewage sludge. Since under environmentally relevant conditions ionic and non-ionic molecules can be found at the same time, 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 melamine[13] 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 atm*m3/mol at 25 °C, which according to him equals ca. 2.0e-08 Pa*m³/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 atm*m³/mol (@20 °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 atm*m3/mol at 25 °C, which equals ca. 1.89e-08 Pa*m³/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 7: 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*m³/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 °C by applying the Arrhenius equation.

Commonly, slightly volatile substances are defined by falling below the threshold of 0.1 Pa*m³/mol, whereas highly volatile substance are expected to exceed the threshold

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[13] https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/5/3
Date of access 18.08.2022

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, melamine 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 8: 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 may not retain any relevant amounts of melamine to be found 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.

The previous considerations refer to treatment processes typically used in municipal sewage treatment plants, which are set up to treat sewage predominantly originating from households. It is acknowledged that sewage treatment plants that are specifically designed to treat sewage predominantly originating from industrial sites may achieve a higher removal efficiency. Such industrial sewage treatment plants are specifically designed to reflect the local situation and site specific legal requirements. In addition, due to the specific composition of the industrial sewage, formation of microorganism populations adapted to degrade melamine in industrial sewage treatment plants is likely. Although such industrial sewage treatment plants may be relevant for melamine, no information is available about removal efficiency in such plants that would allow general conclusions about the removal efficiency to refine the release estimates. It is therefore assumed that manufacture and industrial applications of melamine or its precursors will contribute to distribution of melamine in the environment.

Published studies from the scientific literature indicate that there are techniques available for removal of melamine from raw and sewage water (i.e. Cook et al., 1985; Dodge et al., 2012; Hatakeyama and Takagi, 2016; Jutzi et al., 1982; Shelton et al., 1997; Takagi et al., 2012; Tolleson et al., 2009). These studies on the one hand mentioned potential for removal due to isolated bacterial strains or removal via abiotic hydrolytic degradation under extreme conditions (i.e. around pH 14, temperature 100 °C) that are expected to be hardly implemented the majority of municipal and industrial sewage treatment plants, where sewage containing melamine and/or its precursors are treated. Patents describing treatment methods for waters containing melamine are available and there is evidence that advanced treatment techniques such as reversed osmosis or water filtration through active carbon are capable of removing melamine from water.

However, the first method produces high concentrated residuals, that have to be treated separately. This should prevent release to sewage at another treatment stage or sewage treatment installation. The removal potential of the activated carbon methods (i.e. described by Hynes et al., 2020; Piai et al., 2021; Winzenbacher et al., 2015; Xu et al., 2020), however almost exclusively referred to investigations to conclude on optimal conditions for maximised removal efficiency. Those lab-scale experiments always applied optimal pH, “virgin” activated / specifically engineered carbon material and melamine as single substance to be retained.

Therefore, the results of those publications can be seen as “best case” situation – but often there is no evidence that the results from those experiments were verified in a test-size scale, nor tested to treat sewages from real installations. In reality the removal efficiency to be observed for melamine is expected to be lower, as the constituents of mixed sewage compete for the adsorption traps of the activated carbon. Availability of “virgin” activated carbon for high removal efficiency of melamine in sewage treatment installations is not the common situation, too. Depending on the loading of the activated carbon material in the filter bed, regeneration processes are needed to desorb the retained chemicals. But regeneration and thereof aging of the active carbon results in a decrease for the adsorption potential, especially for polar substances like melamine. This was verified by the experiments of Winzenbacher et al.

Winzenbacher et al. studied the removal potential for melamine via advance water treatment by ozonisation. Samples containing melamine (start concentration about 1100 mg/L) were exposed to different dosages of ozone (1, 2 and 3 mg ozone/L) for a contact period of at least 10 minutes. In none of the tests a relevant decrease of the melamine concentration was observed. In addition, the authors found that a relevant removal of melamine from water with granular activated carbon (GAC) can only be achieved in case the GAC was not pre-charged ("virgin GAC") (Winzenbacher et al., 2015).

Therefore, from these results less effective removal is expect for real-life conditions in case of co-occurrence of melamine to other substances in the water to be treated, which compete with melamine for adsorption traps at the GAC, or as result of "aging" of GAC from regeneration.

The water treatment processes ozonisation and GAC adsorption investigated in experiments of Winzenbacher et al. (2015) are used in drinking water purification. This information indicates that removal rates of melamine achieved by applying ozonisation or adsorption at GAC will be lower under real life conditions than in laboratory scale and might serve as an explanation for the findings of melamine in purified drinking water.

The publication of Piai et al. and references contained in there reflect that melamine is poorly removed from water in conventional wastewater treatment plants which have to be expected as the dominant type for treatment in the EU when it comes to treatment of sewage predominantly originating from households. The poor removal of melamine would be due to its low biodegradability by and low tendency for adsorption to conventional activated sludge. The authors describe conditions under which a reactivation of granular activated carbon adsorptive capacity for melamine was restored to 28% and recommend the methodology to be used in industrial waste water treatment plants. (Piai et al., 2021).

Distribution processes between environmental compartments

The ECHA dissemination website[14] contains the outcome of an environmental distribution prediction according to MacKay, Level I. The results of the registrants' QSAR estimates are presented in Table 9.

Table 9: 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

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[14] https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/5/4
Date of access 18.08.2022

(*) 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:

Table 10: 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 10**Table 10**, therefore these were not displayed again.

Table 11: 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 12: 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 13: 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

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 12). The high rate of occurrence for melamine in soil that is predicted by the fugacity model in EPIsuite as shown in Table 10, Table 11 and Table 13 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.

Distribution behaviour of a substance is an intrinsic, substance specific property, contributing to the overall hazard profile of the substance.

In contrast, dilution is a non-substance specific factor which indeed has an influence on the environmental concentration (exposure) to be found at the end. But dilution is a factor that is considerably influenced by local, highly variable conditions during the assessment period, such as overall precipitation, amount of substance released to the environment, volume flow of receiving waters etc. Dilution is not an intrinsic property of the substance and therefore dilution of melamine released to the aquatic environment is not relevant for the process of identifying melamine as SVHC.

In their report, (Crookes and Fisk, 2018) modelled a simple system where a substance is continuously input to an environmental compartment. They show that for a fixed input rate, the expected steady state concentration and time to reach steady state increase with higher persistency (half-life) of a substance. Their modelling showed that substances that are both persistent and mobile in the environment have the potential to be transported long distances from the point of emission. If such substances accumulate over time in remote regions, they can reach levels that may have effects on both ecosystems and human health. The potential for long-range transport is discussed in section 3.3 below.

3.2.4 Field data

Findings in drinking water

There is no universal conclusion to be drawn about the most dominant source of drinking water because sources a highly impacted on local and regional level by factors such as geological conditions, amount of precipitation, availability of water sources in general etc. For example, about 60 percent of the drinking water in the Netherlands are sourced from groundwater, whereas the remaining 40 percent are produced from surface water[15]. In Germany about 70 percent of the drinking water are produced from groundwater and about 13 percent are directly sourced from surface waters (lakes, river, water supply dams), the remaining amounts are produced by processes resembling natural processes for groundwater formation, namely vertical soil passage or river bank filtration[16].

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 authors developed a method based on HILIC (hydrophilic interaction liquid chromatography) hyphenated with HRMS, with an LOD = 1.19 µg/L for melamine. The samples were taken in The Netherlands and in Flanders (Belgium), without disclosure of the exact sampling locations.

Zhu and Kannan (2020) reported the occurrence and spatial distribution of melamine and its derivatives in waters in New York State, USA, sampled between 2015 and 2019. The authors applied a HILIC-MS/MS method for sample analysis with an quantification limit of 5 ng/L. Field, travel, procedural, and solvent blanks were analyzed for monitoring background levels of contamination to assure validity of analysis. For this study 223 water samples were analysed for melamine and derivatives. For tap water samples (*n*=70), c_{median} amounted 19 ng/l, c_{mean} amounted 33 ng/l, whereas c_{max} was 188 ng/L with a detection frequency of 100 %.

Findings in the aquatic environment

Kiefer et al. (2021) used liquid chromatography high-resolution tandem mass spectrometry (LC-HRMS/MS) to investigate Swiss groundwater more comprehensivly. Therefore groundwater sampling site were were classified “as having high or low urban or agricultural influence based on 498 target compounds associated with either urban or agricultural sources”. In a second step, all LC-HRMS/MS were tried to assign to a potential origin. A most intensive nontarget signals were automatically annotated and suspect screeing was performed. In the scope of the sample analysis, the authors used a C18 column for separation. Thereby the gradient elution started with 100% water (containing

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[15] Source of information: https://www.atlasleefomgeving.nl/en/explore/water/drinking-water; (RIVM, The Netherlands; website visited 07.11.2022)

[16] Source of information: https://www.umweltbundesamt.de/themen/wasser/trinkwasser; (German Environment Agency; website visited 07.11.2022)

0.1% formic acid) to achieve an optimal retention of polar compounds. Within the analysis of the 498 target compounds, melamine (LOQ = 5 ng/L) was reported in 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. The authors used LC-HRMS applying three different chromatographic conditions, i.e. reverse phase (RP), hydrophilic interaction liquid chromatography (HILIC) and mixed mode (MM). Melamine (HILIC) 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 used in total five different analytical LC-MC/MS methods for the analysis of 84 trace organic chemicals (TOrCs), following German norms DIN 38407-36 and DIN 38407-47. The authors used for the analysis of melamines (melamine and HHHM) an Restek Ultra Aqueous C18 column. The gradient elution started with 100% water (5 mmol/L ammonium acetate). 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. Samples were analysed with LC-MS/MS in a suspect and non-target screening approach. Thereby a HPLC equipped with a XBridge C18 column and using a water/methanol gradient as mobile phase were used for separation, resulting in a retention time (RT) of 0.8 min for melamine. Melamine (LOQ = 70 ng/L) was found with a detection frequency of 100 % on a median concentration of 11 µg/L (c_{max} = 94 µg/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 (LOQ = 0.56 µg/L, *n*=7) in the Netherlands and in Flanders (Belgium). The exact locations of the sampling points were not disclosed in the publication. For the analysis, the authors used a combined target/nontarget screening method based on hydrophilic interaction LC coupled to high-resolution MS.

Broneder et al. (2022) collected information and monitoring data on the fate of melamine in Germany and the Netherlands commissioned by the European Melamine Producers Association (EMPA). The aim of the study was to collect above information and to derive a mass flow of the substances. The authors summarize that data indicate that melamine is detected in all or nearly all surface water samples such as in the rivers Rhine and Maas or in North-Rhine Westphalia. However, according to other monitoring data, melamine is not detected in all surface water samples: Broneder et al. (2022) reflect that according to data from the Norman Empodat data base, melamine was <u>not detected</u> in in 61 out of 673 surface water samples (9.1%) in Germany and <u>not detected</u> in 8 out of 325 surface water samples (2.5 %) in the Netherlands. And according to Kolkman et al. (2021) melamine was not detected in 1 out of 7 samples (14.3%) of the samples in surfaces water sampled in the Netherlands. Broneder et al. concluded: while melamine is frequently detected in German and Dutch surface waters, these findings demonstrate that melamine is not always present in the surface waters of these two countries (above the corresponding LODs) and that melamine is not a ubiquitous substance in surface waters in Germany and the Netherlands.
Concerning findings in groundwater and drinking water, the authors confirm the limited availability of data. However, melamine can be detected in some groundwater bodies and is present in drinking water at different locations (Broneder et al., 2022).

Similar high detection rates above LOQ are reported in the Europe-wide surface water data in the NORMAN Empodat data base[17] . There melamine was detected above LOQ in 958 cases and in 267 below LOQ in 267 cases in 10 countries, including Bulgaria, Croatia, Czech Republic, Germany, Hungary, Netherland, Ukraine, Romania, Serbia, and Slovakia. Thus, even if the statistical analysis may inflate the reported quantitative analysis of Melamine in Neuwald et al. (2022) this does not rebut evidence of a wide environmental distribution of the substance, and clearly shows that wildlife and humans are exposed to Melamine in Europe in a wide spread manner.

Neuwald et al. (2022) analysed 34 selected substances for which they assume these can be regarded as PMT/ vPvM substances. Those 34 substances were analysed in 16 surface water, 16 bank filtrate, 7 groundwater, and 7 raw water samples (n = 46) collected in Germany. The authors applied HILIC chromatography with electrospray ionisation (ESI) of positive polarity with an LOQ of 0.1 µg/L and a LOD of 0.033 µg/L. Melamine was found in a frequency of detection of 63% (29 of 46 samples), whereof 5 samples were below the LOQ.. The calculated median concentration was either 0.12 µg/L (Kaplan−Meier statistical approach) or 0.9 µg/L (according to Hites et al.(2019)). The highest concentration found in groundwater was 5.944 µg/L. The authors further compared the concentrations in surface water and bank filtrate. In raw water from river bank-filtrate concentrations of 4.1807 µg/L were found and melamine concentrations in surface waters up to 4.8411 µg/L. In the majority of cases the sampling sites had local relationships (e.g. being influenced by a common water body) and in some cases a correlation between the values found at the corresponding sampling sites was observed. For melamine, median concentrations in bank filtrate were at least order of magnitude lower than in surface water, but the difference were not shown to be statistically significant. The authors reflect, that a lack of

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[17] Source: EMPODAT database https://www.norman-network.com/nds/empodat/; database assessed 07.11.2022

statistical significance in the difference between surface water and bank filtrate concentration would be the first indication for insufficient removal, which would then have to be confirmed in dedicated studies of connected samples.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 14: 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 %

With all the information available from the various monitoring studies for drinking water and the aquatic environment it is evident, that melamine can be found in surface waters and groundwater bodies already in a wide spread manner. While monitoring studies often can verify the occurrence of the substance, the initial source leading to release into the environment might be identified only in exceptional cases. Several substances are suspect of being a precursor and their degradation may be the reason for findings of melamine.

For example, the applications of the active substance cyromazine might contribute to findings of melamine in the groundwater bodies, whereas the degradation of the substance Hexa(methoxymethyl)melamine (HMMM) in the environment might contribute to findings of melamine in groundwater and surface water as well. HMMM is the final etherification product from reactions of melamine, formaldehyde and methanol. Various studies conclude from findings of HMMM in aquatic samples as a proxy for the amount of particles from tyre wear into the water body. However, HMMM as substance is not registered under REACH, but resins made from HMMM seem to be a quite common for applications such as water-based and solvent-based lacquers.

If and which precursors contributing to the presence of melamine need to be explored in any potential follow-up regulatory processes. With regard to the SVHC assessment the presence of melamine in water bodies , as evidenced by monitoring data, indicates that melamine – irrespective of its origin – enters the aquatic environment and persists there.

3.2.5 Summary and discussion of environmental distribution

The available information on adsorption/desorption behaviour of melamine 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 melamine 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 melamine 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. However, it is not obvious in many cases whether releases of melamine into the environment result from manufacture and application of melamine, or from the release of precursors that are degraded into melamine as a stable transformation.

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 Kow < 5 or log Koc < 5 and log Kaw in the range <-3 tend to be mobile in water. Furthermore, they found that the overall CTD for substances with log Kaw down to around -3 is governed mainly by transport via the atmosphere, but at lower log Kaw 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 melamine 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 ($DegT_{50}$) 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 $DegT_{50}$ 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 15: results of LRTP modelling applying different scenarios

Scenario	DegT50(water) for calculation	DegT50(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': DegT50(water) / DegT50(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) melamine 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 melamine 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.

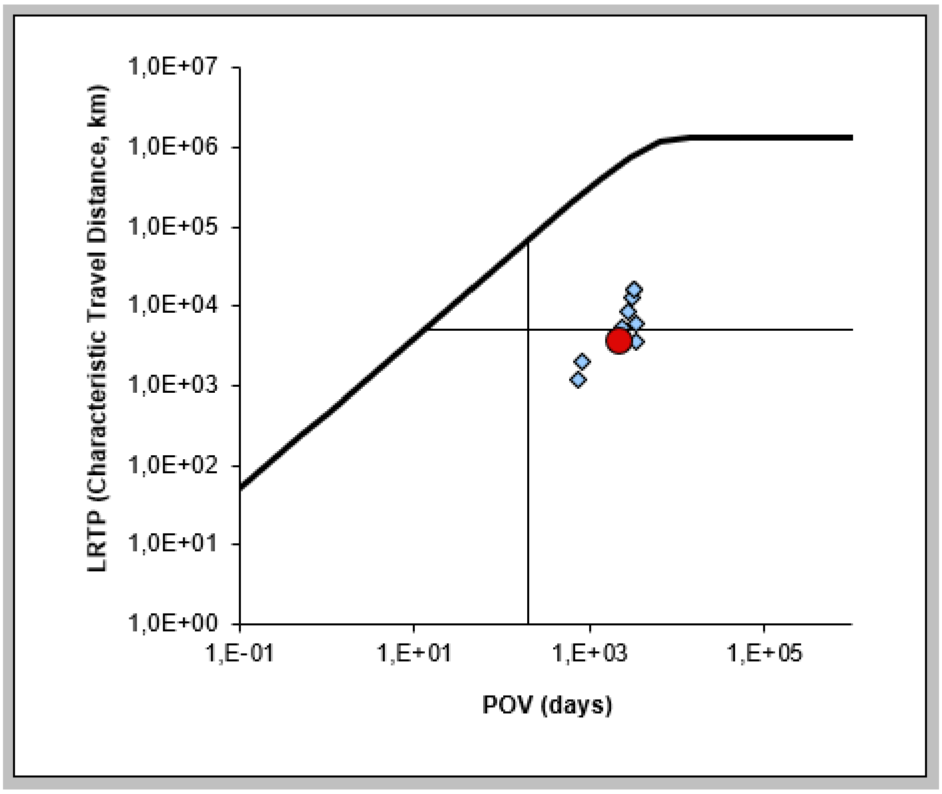

Figure 2: Graphical output for CTD of melamine assuming substance being "not biodegradable"; output in comparison to 'Generic PCB homologues'.

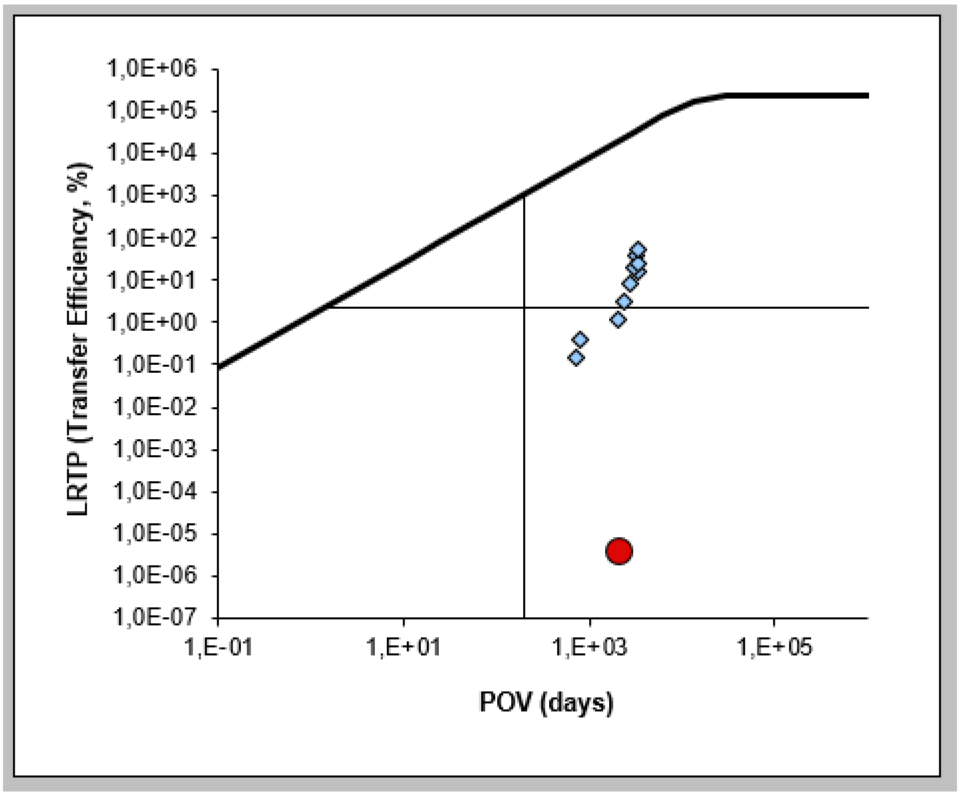

Figure 3: Graphical output for Transfer Efficiency of melamine assuming substance being "not biodegradable"; output in comparison to 'Generic PCB homologues'

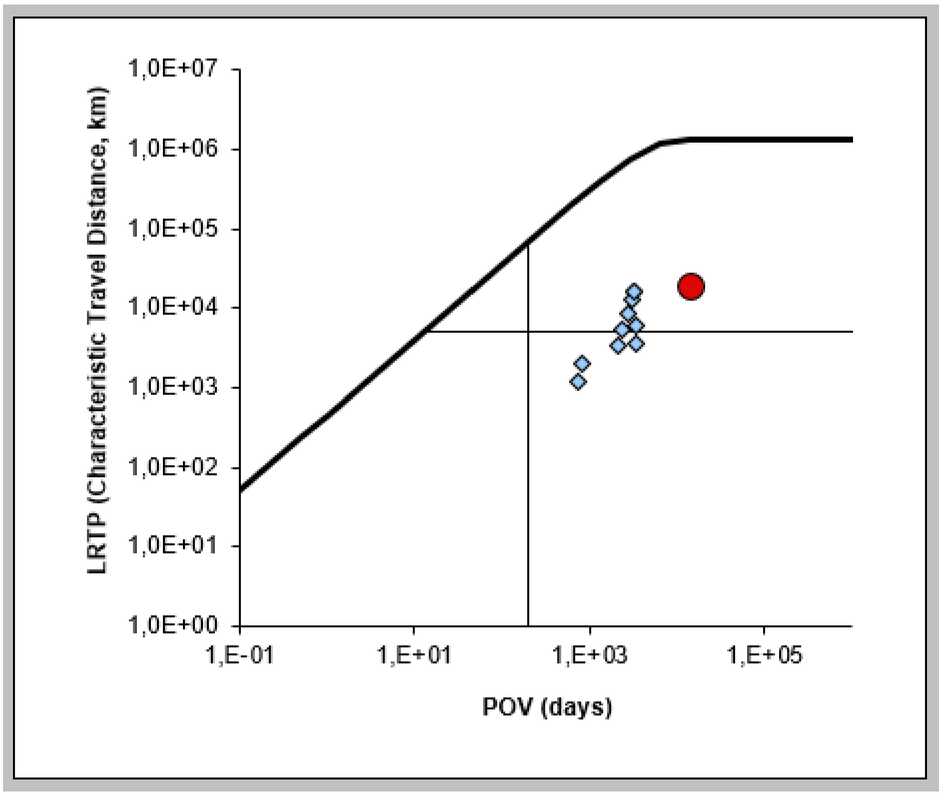

Figure 4: Graphical output for CTD of melamine assuming substance for scenario "upper limit"; output in comparison to 'Generic PCB homologues'.

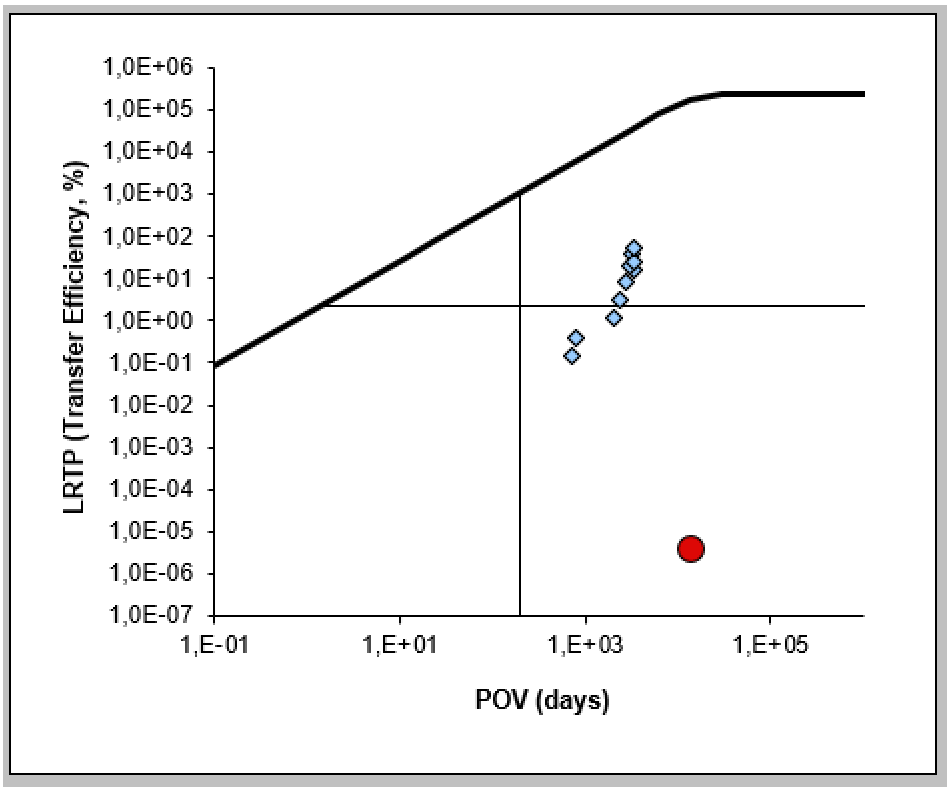

Figure 5: Graphical output for Transfer Efficiency of melamine assuming substance for scenario "upper limit"; output in comparison to 'Generic PCB homologues'

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 melamine'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 website[18].

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 ^{14}C-melamine in *Pimephales promelas,* ^{14}C-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,* ^{14}C-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 (Kow) and air-water (Kaw) on ECHA dissemination site, octanol-air partition coefficient (Koa) for melamine was

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[18] 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 Kow on screening level. According to ECHA R.11, substances with a log Koa ≥ 5 in combination with a log Kow ≥ 2 have the potential to biomagnify, while log Kow below 2 indicates a low biomagnification potential as the substances are quickly eliminated by the urinary excretion.

The calculated log Koa for melamine amounted 11.12, the reported log Kow for melamine is -1.22. Considering the log Koa and log Kow 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 Kow 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 Kow cannot be used as descriptor for assessing aquatic bioaccumulation on screening level according to ECHA R.11. Melamine has a calculated log Koa of 11.12 and a reported log Kow of -1.22, indicating a low potential for terrestrial bioaccumulation. However, as melamine is ionisable under environmentally relevant pH conditions, low Kow 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*m^3/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. The TDI was calculated without consideration of mixture toxicity to cyanuric acid as "the potential of melamine to form crystals is increased by concomitant exposure to cyanuric acid, and therefore the TDI is not appropriate for protection of consumer health in the presence of such concomitant exposure." (EFSA Scientific opinion; EFSA Journal 2010; 8(4):1573).

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 alkalinisation) (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. ammelide and ammeline 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. Furthermore, an assessment regarding endocrine disrupting properties is ongoing.

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 website[19] 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.

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

Table 16: Acute fish tests

Test design	**Effects**	**Comments**[20]	**Source**
O.mykiss (Rainbow trout) Exposure via water, duration 28 d (also examined after 96 h), rainbow trout fingerlings, semi-static, 10 fish per treatment, Conc.: 0, 750, 3000 ppm, no analytics	LC50 (96 h) > 3000 mg/L (n)	Klimisch 2	ECHA dissemination website, report dated 1984
Poecilia reticulata (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
Leuciscus idus (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
Oryzias latipes (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
Poecilia reticulata (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
Leuciscus idus melanotus (Ide) Duration 48 h	LC50 > 500 mg/L	Klimisch 3 (only few details known, short duration)	ECHA dissemination website
Poecilia reticulata (Guppy) Duration 96 h	LC50 > 3000 mg/L	Klimisch 4 (only few details known)	ECHA dissemination website
Clarius batrachus (Walking catfish) Feeding study Body weight: 80 – 140 g, Duration 2 weeks, flow-through, 10 fish per treatment,	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	Purity not specified Klimisch 2	(Pirarat et al., 2012)

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

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	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 - 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: *Oreochromis* spp. (Tilapia), *Oncorhynchus mykiss* (Rainbow trout), *Ictalurus punctatus* (Channel catfish), 6 fish of each species above; *Salmo salar* (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)
O. mykiss (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 site[21] performed with fathead minnow according to OECD TG 210 and duration of 36 d the LOECs for survival

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

and growth (based on reduced length) 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).

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 17: Chronic fish tests

Test design	**Effects**	**Comments** [22]	**Source**
P.promelas (Fathead minnow) Fish early life-stage (FELS) study (OECD TG 210), with GLP Test started with eggs < 4 h old, 100 fertilised eggs per test group, divided in four replicates; flow through, Temp.: 24.5 – 25.4 °C Conc.: 0.618, 1.18, 2.30, 5.25 and 10.1 mg/L melamine (measured) Duration 36 d	Survival and growth (length) sign. decreased: NOEC 5.25 mg/L, LOEC 10.1 mg/L, At 10.1 mg/L two deformed fish, Mortalities appeared in the time after swim-up (day 6) until day 36 (juvenile survival), No effects on time to hatch, hatching success, time to swim-up and larval survival to swim-up. No information for effects on organs was given.	Klimisch 1	ECHA dissemination website, report dated 2015
Jordanella floridae (American flagfish) Long-term toxicity to fish, egg-larval development, no analytical monitoring, semi-static, test started with eggs < 6 h old, Conc.: 100, 180, 320, 560, 1000 mg/L (nom.) Duration: 35 d	Length, weight, hatching, survival, condition: NOEC >= 1000 mg/L (nominal) At 1000 mg/L length and weight were slightly decreased, but not significantly	Klimisch 2	ECHA dissemination website, report dated 1982
O. mykiss (Rainbow trout) Fish, juvenile growth test semi-static test Conc: 750, 1500, 3000 mg/L melamine (nom.) Duration 28 d	Survival and growth (weight) sign. decreased: NOEC 1500 mg/L, LOEC 3000 mg/L (nominal) At 1550 mg/L weight was also reduced (no statistics).	Klimisch 2	ECHA dissemination website, report dated 1984
Oreochromis niloticus (L.) x *O. mossambicus (Peters),* (sex-reversed red tilapia) Based on fish, juvenile growth test, Fingerlings, weight: 5.63 g,	Mortality significantly increased at all doses, LOEC 5 g/kg feed Reduced average body weight, LOEC 5 g/kg feed Reduced specific growth rate (SGR[23]),	Purity 99.5 % Klimisch 2	(Phromkunthong et al., 2013)

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

[23] 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).

25 fish per vessel, three replicates, 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) Duration 8 weeks, Melamine residue measured in a) whole fish b) fillet c) viscera	LOEC 5 g/kg feed Skin discolouration: at 5 g/kg feed (1-10%), at 10 g/kg (10-25 %), at 15 g/kg (>25 % abnormality) Sluggish swimming behavior at 10 g/kg and higher Anorexia at all doses Fin erosion (dose not specified) **Kidney**, **liver** and **gills**: dose dependent histological changes: - enlargement of renal tubules at 10 g/kg feed - vacuolisation of liver hepatocytes at 5 g/kg feed - 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 ≥ 15 g/kg feed; detachment of respiratory epithelium at ≥ 25 g/kg feed (no statistics) 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): 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		
Cromileptes altivelis (Humpback grouper) Juveniles Weight 5.6 g (2 weeks before exposure begin) Dose: 167 mg/kg feed (200 mg melamine + 1 kg pellet), fed 2 times daily ad libitum Two tests: test 1: 20 fish per tank, test 2: 30 fish per tank Duration of both tests: 3 months	At 167 mg/kg feed: 80 % mortality after 3 months exposure in both groups, Mortality increased with exposure time, No mortality in controls Histology: swollen kidneys, necrosis of renal tubules, some of them showed crystal like structures	Purity not specified Klimisch 2	(Mahardika et al., 2017)
Lates calcarifer Bloch (Asian sea bass) Fingerlings (mean weight 8.62 g), Feeding study, Dose: 10 g/kg feed, Duration 12 weeks	At 10 g/kg feed: - No effect on survival - Sign. reduced specific growth rate - Sign. reduced weight (42 % of control fish) - Histology: Kidney: enlargement of renal tubules, degenerated tubular epithelium, pyknotic nuclei, melano-macrophages	Purity 99.5 % Klimisch 2	(Phromkunthong et al., 2015)

Melamine residue in fillet measured Co-exposure melamine and cyanuric acid: Each substance at the doses 2.5, 5, 7.5, 10 g/kg diet	no crystals after melamine only diet Liver: distinctive vacuolisation - Hematology: Erythrocytes, leukocytes, hemoglobin, hematocrit sign. decreased - Antioxidant enzymes: GPx sign. decreased in liver, not in kidney; CAT not affected in both - Melamine content in fillet: 114.3 mg/kg - Crystal formation: Co-exposure melamine and cyanuric acid: At all doses irregular golden-brown or needle-like crystals were found within the lumina of the renal tubules No crystals were found after melamine exposure only		
Pelteobagrus vachelli (Darkbarbel catfish), 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	No effect on mortality Specific growth rate (SGR) sign. decreased at all doses, LOEC 2 g/kg feed Skin: abnormal lightness at 5 and 10 g/kg feed, LOEC 5 g/kg feed, statistically significant decreased content of mela**n**in (pigment in the epidermis) at the same concentrations	Purity 99.5 % Klimisch 2	(Jipeng et al., 2011)
Clarias gariepinus (African catfish) Juveniles (weight 15.1 g), Feeding study Dose 3 g/kg feed (at 3 % of fish body weight), Duration 45 d	At 3 g/kg feed: - Neurotoxicity: Acetylcholinesterase activity in brain sign. decreased - Antioxidant enzymes in brain: SOD, GPx, CAT activities sign. decreased (superoxide dismutase, glutathione peroxidase, catalase) MDA level increased (malondialdehyde, biomarker of lipid peroxidation in cells) - 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)	Purity 99.8 % Klimisch 2	(Iheanacho et al., 2020)
Clarias gariepinus (African catfish) Juveniles (weight 15.1 g), Feeding study at 3 % of body weight Dose 3 g/kg feed, Duration 45 d, followed by depuration period of 30 d	At 3 g/kg feed: - Weight sign. decreased after 45 d - Hematology: Erythrocyte, leukocyte count and hemoglobin sign. decreased PCV (packed cell volume) sign. increased - Antioxidant enzymes: Liver: SOD, GPx activity sign. decreased	Purity 99.8 % Klimisch 2	(Iheanacho et al., 2021)

	Gill: SOD, GPx activity no effect No effects on CAT activity in liver and gills Gills and liver: MDA level sign. increased - Effects on weight, hematology and enzymes resolved during depuration period		
O.mykiss (Rainbow trout) Adult females, Body weight about 350 g and 32 cm length, Feeding study, Doses: 250, 500, 1000 mg/kg feed, feed at 1% bw of fish, 2.5, 5, and 10 mg/kg bw Additional co-exposure to cyanuric acid at the same doses 5 animals per treatment, without replicates Duration 8 weeks	Mortality, growth: no effects No formation of crystal complexes Oxidative stress: ▪ Glutathione peroxidase activity enhanced at 5 and 10 mg/kg bw in the liver ▪ 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 Melamine-cyanurate complexes after co-exposure to cyanurate and melamine at the middle and high doses	Melamine purity 99% Klimisch 3 (not enough animals per treatment, no replicates), however information is plausible	(Pacini et al., 2013)
O.mykiss, Adult females, Body weight about 351 g, total length 32 cm; Feeding study, Doses: 250, 500 and 1000 mg/kg feed, Fed at 1 % of bw per day, 2.5, 5 and 10 mg/kg bw, Duration 10 weeks, Aim of the test was crystal analysis and biochemistry	Oxidative stress: ▪ 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 No crystals were seen in fish exposed to melamine alone	Klimisch 3 (missing information)	(Pacini et al., 2014)
Ictalurus punctatus (Catfish), (warm water species, 23.9 °C) *O.mykiss ,* (Rainbow trout), (cold water species, 12.2 °C) Only co-exposure to melamine and cyanuric acid Exposure 14 d (dose of melamine and cyanuric acid each: 0.5, 1, 2.5, 5, 10, 20 mg/kg bw) Exposure 28 days of catfish (6 animals), (dose of melamine and cyanuric acid each: 0.1 mg/kg bw, comparable to 2.5 ppm of each substance in feed, fed at 4 % of bw per day) Additional sequential co-exposure (not described here)	Co-exposure to melamine and cyanuric acid: No crystals were observed in catfish after co-exposure to melamine and cyanuric acid at 0.1 mg/kg bw after 28 d NOAEL (14 d repeated dose) for both species was 0.5 mg/kg bw co-exposure At 1 mg/kg co-exposure (14 day repeated dose) one third of fish of both species had crystals in the kidney (intensity: single) The intensity of crystals per histological section was dose-dependent and varied in species: in catfish at 2.5 mg/kg few, in trout at 2.5 mg/kg moderate to extensive (trout)	2	(Reimschuessel et al., 2010b)

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 website[24], reports dated 1978 and 1988).

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

Table 18: Acute Daphnia tests

Test design	**Effects**	**Comments[25]**	**Source**
Daphnia magna 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
Daphnia magna 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 website[26] 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

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[24] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/6/2/4

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

[26] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/6/2/5

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 19: Chronic invertebrate tests

Test design	**Effects**	**Comments** [27]	**Source**
Daphnia magna 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
Daphnia magna 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 Daphnia 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.)	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)	Klimisch 3 (part reproduction test, due to less sensitive test design, see column 1) Klimisch 2 (part mortality test)	ECHA dissemination website, report dated 1978
Litopenaeus vannamei (Pacific white shrimp) Feeding study	At 10.1 g/kg feed: Survival, growth and specific growth rate sign. reduced	Purity 99.5 % Klimisch 2	(Nuntapong et al., 2019)

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

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	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		

5.1.3 Algae and aquatic plants

There are two algae studies available on the ECHA dissemination website[28] 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 20: Algae tests

Test design	Effects	Comments[29]	Source
Pseudokirchneriella subcapitata 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
Scenedesmus pannonicus 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

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[28] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15978/6/2/6

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

5.2 Terrestrial compartment

Birds:

Egg laying hens (ISA Brown) were exposed to the substance for 5 weeks (Suchy et al., 2014). The hens were 36 weeks old. There were in the exposure group, as well as in the control group 10 hens. Before the exposure experiment the hens were fed a commercial diet. During exposure the hens received the same diet containing 100 mg Melamine per kg feed. Eggs from each layer were collected each week, in total 30 eggs per week, in order to evaluate total weight, as well as yolk, albumen and shell weight. Furthermore egg shell breaking strength was measured, and haematological and biochemical examination was done. At last tissue samples were collected.

Effects (at 100 mg/kg feed):
- There were no effects on mortality, production of eggs and body weight.

- Distribution of Melamine and cyanuric acid in tissues:
Melamine was detected in several tissues of exposed hens, but not in control hens: Kidneys contained the highest amount (7.43 mg/kg d.w.), followed by breast muscle (3.88 mg/kg d.w.) and liver (3.11 mg/kg d.w.).
Cyanuric acid was found in the liver (6.99 mg/kg d.w.) and kidneys (6.26 mg/kg d.w.) of Melamine exposed hens, but not in control hens. The authors of the study were of the opinion that biotransformation of melamine to cyanuric acid took place primarily in the liver, and elimination proceeded through the kidneys.

- Egg shell weight and egg shell strength were significantly decreased after 3 and 4 weeks. After 5 weeks both values were lower than in control too, but not significantly decreased. Egg yolk weight was significantly increased after 4 and 5 weeks exposure. The total egg weight and albumen weight were increased at week 4 and 5, but not significantly.

- Haematology: The number of erythrocytes increased significantly, but the mean corpuscular haemoglobin concentration (MCH) decreased significantly. No change of other parameters.

Table 21: Test on birds (egg laying hens)

Test design	Effects	Comments[30]	Source
Egg laying hens (ISA Brown), Age 36 weeks, Exposure for 5 weeks, 10 hens in treatment and control group Dosis: 100 mg/kg feed	- Melamine and cyanuric acid were detected in the liver and kidneys, but none of both substances in control animals - significantly decreased egg shell weight and egg shell strength after 3 and 4 weeks, also decreased at week 5, but not significantly. - sign. increased yolk weight at week 4 and 5 - sign. decreased mean	Klimisch 2	(Suchy et al., 2014)

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

	corpuscular haemoglobin concentration (MCH), number of erythrocytes sign. increased		

5.3 Summary and discussion of the environmental hazard assessment

Melamine 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.

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).

In a study with birds (egg laying hens) at 100 mg Melamine/kg feed the egg shell strength and egg shell weight were significantly decreased, the haemoglobin concentration was significantly decreased, whereas the number of erythrocytes was significantly increased.

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, 2017b) 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 melamine 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 DT_{50} 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, melamine is assessed of being of low volatility. It will remain in the water phase.

Information included on the ECHA dissemination website[31] 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 pKb1 of 7.3, non-ionic and ionic forms of the molecule occur under environmental conditions. It has to be considered, that the QSAR calculations normally refer to the non-ionic form of a substance. Claßen (2019) investgated the experimental and calculated log Koc of a cationic substance (Claßen, 2019). The author show that the experimental determined log Koc value for the ionic forms of the model cation was 1.6 log units higher compared to the calculated log Koc for the non-ionic forms of the molecule using EPISuite Software (EPIWEB Version 4.1). Assuming that the same difference in log Koc units applies to the non-ionic and cationic forms of melamine, a log Koc of 3.11 (based on KOCWIN v2.00) - 3.41 (based on experimental data) is estimated based on that study.

Melamine has, therefore, a low tendency to adsorb to organic matter and is expected to remain in the water phase, once the substance emerges in the aquatic environment - regardless whether this results directly from releases related to the life cycle of the substance as such, or if the occurrence of melamine is the result of releases and degradation of precursor substances.

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

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[31] https://echa.europa.eu/registration-dossier/-/registered-dossier/15978/5/5/2

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 emerges in the environment, the substance will be preferentially distributed to the aquatic phase and therefore contaminates unevitebly drinking water ressources.

Due its persistency and mobility, Melamine is difficult to remove from drinking water resources, as, melamine will bypass natural barriers e.g. in river bank filtration {Neuwald, 2022 #89} but also filters with GAC as used in drinking water utilities (Winzenbacher et al., 2015).

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.

Melamine 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 - melamine 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.

In an extended one-generation reproductive toxicity study conducted in rats, 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. Furthermore, an assessment on endocrine disrupting properties for melamine is ongoing.

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 municipal sewage treatment plants dealing with mixed sewage that predominantly originates from households. 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 (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 by the treatment processes in such municipal sewage treatment plants. It is acknowledged that sewage treatment plants that are specifically designed to treat sewage predominantly originating from industrial sites may achieve a higher removal efficiency. Such industrial sewage treatment plants are specifically designed to reflect the local situation and site specific legal requirements. Although such industrial sewage treatment plants may be relevant for melamine, no information is available about removal efficiency in such plants that would allow general conclusions about the removal efficiency to refine the release estimates. It is therefore assumed that manufacture and industrial applications of melamine or its precursors will contribute to distribution of melamine in the environment.

Once released, melamine remains in the aquatic environment due to its persistency and mobility. For the concerns being described for identifying melamine as SVHC according to Article 57 (f) it is irrelevant whether the release to the environment is a direct result from the life cycle of the substance melamine as such, or whether the appearance of melamine in the environment results from the releases and degradation of precursor substances being degraded into melamine.

Due to the high persistency, the amount of melamine present in the environment is expected to increase over time if continuous exposure occurs. In addition, local concentrations may increase temporarily or permanently due to aridity periods e.g. as result of climate change

Melamine is already present in the aquatic environment. The publication of Broneder et al. (2022) reflect that according to data from the Norman Empodat data base, melamine only was detected in in 612 out of 673 samples (91 %) in Germany and detected in 317 out of 325 samples (97.5 %) in the Netherlands. Broeder et al. also concluded: "The available data also shows that although melamine is detected in all or almost all surface water samples such as in the rivers Rhine and Maas or in North-Rhine Westphalia, melamine is not present in all surface water samples (above the corresponding LODs). It can therefore be concluded, that melamine is not a ubiquitous substance in surface waters in Germany and the Netherlands".

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.

In the NORMAN Empodat data base[32] melamine was detected above LOQ in 958 cases and below LOQ in 267 cases in 10 countries, indicating a widespread distribution in the environment.

Due to the high persistency, the amountof melamine present in the environment is expected to increase over time if continuous exposure occurs. In addition, local concentrations may increase temporarily or permanently due to aridity periods e.g. as result of climate change.

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

Melamine is evaluated as a very persistent (section 3.1.1, section 3.1.2 Biodegradation) and very mobile (3.2) substance. Once, melamine emerges in the aquatic compartment and is widely distributed in the environment, it is difficult to be removed from there.

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 difficult to be removed from water with natural (or even conventional) water purification techniques (please see section 3.2.4 Field data).

One report is available which estimates the behaviour of melamine in drinking water purification steps.

Neuwald et al. (2022) found that the median concentrations of melamine in bank filtrate were at least an order of magnitude lower than in surface water, but the differences were not shown to be statistically significant. The authors reflect, that a lack of statistical significance in the difference between surface water and bank filtrate concentration would be the first indication for insufficient removal. Further the authors assessed removal capacities of ozonation and activated carbon (AC) filtration are used as advanced treatment methods and concluded that melamine has limited removal potential through fresh or biologically AC.

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

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[32] Source: https://www.norman-network.com/nds/empodat/; (database assessed 07.11.2022)

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 is a concern as it may cause effects if critical dose levels are exceeded.

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).In an extended one-generation reproductive toxicity study conducted in rats, 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. Furthermore, an assessment on endocrine disrupting properties for melamine is ongoing.

6.3.2.4 Human exposure

As supporting information, ECHA notes that 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.

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 (persistency, mobility and potential for being transported in the water phase over long distances) it is not possible to assess its (local) environmental concentration with sufficient certainty and to consier effect concentration limits for the environment by the means of standardised acute and chronic ecotoxicological tests and the assessment criteria investigated within (in short: no safe concentration limits can be derived).

One aspect that adds to the concern that the effects in the environment might currently be underestimated 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.

In an extended one-generation reproductive toxicity study conducted in rats, 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. Furthermore, an assessment on endocrine disrupting properties for melamine is ongoing. This information can also be of relevance for other environmental animals, and may be connected to yet unknown effects in the environment.

The observed testicular and sperm effects of melamine identified in experimental animals (rats) are considered relevant for identification of probable serious effects on the environment. They can impact reproduction and can have an effect on the population level. Rats are rodents and rodents represent approximately 42% of all mammalian species. Rodents play an important role in the environment, for example in the dispersal of seed and spores, pollination, energy and nutrient cycling, modification of plant succession and species composition. Rodents are a food source for many predators (Witmer and Shiels, 2017).

In an extended one-generation reproductive toxicity study conducted in rats, 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. Furthermore, an assessment on endocrine disrupting properties for melamine is ongoing. This information can also be of relevance for other environmental animals, and may be connected to yet unknown effects in the environment.

In a study with birds effects on egg shell strength and egg shell weight were seen, that both decreased significantly after melamine exposure. These effects are important as they might be relevant for populations. If an egg shell is weakened, the egg may break inside a bird before it is laid and can cause the death of the bird. Once laid, the egg may break while the chick is still developing and cause the death of the chick.

In exposed hens Melamine as well as cyanuric acid were detected in the liver and kidneys. The study authors suggested that Melamine was biotransformed to cyanuric acid.

Hence, the substance properties raise the concern that effects like described above or yet unknown effects could appear in the environment and lead to irreversible 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.

Exposure to humans might occur via consumption and use of contaminated drinking water. 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.

This societal concern is further confirmed by reports that extraction of water from the Rhine and Meuse rivers was stopped due to the presence of melamine (Broneder et al., 2022).

6.3.2.7 Increasing exposure of humans and environmental organisms due to increasing and wide spread presence in the aquatic environment

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 melamine 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 melamine’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 releases of melamine to the environment or its precursors continue, 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 melamine 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 appearance in 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 occurrence 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). 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 environment[33]. 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 during co-exposure with cyanuric acid involves the dose-dependent formation of crystals Crystals or stones composed of melamine and cyanuric acid are highly nepohrotoxic. Thus, effects occur at lower melamine concentrations following co-exposure to melamine and cyanuric acid.

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, ammelide 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 emissions 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.

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[33] https://echa.europa.eu/registration-dossier/-/registered-dossier/15028/3/1/6; https://echa.europa.eu/registration-dossier/-/registered-dossier/14822/3/1/6

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 initial 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 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 melamine 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 experimental studies and QSAR predictions.

The overall weight of evidence shows, that degradability of melamine under environmental conditions is low: 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. Additional studies with cultures of single species of bacteria or in wastewater treatment plants treating industrial effluent indicate that melamine might be degradable under specific conditions however these conditions are considered not representative of either municipal sewage treatment plants or environmental conditions and therefore are not applicable by their own for persistency assessment under REACH, but might be applied for refinement of environmental exposure estimates.

Considering the data on abiotic and biotic degradation, the half-life of melamine in water is >60 days.

Volatility, water solubility, adsorption, distribution in the environment:

Melamine has an experimentally derived log K_{OC} of 1.81 (pH value in experiment not disclosed) indicating a low potential for adsorption on organic matter and clay minerals in the environment. The QSAR estimate for the non-ionic molecule results in a log K_{OC} of 1.51 (KOCwin from EPIsuite was applied). As melamine has a pKb1 (base dissociation constant) of 7.3, non-negligible quantities of non-ionic and ionic forms of the molecule occur under environmentally relevant conditions. For the cationic forms of melamine, a higher log K_{OC}. Thus, for the ionic melamine forms, adsorption on soil constituents such as organic matter or clay minerals is expected to be higher when compared to the non-ionic form.

As the data indicates, that under environmentally relevant conditions ionic and non-ionic molecules can be found in non-negligible quantities at the same time, the log K_{OC} of 1.81 of the non-ionic form is used in the assessment of environmental fate and behaviour of melamine.

The substance 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 physical-chemical substance properties indicate that melamine 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 make 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 tends to remain in the water phase.

Because of melamine's intristic property of high water solubility, low volatility from water and low potential for adsorption, water will be the dominant transport medium in the environment once melamine emerges in the environment. In combination with another intrinsic property, 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.

Distribution of melamine in the environment is influenced by the intrinsic properties of the substance, in addition to the properties and conditions of the environment. Therefore, it is not of relevance for the purpose of SVHC identification whether the initial release into the environment results from manufacture or application processes of melamine as such, or if precursors are released, that are degraded into melamine.

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. Although the exact mechanisms have not been fully elucidated, it is thought that the intrinsic structural properties of melamine allow for hydrogen bonding with urinary uric acid to form a crystalline lattice structure (melamine–uric acid complexes). 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 CLP[34]. This classification confirms that melamine has probable effects for human health.

The observed testicular and sperm effects of melamine in an extended one-generation reproductive toxicity study conducted in rats are additional probable effects of melamine.

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[34] 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.

These effects are serious for human health because they fulfil the criteria for classification (STOT RE, carcinogenicity). According to the RAC opinion, classification as STOT RE is required based on the potential to cause acute and chronic renal diseases in humans. RAC also notes that the mode of action of carcinogenesis established in experimental animals cannot be disregarded as potentially relevant in humans. Thus, melamine fulfils the criteria for classification as a suspected human carcinogen. An additional harmonised classification may be warranted based on the observed effects on reproductive toxicity. These effects are also significant when combined with environmental fate properties leading to irreversible and increasing presence in the environment for the reasons described below.

Ecotoxicity:

The observed testicular and sperm effects of melamine identified in experimental animals are probable effects for all mammals and they are serious because they can have an effect at population level.

There is also scientific evidence of lethal and sub-lethal effects on fish (mortality and growth) and sublethal effects on aquatic invertebrates (mobility, poor condition) and terrestric birds (decreased egg shell strength). These effects are serious because they also can have an effect on population level.

These effects are also significant when combined with environmental fate properties leading to irreversible and increasing presence in the environment for the reasons described below.

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 a potential to cause an irreversible presence in the aquatic environment, together with a widespread contamination of the aquatic environment.

Due to its low tendency for adsorption, melamine 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 municipal sewage treatment plants dealing with mixed sewage that predominantly originates from households. Thus, the overall amount of melamine emitted from production and use is, if at all, only marginally reduced by the treatment processes in such municipal sewage treatment plants.

It is acknowledged that sewage treatment plants that are specifically designed to treat sewage predominantly originating from industrial sites may achieve a higher removal efficiency. Such industrial sewage treatment plants are specifically designed to reflect the local situation and site specific legal requirements. Although such industrial sewage treatment plants may be relevant for melamine, no information is available about removal efficiency in such plants that would allow general conclusions about the removal efficiency to refine the release estimates. It is therefore assumed that manufacture and industrial applications of melamine or its precursors will contribute to distribution of melamine in the environment.

Once released, melamine may remain in the environment for a long time due to its persistency.

Supporting monitoring data already confirmed occurrence of melamine in various rivers (e.g. Rhine, Meuse, Mulde, Danube), where sampling points do not influence each other because as they are located in different river catchment areas. 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.

In the NORMAN Empodat data base[35] melamine was detected above LOQ in 958 cases and below LOQ in 267 cases in 10 countries, indicating a widespread distribution in the environment.

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 intrinsic properties of melamine are likely to cause a transport across water bodies to pristine oceans and groundwaters, raising the concern that melamine might also occur at remote locations from the origin of release.

Melamine stays in the environment even if emissions from manufacture or application of the substance or its precursors have already ceased, as can be concluded from the substance's intrinsic properties and supported by the recurring findings in groundwater samples.

Due to the high persistency, the amount of melamine present in the environment is expected to increase over time if continuous exposure occurs. In addition, local concentrations may increase temporarily or permanently due to aridity periods e.g. as result of climate change.

Decontamination from the aquatic environment and from drinking water resources

Sources of emission in the vicinity are seldom apparent. Once, melamine emerges in the aquatic compartment as direct consequence of its use pattern or as result of the degradation of precursor substances and it is widely distributed in the environment in the follow-up,, it is difficult to be removed from there. This results from the recalcitrance 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 drinking 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.

Increasing exposure of humans and environmental organisms as a result of increasing presence in the aquatic environment and wide distribution

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[35] Source: https://www.norman-network.com/nds/empodat/; (database assessed 07.11.2022)

Melamine is already present in surface waters. Computational models predict that, once occurring in the environment as result of releases that are directly associated with manufacture and use of melamine, or as result of releases and degradation of precursor substances, melamine is able to remain in the environment for several years or maybe even decades and is able to reach remote regions. This is due to melamine's intrinsic property to disperse in the aquatic environment over several thousand kilometres. This means, that detection of melamine and undesirable effects may occur far away from the point of the 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 can be expected to increase over time. This can 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 melamine has probable serious effects, the occurrence of melamine in the environment should be prevented.

Human Health effects:

Melamine is classified as STOT RE 2, H373 (urinary tract), and Carc. 2, H351, and therefore its presence in drinking water is a concern as it may cause effects if critical dose levels are exceeded.

In an extended one-generation reproductive toxicity study conducted in rats, 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. Furthermore, an assessment on endocrine disrupting properties for melamine is ongoing.

Human exposure:

As supporting information, ECHA notes that 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.

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 and potential for being transported in the water phase over long distances) it is not possible to assess its (local) environmental concentration with sufficient certainty and to consider effect concentration limits for the environment by the means of standardised acute and chronic ecotoxicological tests and the assessment criteria investigated within (in short no safe concentration limits can be derived).

One aspects that adds to the concern that the effects in the environment might currently be underestimated are sublethal effects in several species. Although available acute aquatic studies in fish do not show effects on mortality, sub-lethal effects in acute and chronic studies 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.

In an extended one-generation reproductive toxicity study conducted in rats, 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. Furthermore, an assessment on endocrine disrupting properties for melamine is ongoing. This information can also be of relevance for other environmental animals, and may be connected to yet unknown effects in the environment.

In a study with birds effects on egg shell strength and egg shell weight were seen, that were both significantly decreased after melamine exposure (100 mg/kg feed). These effects are important as they might be relevant for populations. In the exposed hens Melamine as well as cyanuric acid were detected in the liver and kidneys. The study authors suggested that Melamine was biotransformed to cyanuric acid. This study on birds is used as supporting information in the assessment of environmental effects of melamine.

Hence, the substance properties raise the concern that effects like decribed above or yet unknown effects could appear in the environment and lead to irreversible population-relevant effects, due to long-term exposure over the whole life and over several generations, continuous exposure via water and potentially increasing concentrations, keeping in mind potential reproductive toxicity and potential endocrine effects.

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 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.

This societal concern is further confirmed by reports that extraction of water from the Rhine and Meuse rivers was stopped due to the presence of melamine.

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. For example 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, which is linked to the formation of highly nephrotoxic melamine–cyanuric acid crystals/stones. 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).

Therefore, appearance of undesirable effects due to additive or even synergistic mode of action cannot be excluded which adds to the concern that no safe concentration can be derived.

Overall evaluation of the concerns and summary with regard to the equivalent level of concern:

The persistency, mobility and toxicity (specific target organ toxicity after repeated exposure and carcinogenicity) and the irreversibility of the contamination of the aquatic compartment compromise the quality of drinking water resources and give rise to the concern of increasing and wide spread 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.

Based on these concerns the level of concern is considered very high in particular due to the combination of the above-mentioned concern which can be clustered into the following concern elements:

- Irreversible, wide spread and increasing presence in the environment, (limited potential for removal of melamine from the aquatic environment and drinking water resources, persistency in the environment, potential to be transported to remote areas and continuous emission due to low degradation in sewage treatment plants),

- Increasing exposure of humans and environmental organisms as a result of the irreversible, wide spread an increasing presence in the environment (increasing contamination of drinking water resources, long-term exposure, exposure of remote areas),
- Concern about the effects observed and other potential effects (e.g., combined effects from concomitant exposure to similar substances)
- The combination of these concern elements results in a potential for:
- Environmental and 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 which are used as a source of drinking water

Conclusion

The combination of melamine's substance properties causes very 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 and toxicity. The combination of persistence and mobility of melamine lead to the difficulty of remediation and water purification. There is scientific evidence that there are probable serious effects for human health and the environment that may occur with increasing concentrations in the environment. These probable serious effects to human health are urinary tract toxicity, carcinogenicity and reproductive toxicity and the probable serious effects to the environment are sub-lethal effects on fish and aquatic invertebrates and reproductive toxicity in rats and other mammals.

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