National Institute for Public Health
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A proposal in accordance with the methodology of the Water Framework Directive

RIVM Letter report 2018-0077

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Colophon

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Synopsis

Water quality standards for melamine
A proposal in accordance with the methodology of the Water Framework Directive

RIVM proposes water quality standards for melamine. Melamine is an industrial compound primarily used for the production of plastics. The substance was found in Dutch surface waters on multiple occasions and the standards can be used to evaluate the environmental risks.

Melamine does not accumulate in fish and exposure of humans or animals via this route is not relevant for the derivation of water quality standards. For direct effects on water organisms RIVM determined a safe concentration of 525 microgram per liter for long term exposure.The proposed standard for short term concentration peaks is 6 milligram per liter. Measured concentrations in Dutch surface waters are well below these levels.

RIVM also derived an indicative quality standard for surface waters intended for drinking water production. This standard of 50 microgram per liter is based on an indicative drinking water limit derived earlier by RIVM. The value is indicative because simultaneous exposure to structure analogues of melamine is not taken into account. RIVM advises to further investigate the risks of co-exposure to melamine and related substances.

Keywords: melamine; water quality standards; Water Framework Directive

Publiekssamenvatting

Waterkwaliteitsnormen voor melamine
Een voorstel volgens de methodiek van de Kaderrichtlijn Water

Het RIVM doet een voorstel voor waterkwaliteitsnormen voor melamine. Melamine is een industriële stof die vooral wordt gebruikt als grondstof voor kunststoffen. De stof is meerdere malen in Nederlands oppervlaktewater gevonden en de normen kunnen worden gebruikt om de risico’s voor het milieu te beoordelen.

Melamine hoopt zich niet op in vis. De blootstelling van mensen of dieren via deze route is niet relevant om de waterkwaliteitsnormen te bepalen. Voor de directe effecten op waterorganismen heeft het RIVM berekend dat een concentratie van 525 microgram per liter veilig is als zij langdurig worden blootgesteld. De voorgestelde norm voor kortdurende concentratiepieken is 6 milligram per liter. De gemeten concentraties in Nederlandse wateren zijn ruim lager dan deze waarden.

Het RIVM heeft ook een indicatieve norm afgeleid voor oppervlaktewater dat wordt gebruikt voor de productie van drinkwater. Deze bedraagt 50 microgram per liter en is gebaseerd op een eerder door het RIVM afgeleide voorlopige richtwaarde voor drinkwater. Het betreft een voorlopige richtwaarde, omdat geen rekening is gehouden met gelijktijdige blootstelling aan stoffen die aan melamine verwant zijn. Het RIVM beveelt aan om uitvoeriger te onderzoeken of melamine en soortgelijke stoffen gelijktijdig voorkomen en wat daarvan de risico’s zijn.

Kernwoorden: melamine; waterkwaliteitsnormen; Kaderrichtlijn water

Contents

Summary

In this report, water quality standards for melamine are derived according to the methodology of the Water Framework Directive. Melamine is frequently found at drinking water intake points in the Netherlands. The Ministry of Infrastructure and Water Management assigned RIVM to derive quality standards for surface water. These can be used in the context of discharge permitting and to evaluate the environmental risks of diffuse emissions of melamine.

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton. It has many industrial applications and is used in consumer and commercial products. Globally, melamine is used primarily in the synthesis of melamine–formaldehyde resins for the manufacture of laminates, plastics, and moulding compounds used for dishware and kitchenware. Melamine is also a metabolite of the insecticide cyromazine.

Because melamine does not accumulate in fish, direct ecotoxicity is the only relevant route for generic surface water quality standards. The proposed freshwater quality standard for long-term exposure, expressed as an annual average concentration (AA-EQS), is 0.525 mg/L, the proposed standard for concentration peaks (MAC-EQS) is 6.0 mg/L. Monitoring information from Dutch surface waters over 2016 indicate that these values are not exceeded.

In addition to the generic water quality standards, a quality standard for surface water for drinking water abstraction is presented, based on the provisional drinking water limit derived earlier by RIVM. This limit of 50 µg/L is indicative because simultaneous exposure to melamine related compounds was not taken into account. The presence of structure analogues may contribute to the toxicity due to the increased potential for formation of urinary crystals. RIVM advises to further investigate the risks associated with the occurrence of melamine and structure analogues.

1 Introduction

1.1 Background of this report

In this report a proposal is made for environmental quality standards (EQSs) for melamine in surface water. The compound is frequently found at drinking water intake points in the Netherlands (RIWA-Maas, 2017; RIWA-Rijn, 2017). A provisional drinking water limit was derived by RIVM in 2016 (Mengelers et al., 2016), but melamine is not included in European or Dutch national legislation in the context of the Water Framework Directive (WFD; (EC, 2000), and surface water quality standards have not been set to date. The Ministry of Infrastructure and Water Management assigned RIVM to derive EQSs for surface water according to the WFD-methodology. These can be used in the context of discharge permitting and to evaluate the environmental risks of diffuse emissions of melamine resulting from e.g., consumer uses.

1.2 Standards considered

Under the WFD, the following types of EQSs are derived to cover both long- and short-term effects resulting from exposure (EC, 2000, 2011):

- Annual Average EQS (AA-EQS) – a long-term standard, expressed as an annual average concentration (AA-EQS) and normally based on chronic toxicity data which should protect the ecosystem against adverse effects resulting from long-term exposure.
- The AA-EQS should not result in risks due to secondary poisoning and/or risks for human health aspects. These aspects are therefore also addressed in the AA-EQS, when triggered by the characteristics of the compound (i.e. human toxicology and/or potential to bioaccumulate). Separate AA-EQSs are derived for the freshwater and saltwater environment.
- Maximum Acceptable Concentration EQS (MAC-EQS) for aquatic ecosystems – the concentration protecting aquatic ecosystems from effects due to short-term exposure or concentration peaks. The MAC-EQS is derived for freshwater and saltwater ecosystems, and is based on direct ecotoxicity only.
- Quality standard for surface water that is used for drinking water abstraction ($QS_{dw, hh}$). This is the concentration in surface water that meets the requirements for use of surface water for drinking water production. The $QS_{dw, hh}$ specifically refers to locations that are used for drinking water abstraction.

Table 1 summarises the different types of WFD-water quality standards.

Table 1. Overview of the different types of WFD-quality standards for freshwater (fw), saltwater (sw) and surface water used for drinking water (dw).

Type of QS	Protection aim	Terminology for temporary standard[1]	Notes	Final selected quality standard
long-term	Water organisms	$QS_{fw, eco}$ $QS_{sw, eco}$	Refers to direct ecotoxicity	lowest water-based QS is selected as $AA\text{-}EQS_{fw}$ and $AA\text{-}EQS_{sw}$
	Predators (secondary poisoning)	$QS_{biota, secpois, fw}$ $QS_{biota, secpois, sw}$	QS for fresh- or saltwater expressed as concentration in biota, converted to corresponding concentration in water	
		$QS_{fw, secpois}$ $QS_{sw, secpois}$		
	Human health (consumption of fishery products)	$QS_{biota, hh food}$	QS for water expressed as concentration in biota, converted to corresponding concentration in water; valid for fresh- and saltwater	
		$QS_{water, hh food}$		
short-term	Water organisms	$MAC\text{-}QS_{fw, eco}$ $MAC\text{-}QS_{sw, eco}$	Refers to direct ecotoxicity; check with $QS_{fw, eco}$ and $QS_{sw, eco}$	$MAC\text{-}EQS_{fw}$ $MAC\text{-}EQS_{sw}$
dw	Human health (drinking water)		Relates to surface water used for abstraction of drinking water	$QS_{dw, hh}$

1: The subscript "fw" refers to the freshwater, "sw" to saltwater; subscript "water" is used for all waters, including marine.

For the purpose of national environmental quality policy, e.g., groundwater assessment or specific policy measures, two additional risk limits are derived:

- Negligible Concentration (NC) – the concentration in fresh- and saltwater at which effects to ecosystems are expected to be negligible and functional properties of ecosystems are safeguarded fully. It defines a safety margin which should exclude combination toxicity. The NC is derived by dividing the AA-EQS by a factor of 100, in line with (VROM, 1999, 2004). The NC for freshwater can be used for groundwater assessment as well.
- Serious Risk Concentration for ecosystems (SRC_{eco}) – the concentration in water at which possibly serious ecotoxicological effects are to be expected. The SRC_{eco} is valid for the freshwater and saltwater compartment, and can also be used for groundwater.

1.3 Methodology

1.3.1 Guidance documents

The methodology is in accordance with the European guidance document for derivation of environmental quality standards under the WFD (EC, 2011). This document is further referred to as the WFD-guidance. Additional guidance on data collection, study evaluation, data treatment and derivation of risk limits that are specific for the Netherlands, such as the NC and SRC, can be found in an RIVM-guidance document (RIVM, 2015).

1.3.2 Data sources

For the derivation of the $QS_{dw,\ hh}$ for surface water at drinking water abstraction points, the recent evaluation by RIVM was used (Mengelers et al., 2016). The provisional drinking water limit from this document is used to derive a provisional $QS_{dw,\ hh}$, and the Tolerable Daily Intake (TDI) is used to evaluate the relevance of human fish consumption (see 3.1). It is noted that future changes in the TDI or drinking water limit may have an effect on these aspects.

Several sources were used to retrieve bioaccumulation and ecotoxicity data. Melamine is registered under the European REACH regulation (EC, 2006), and summaries on aquatic ecotoxicity and bioaccumulation in fish are accessible via the website of the European Chemicals Agency (ECHA, 2018). For this evaluation, original study reports on algae, daphnids and fish included in the REACH dossier were made available to RIVM by the REACH registration holder, OCI Nitrogen, Geleen, the Netherlands. The evaluation of melamine by Environment and Climate Change Canada (ECCC, 2016) was checked for additional relevant data, as was the OECD SIDS evaluation (OECD, 1998a), but the ecotoxicity studies in this latter document are also included in the REACH dossier. In addition, the Draft Assessment Report (DAR) and Competent Authority Report (CAR) prepared for the European evaluation of cyromazine as active substance in plant protection products and biocides were consulted for relevant data on its metabolite melamine (EC, 2007, 2016; EFSA, 2008). The US EPA Ecotox database (US EPA, 2018) was searched for relevant references and a literature search was performed using Scopus®. As most aquatic ecotoxicity studies with melamine concern feeding experiments, this resulted in only one relevant reference (Wang et al., 2011) which is also included in ECCC (2016).

1.3.3 Data evaluation

The studies from the REACH dossier were evaluated according to the procedures of the WFD- and RIVM-guidance. Reliability indices (Ri) were assigned according to Klimisch et al. (1997), taking into account the criteria for reporting and evaluating ecotoxicity data as developed by Moermond et al. (2016). The aquatic ecotoxicity summaries in the DAR contain detailed information on test methods. For studies that were accepted in the DAR, the results were adopted as reliable without restrictions (Ri 1) in case effect percentages could be checked in the summary (e.g., numbers of immobilised daphnids). Reliable with restrictions (Ri 2) was assigned if only the effect value itself (NOEC, EC50, etc.) was given. An exception was made for the older algae studies. The effect values of these studies were not taken over as such, but re-evaluated according to current methods, because the data treatment according to the most recent OECD-guideline (OECD, 2011) is different from the methods used when these studies were performed and reported. Because of the high solubility and expected stability of melamine in water (see 2.3), the absence of analytical verification of test concentrations was considered not a sole reason to reject study results.

1.3.4 Data treatment

According to the WFD-guidance, a single endpoint per species is presented based on the lowest relevant endpoint observed. If multiple reliable values are available for the same species and the same endpoint originating from similar tests, the geometric mean is taken. Unbound values are not used for EQS-derivation, but are included in the tables to show that a particular taxon has been tested. If endpoints are available from multiple tests with different durations, preference is given to the endpoints from tests that followed the minimum test duration as specified in the guideline, e.g., 72 hours for algae, 48 hours for daphnids, 96 hours for fish. If lower effect values are available from test that are shorter than the prescribed duration, the higher values obtained with the minimum prescribed test duration are preferred.

1.4 Status of the results

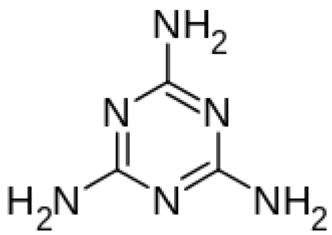
The results presented in this report have been discussed by the members of the Scientific Advisory Group for standard setting for water and air in the Netherlands (*WK-normstelling water en lucht*). It should be noted that the proposed standards in this report are scientifically derived values, based on (eco)toxicological, fate and physico-chemical data. They serve as advisory values for the Dutch Ministry of Infrastructure and Water Management, that is responsible for setting EQSs. The values presented in this report should thus be considered as advisory values that do not have an official status yet.

2 Information on the substance

2.1 Identity

The identity of melamine is summarised in Table 2.

Table 2. Identity of melamine

Name	melamine
Chemical name	1,3,5-triazine-2,4,6-triamine
CAS number	108-78-1
EC number	203-615-4
Molecular formula	$C_3H_6N_6$
Structural formula	
SMILES code	NC1=NC(N)=NC(N)=N1

2.2 Production, use and emissions

2.2.1 Industrial and domestic applications

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton. It is made from dicyandiamide, hydrogen cyanide, or urea. Modern commercial production of melamine typically employs urea as a starting material. Urea is broken down to cyanuric acid, which then can be reacted to form melamine. Melamine has a variety of industrial and domestic uses, the information below is mainly taken from ECCC (2016). It is used in paints and coatings in consumer and commercial products, in foam seating and bedding and it has applications as a plasticiser in concrete and in automobile brake tubes and hoses, in thermally-fused melamine paper and shelves, whiteboards and flakeboards, paints, sealants for mechanical, electrical and plumbing applications, and in inkjet ink. Globally, melamine is used primarily in the synthesis of melamine–formaldehyde resins for the manufacture of laminates, plastics, coatings, commercial filters, glues or adhesives, and moulding compounds (dishware and kitchenware). Melamine resins have a good heat and fire resistance, melamine is used as flame retardant itself or used for the production of other flame-retardants, such as melamine cyanurate, melamine phosphate, melamine polyphosphate, and melamine pyrophosphate. Melamine foam is also used as abrasive cleaner. The foam has a microporous open-cell structure that is very hard, working like extremely fine sandpaper.

According to the REACH-dossier (ECHA, 2018), release to the environment can occur upon manufacturing and formulation of mixtures, and in the production of articles. Other release to the environment is likely to occur from indoor use (e.g., machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), and indoor and outdoor use in long-life materials (e.g., metal, wooden and plastic construction and building materials, flooring, furniture, toys, construction materials, curtains, foot-wear, leather products, paper and cardboard products, electronic equipment).

2.2.2 *Agriculture*

According to WHO (2009), melamine is reportedly used as fertiliser because of its high nitrogen content, but it is not produced for that purpose. Melamine is a major soil metabolite of the insecticide cyromazine, which is approved for use in plant protection products and biocidal products in Europe. In laboratory soil degradation studies with cyromazine, residues of melamine amount to >70% of the applied parent (EC, 2016; EFSA, 2008). Melamine is also a major metabolite of cyromazine in animals and crops (FAO, 2007). Cyromazine is a cyclopropyl derivative of melamine and is used as insect growth regulator. The exact mode of action is unknown, but may be related to interaction with the development hormone, 20-hydroxyecdysone (Van de Wouw et al., 2006). Cyromazine is included in the regular monitoring of Dutch water managers, the Pesticide Atlas[1] shows a trend towards decreasing concentrations over time, with an overall average concentration of 65 ng/L in 2016. Maximum concentrations of melamine at drinking water intake points in 2016 were about 90 times higher (5.8 µg/L, see 2.4). This indicates that agricultural use of cyromazine is most likely not a major contributing factor to the observed concentrations of melamine in surface water.

2.2.3 *Misuse*

In recent years, melamine has become known from scandals with infant formula and petfood. In China, water has been added to raw milk to increase its volume and this dilution decreased the protein concentration in the milk. Companies that use the milk for further production (e.g., powdered infant formula) normally check the protein level through a test measuring nitrogen content. The addition of melamine increases the nitrogen content of the milk and therefore its apparent protein content[2]. The addition of melamine resulted in several deaths and numerous hospitalised infants (WHO, 2008, 2009). These illegal practises have not occurred in the Netherlands and therefore have no relationship with the occurrence of melamine in Dutch surface waters.

2.2.4 *Related compounds*

Cyanuric acid (see Figure 1) may be produced as a by-product in melamine synthesis. It is also found in swimming pool water as the dissociation product of the dichloroisocyanurates used for water disinfection (Tolleson et al., 2009), but these compounds are not approved for biocidal use in Europe. The approved feed additive biuret can also contain impurities such as melamine and cyanuric acid (EFSA, 2010). Ammelide and ammeline are produced as by-products of melamine synthesis or by the microbial degradation of melamine (see 2.3.2). Ammelide is pre-registered under REACH[3]. In the USA, ammeline is used in lubricating greases (Tolleson et al., 2009), but this compound is not registered under REACH.

[1] http://www.bestrijdingsmiddelenatlas.nl/
[2] http://www.who.int/csr/media/faq/QAmelamine/en/
[3] https://echa.europa.eu/substance-information/-/substanceinfo/100.010.416

2.3 Physico-chemical properties, fate and behaviour

Selected physico-chemical and environmental properties of melamine are summarised below.

Table 3. Physico-chemical properties of melamine

Parameter	Value	Reference
Molecular weight	126.12 g/mol	
Water solubility	3230 mg/L (20 °C, exp.[a]) 3480 mg/L (20 °C, pH 7.7, exp.)	US EPA (2002-2012) ECHA (2018)
Dissociation pKa[c] pKa2 pKb1 pKb2	 5.35 5 (25 °C) 5.04 (handbook value) 0 (handbook value) 7.3 (exp.) 5.3 (est.) 8.96 (handbook value) 11.4 (exp.) 14 (handbook value)	(Tolleson et al., 2009) ECCC (2016) ECHA (2018) ECHA (2018) ECCC (2016), ECHA (2018) ECCC (2016) ECHA (2018) ECCC (2016), ECHA (2018) ECHA (2018)
log Kow	-1.37 (exp.) -1.2 (22°C, pH 8, exp., shake flask)	Biobyte (2006), US EPA (2002-2012) ECHA (2018)
Melting point	133 °C (est.) 361 °C (exp.)	US EPA (2002-2012) ECHA (2018)
Boiling point	330 °C (est.)	US EPA (2002-2012)
Vapour pressure	4.79×10^{-8} Pa (20 °C, exp.)	US EPA (2002-2012)
Henry's law constant	1.86×10^{-9} Pa.m^3/mol (exp.)	US EPA (2002-2012)

a: exp. = experimental value
b: est. = estimated value
c: probably pKb (see 2.3.1)

2.3.1 Dissociation

Melamine is an organic base. Contradictory information about the dissociation was obtained from the literature. In one of the entries of the REACH-dossier (ECHA, 2018), it is stated that 'the molecule is neutral in the pH range 6 to 13, it is simple protonated in the range 1 to 4, another reported range is: 0.3 to 3'. EFSA (2010) cites a reference stating that 'below pH 6 melamine is converted from the uncharged free amine form to the melamine ammonium cation', and ECCC (2016) concludes that 'although the empirical and modelling data indicate that melamine exists in both the neutral and ionized forms at environmentally relevant pH, the available weight of evidence suggests that melamine will predominantly exist (>~90%) in the neutral form under typical environmental pH.'

However, estimations with MarvinSketch[4] indicate that pKa's are 1.84 and 8.56. At pH 8.56, the proportion of neutral melamine is 50% and this increases to 100% at pH 11.8. Between pH 1.84 and 8.56, one N-atom in the triazine ring is protonated, below pH 1.84 there are two protonated N-atoms. The dicrepancy may result from a mis-interpretation of the pK-value of 5 being an acid dissociation constant instead of a base dissociation constant. For the present evaluation, it is assumed that at environmentally relevant pH, melamine is present in ionised form. In general, ionised molecules are considered as less toxic because they pass membranes less easily than neutral ones. Exceptions are molecules that are actively transported, e.g., via ion channels, but this is not the case for melamine.

2.3.2 *Degradation, mobility and partitioning*

Melamine is not readily biodegradable (Pagga, 1991; Taeger, 1992a,b), there are some indications that acclimation of microorganisms may occur under continuous exposure in industrial waste water treatment conditions (ECCC, 2016). Tolleson et al. (2009) cite some references demonstrating that melamine can be metabolised by at least two strains of bacteria (*Pseudomonas* strain A and *Klebsiella terragena*) into carbon dioxide and ammonia. The pathway is shown in Figure 1.

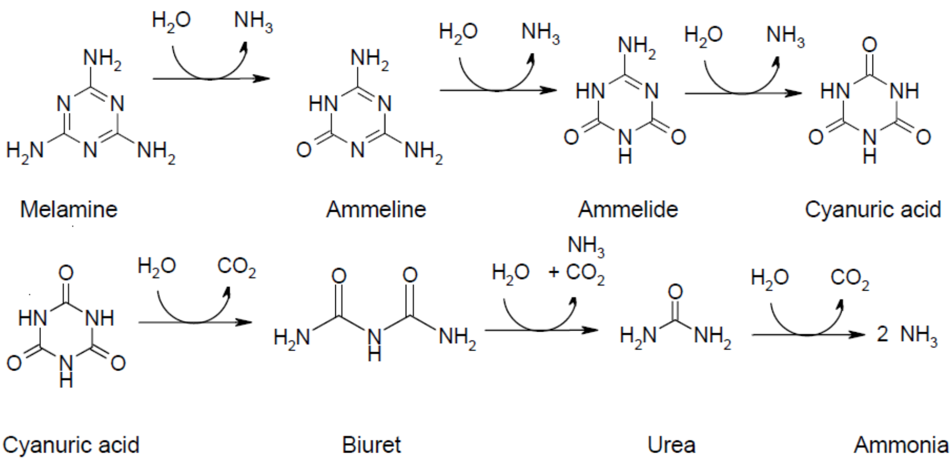


Figure 1. Melamine metabolic breakdown pathway in Pseudomonas strain A and Klebsiella terragena. Taken from Tolleson et al. (2009).

Half-life times for aerobic degradation of melamine in soil in the range of 46-211 days at 20 °C are reported by EFSA (2008). Information on behaviour in water is scarce, the REACH-dossier and OECD SIDS cite a chemical handbook and state that melamine is hydrolysed in mineral acid or inorganic alkali. Hydrolysis proceeds stepwise, with loss of one, two, or all three amino groups, i.e., producing ammeline, ammelide and cyanuric acid (ECHA, 2018; OECD, 1998a), see also Figure 1.

Organic-carbon normalised Freundlich partitioning coefficients of 54 to 423 L/kg (1/n 0.71-0.83) were obtained in sorption studies, sorption is pH dependent (EFSA, 2008). The lower Koc-values 54 and 97 L/kg are

[4] https://chemaxon.com/products/marvin

reported for pH values of 7.3 and 7.5, respectively, higher Koc-values of 154, 371 and 423 L/kg are reported for pH 6.8, 4.2 and 5.9 respectively (pH values determined in H_2O). The higher sorption at low pH may be explained by the affinity for ionised molecules in soils with a higher cation exchange capacity.

Level III fugacity modelling with EpiWin (US EPA, 2002-2012) shows that upon 100% release to water, 99.7% remains in water and 0.34% in sediment, while distribution to soil and air are negligible. If 100% release to soil is assumed, 88.3% is estimated to remain in soil, and 11.6% is distributed to water.

2.4 Environmental concentrations

Melamine is not included in regular monitoring by Dutch water managers, but is monitored by drinking water companies at drinking water intake points along the rivers Rhine and Meuse. In the Rhine area, annual average concentrations in 2016 were 1.36 µg/L at Lobith, 1.33 µg/L at Nieuwegein, and 0.97 µg/L at Andijk. Maximum concentrations were 2.3 µg/L at Lobith in October-November, 2.8 µg/L at Nieuwegein in December and 1.6 µg/L at Andijk in December (RIWA-Rijn, 2017). For the Meuse-area, RIWA-Maas (2017) reports maximum concentrations in 2016 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.

2.5 Classification and hazardous properties

There is no harmonised classification available for melamine. In the REACH registration dossier, melamine is not classified for any hazard (ECHA, 2018). In the inventory for notified classifications (ECHA, 2018) that includes classification by more notifiers than the joint entry of the REACH registration, the majority (613 notifiers) of notifications also submitted no classification for melamine. There are however 26 notifiers that submitted some kind of hazard classification like skin sensitisation, eye irritation, aquatic acute and chronic toxicity and there are also two notifiers that classified for Carc. 2, H351. The latter is a trigger for the derivation of a $QS_{water,\ hh\ food}$ for human fish consumption. Furthermore, melamine is included in the registry of harmonised classification and labelling (CLH) intentions[5]. A future entry on carcinogenicity in Annex VI of the CLP Regulation is proposed by the dossier submitter Germany, but the actual proposal has not been submitted yet and the level that will be proposed for the carcinogenicity classification is unknown. Because of the classification intention for carcinogenicity, melamine is listed as potential substance of very high concern ('potentieel Zeer Zorgwekkende Stof') within the context of the Dutch national substances policy (RIVM, 2018). It is expected that the European CLH process will be finished in 2019. Altogether, derivation of a $QS_{water,\ hh\ food}$ for human fish consumption is considered to be triggered, the relevance of this route is further discussed in section 3.1.

[5] https://echa.europa.eu/nl/registry-current-classification-and-labelling-intentions/-/substance-rev/17834/term

2.6 Human toxicological threshold and drinking water limit

In 2016, RIVM was requested by the Human Environment and Transport Inspectorate (*Inspectie Leefomgeving en Transport*, ILT) to evaluate the risks of melamine in drinking water. This advice is used to determine the relevance of human fish consumption (see 3.1) and for the derivation of the quality standard for surface water that is used for drinking water abstraction ($QS_{dw, hh}$; see 3.6).

In the RIVM-advice, Mengelers et al. (2016) present an overview of previously derived human toxicological limit values which is summarised here. In 2009 and 2010, the World Health Organization (WHO) and the European Food Safety Authority (EFSA) established a tolerable daily intake (TDI) for melamine of 0.2 mg/kg bodyweight per day whichare both based on a dietary 13-weeks study with rats. From this study, EFSA derived a benchmark dose for 10% increase in urinary bladder crystals (BMD10) of 41 mg/kg bw per day with a lower limit of the 90% confidence interval (BMDL10) of 19 mg/kg bw per day. The TDI was derived by applying an assessment factor (AF) of 100 to the latter value (EFSA, 2010). The WHO derived a higher BMDL10 of 35 mg/kg bw per day, but arrived at the same TDI because an AF of 200 was applied, including an AF of 2 to account for sensitivity of children (WHO, 2009). For children of 1 year old and under, the United States Food and Drug Administration (US FDA) decided in 2008 to apply an additional AF of 10 to the earlier established TDI of 0.63 mg/kg bw per day, resulting in a value of 0.063 mg/kg bw per day[6]. The original TDI was also based on the afore mentioned rat study (FDA, 2008). In addtion to the values of WHO, EFSA and US FDA, Mengelers et al. (2016) cite human toxicological reference values from the open literature of 0.008 and 0.13 mg/kg bw per day, based on a BMDL5-value of 16 mg/kg bw per day with an AF of 1000, and a BMDL10 of 38 mg/kg bw per day with an AF of 300, respectively.

None of the above mentioned TDIs was derived in accordance with the latest insights of EFSA on benchmark dose modelling. Therefore, Mengelers et al. (2016) re-analysed the data of the rat-study according to the then available draft EFSA-guidance. The newly derived BMDL10 is 16 mg/kg bw per day, which is not very different from the previously derived values. An AF of 300 was applied to account for within and between species variation (AF 10 x 10) and for study duration (AF 3). The latter value was applied because in a 2-year chronic study with rats other effects were observed, although at a higher dose. The TDI as established by RIVM is 0.05 mg/kg bw per day. In the meantime, the EFSA guidance on benchmark dose modelling has become definitive (EFSA, 2016), but it was confirmed that this would not lead to a different conclusion (pers. comm. W. Slob, RIVM).

Based on the TDI, a provisional drinking water limit of 50 µg/L was derived by Mengelers et al. (2016) for infants and small children as sensitive group. This value is based on a body weight of 10 kg, a daily water intake of 1 L per child per day, and a maximum contribution of drinking water to the TDI of 10% because of the intake of melamine via other sources (e.g.,

[6] https://wayback.archive-it.org/7993/20170111174251/http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm164520.htm

food, food contact materials). This value is also protective for older children and adults. The value is indicated as 'provisional' because it does not account for mixture toxicity due to the presence of structural related compounds, such as ammelide, ammeline, cyanuric acid, melam and melem. Moreover, a literature screening indicated that there would probably be additional information on the toxicity of melamine and structure analogues.

One of the uncertainties in the published risk assessments is the fact that the presence of cyanuric acid may enhance crystal formation. Combined exposure to melamine and cyanuric acid in livestock, fish, pets and laboratory animals showed higher toxicity compared with melamine or cyanuric acid alone (EFSA, 2010; Hau et al., 2009; WHO, 2009). There is limited evidence that ammelide and ammeline can also form crystals with melamine (EFSA, 2010). In the US FDA interim safety/risk assessment of melamine and its analogues in food for humans of 3 October 2008, an additional safety factor of 10 was applied to cover the uncertainty related to combined exposure (FDA, 2008). Later on, a specific risk assessment was performed for infant formula containing solely melamine or cyanuric acid, resulting in the above mentioned TDI of 0.063 mg/kg bw per day[6]. In that risk assessment, melamine and the analogues were assumed to have equal effect. The lack of data to assess the effects of combined exposure was identified as a major uncertainty[7]. EFSA (2010) states that there is too little information to determine a factor by which the toxicity is increased by co-exposure and concludes that the TDI for melamine is not applicable if there is significant concomitant exposure to cyanuric acid, ammelide or ammeline due to the increased potential for formation of urinary crystals (EFSA, 2010).

To cover mixture toxicity, RIVM recommended to set the 'derogation value' for melamine at 5 µg/L[8], and advised to further investigate the actual presence of structure analogues and their toxicity (Mengelers et al., 2016; Versteegh, 2016). According to recent information from the Dutch Watercycle Research Institute (KWR), the presence of structural analogues with potentially similar effects is confirmed with monitoring data. Disclosure of these data and further toxicological review is needed to derive a scientifically based drinking water limit that covers concurrent exposure to melamine and related compounds.
It should be noted that the considerations for mixture toxicity have been made in the context of drinking water policy. The water quality standards for surface water as derived in the present report only concern melamine. The issue of mixture toxicity assessment in the context of the WFD is discussed in Chapter 4.

[7] https://wayback.archive-it.org/7993/20170111174239/http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm174165.htm

[8] The derogation value is a temporary limit that allows for the intake of surface water for drinking water preparation in situations where concentrations are higher than the signalling value of 1 µg/L. The signalling value is an action limit which triggers further investigations into the potential risks for drinking water quality. In practice, the derogation value means that when surface water concentrations of melamine are at or below 5 µg/L intake is allowed, followed by storage and treatment. The implementation and use of the various drinking water thresholds is outside the scope of this report, more information can be found in Van der Aa et al. (2017).

3 Derivation of environmental quality standards

3.1 Relevance of human fish consumption and secondary poisoning

With a log Kow of -1.37, melamine is not expected to accumulate in fish. This is confirmed by BCF-studies with fish, resulting in BCF-values of 0.11 L/kg for *Oncorhynchus mykiss* and 0.32-0.48 L/kg for *Pimephales promelas* (Lech & Szmania, 1984a,b), details can be found in Appendix 1. In view of this, human fish consumption and secondary poisoning are not considered relevant for derivation of the EQS. Moreover, following the calculation method of the WFD-guidance, a $QS_{water,\ hh\ food}$ would be derived which is much higher than the proposed QS for direct ecotoxicity (see 3.5). Using the TDI of 0.05 mg/kg bw per day, a daily fish consumption of 1.6 g/kg bw per day, an allocation factor of 20% and the highest BCF of 0.48 L/kg, the $QS_{water,\ hh\ food}$ would amount to 13 mg/L. It may be argued that a lower allocation factor of 10% should be used, because other sources contribute to the intake of melamine. Even then, the resulting $QS_{water,\ hh\ food}$ of 6 mg/L would be much higher than the QS for direct ecotoxicity as derived in section 3.5. Therefore, direct ecotoxicity is the only route considered.

3.2 Toxicity to aquatic organisms

3.2.1 *Acute toxicity*

Detailed aquatic toxicity data for melamine are tabulated in Appendix 2. Based on the considerations in section 1.3.3, the selected valid acute freshwater ecotoxicity data per species are summarised in Table 4. No data are available for marine species.

Table 4. Accepted acute ecotoxicity data of melamine for freshwater organisms.

Taxon/species	**L(E)C50 [mg/L]**	**Reference**
Bacteria		
Pseudomonas putida	> 1000	Tillmann (1990)
Protozoa		
Tetrahymena pyriformis	1854	Wang et al. (2011)
Tetrahymena thermophila	1000	Li et al. (2015)
Algae		
Scenedesmus pannonicus	2544[a]	Oldersma & Hanstveit (1982)
Pseudokirchneriella subcapitata	>100	EC (2007)
Crustacea		
Daphnia magna	60	EC (2007)
Pisces		
Oncorhynchus mykiss	>3000	Lech (1985)

a: preferred test duration 90.5 h

The lowest acute test result for *Daphnia magna* is the EC50 for immobilisation of 60 mg/L reported in the DAR (EC, 2007). This study was performed according to OECD guideline 202 (OECD, 1984). Much higher effect values for mortality are reported for mortality in older studies: LC50 2176 mg/L by Adema (1978) and LC50 >1000 mg/L by

Frazier (1988). This may seem strange because for *Daphnia* the difference between immobility and mortality is subtle. Immobility as defined in OECD 202 is the inability to swim within 15 seconds after gentle agitation. It is not clear from the older reports how mortality is determined, but it is likely that a less strict criterion is used for mortality assessment. Slight differences in pH could also be a reason for the difference in toxicity, as this may change the proportion of ionised melamine. Moreover, it should be noted that in the studies reporting high LC50s, poor condition of the animals was observed at all test concentrations, including the lowest tested concentration of 56 mg/L. Therefore, the EC50 of 60 mg/L is considered as most relevant for derivation of the MAC-EQS (see 3.4). For *Oncorhynchus mykiss*, 96-hours LC50s of >120 mg/L and >3000 mg/L are available from studies without mortality at the highest tested concentration (EC, 2007; Lech, 1985). Since both studies are reliable, the higher value is selected. This is only relevant for the derivation of the SRC_{eco} (see 3.7). Apart from *O. mykiss*, additional fish species have been tested in acute studies, but too little information was available to determine the reliability of these tests.

3.2.2 *Chronic toxicity*

The selected valid chronic ecotoxicity data for freshwater organisms are summarised in Table 5. No marine data are available.

Table 5. Accepted chronic ecotoxicity data of melamine for freshwater organisms.

Taxon/species	**NOEC or L(E)C10 [mg/L]**	**Reference**
Bacteria		
Pseudomonas putida	> 1000	Tillmann (1990)
Protozoa		
Tetrahymena pyriformis	216	Wang et al. (2011)
Tetrahymena thermophila	250	Li et al. (2015)
Algae		
Scenedesmus pannonicus	601[a]	Oldersma & Hanstveit (1982)
Crustacea		
Daphnia magna	32	Adema (1978); EC (2007)
Pisces		
Jordanella floridae	≥ 1000[b]	Adema (1982)
Oncorhynchus mykiss	750[c]	Lech (1985)
Pimephales promelas	5.25[b]	Salinas et al. (2015)

a: preferred test duration 90.5 h
b: Early Life Stage test
c: 28-days test

For *D. magna*, 21-days NOECs of ≥ 11 mg/L and 32 mg/L are available (Adema, 1978; Salinas & Fabian, 2015). Since both studies are reliable, the higher value is selected. There is a large difference between the results of the ELS-test with *Jordanella floridae* (no effects at 1000 mg/L) and those for *Pimephales promelas* (NOEC 5.25 mg/L). It should be noted that in the latter study the effect percentages for survival and length at the highest test concentration of 10 mg/L, although significant, were <10% (3.1% and 5.9%). Because no higher concentrations were tested, it cannot be judged if this is biological variation. The significant differences are therefore considered as the onset of substance related effects, and the NOEC is set to the second highest test concentration of 5.25 mg/L. The REACH-dossier reports an actual NOEC of 5.1 mg/L, but this is not in accordance with the data in the study report (Salinas et al., 2015).

3.3 Representation of sensitive taxa

Acute and chronic data are available for bacteria, protozoa, and the base set of algae, crustaceans, and fish. The fact that the related compound cyromazine is an insecticide, may trigger the need for data on aquatic insects. On the other hand, in contrast to melamine, cyromazine has a specific structure with a cyclopropyl-group, similar to another insect growth regulator, the veterinary antiparasiticum dicyclanil (see Figure 2).

Figure 2. Structure of the insecticide cyromazine and antiparasiticum dicyclanil.

In a study on the mode of action of cyromazine, Bel et al. (2000) included dicyclanil and cyromazine derivatives with a cyclopropyl group, which may suggest that the authors expected the cyclopropyl moiety to be of importance. The chronic NOEC of cyromazine for *Daphnia magna* is 310 µg/L (EC, 2007, 2016; EFSA, 2008). This is a factor of 100 lower than the NOEC of melamine for *D. magna* (32 mg/L), indicating that the biological activity of the latter is indeed much lower than that of the parent compound.

In the EFSA conclusion on cyromazine (EFSA, 2008), melamine is considered not relevant for groundwater following the guidance document on the assessment of the relevance of metabolites in groundwater (EC, 2003). According to this guidance document, the relevance assessment should include a screening for biological activity, and the declaration of non-relevance suggests that melamine does not have insecticidal properties. However, it is also indicated in the EFSA-conclusion that the pesticidal activity of melamine is unknown (page 33). In a study into the mechanism of melamine-related formation of renal stones, Chen et al. (2012) showed that melamine causes crystal formation in the Malpighian tubes of *Drosophila melanogaster*. However, dietary exposure was applied in this study, and the relevance of this finding for aquatic organisms remains unclear.

As a triazine, melamine is also related to S-triazine herbicides, such as atrazine, simazine, propazine, and metabolites of these substances, which could be a reason to focus on macrophytes as well. However, it is common knowledge that small changes in chemical structure can lead to differences in biological activity. Therefore, the presence of the chloride, isopropyl- or ethyl groups in these compounds make it hard to extrapolate the herbicidal action to melamine. Moreover, as green algae are present in the dataset, this aspect is considered to be covered.

In summary, melamine is structurally related to compounds with insecticidal and/or herbicidal modes of action, but the insecticidal/herbicidal activity of these compounds is most likely related to the presence of specific functional groups. According to the requirements of the WFD-guidance, the dataset is sufficiently representative for the whole ecosystem.

3.4 Derivation of the MAC-EQS

Acute data are available for bacteria, protozoa, and the acute baseset (algae, crustaceans, and fish). The lowest EC50 is 60 mg/L for *Daphnia magna*. According to the WFD-guidance, an assessment factor of 10 can be used on the lowest acute toxicity endpoint if the variation in the dataset is limited and the standard deviation of the log transformed L(E)C50 values is <0.5, or if the compound has a known mode of toxic action and representative species for most sensitive taxonomic group included in dataset (EC, 2011). Because most test results refer to >-values, it is not possible to test the variation in L(E)C50-values, but the >-values indicate that acute toxicity is relatively low. Therefore, an assessment factor of 10 is used, leading to a MAC-$EQS_{fw, eco}$ of 6 mg/L (6000 μg/L).

Because data for marine species are not available, the MAC-$EQS_{sw, eco}$ is derived with an additional assessment factor of 10 and is 0.6 mg/L (600 μg/L).

3.5 Derivation of the AA-EQS

Chronic data are available for bacteria, protozoa, and the chronic baseset (algae, crustaceans, and fish). The lowest NOEC is 5.25 mg/L for *Pimephales promelas*, and the acutely most sensitive species is also tested chronically. An assessment factor of 10 is used on the lowest NOEC-value, resulting in a $QS_{fw, eco}$ of 0.525 mg/L (525 μg/L). Because data for marine species are not available, the $QS_{sw, eco}$ is derived with an additional assessment factor of 10 and is 0.0525 mg/L (52.5 μg/L).

Because direct ecotoxicity is the only relevant route, the AA-EQS_{fw} is 525 μg/L and the AA-EQS_{sw} is 52.5 μg/L.

The proposed AA-EQS_{fw} and AA-EQS_{sw} are almost similar to the corresponding Predicted No Effect Concentrations (PNECs) of 510 and 51 μg/L from the REACH-dossier. The PNECs are based on the same study, but in the present evaluation the critical NOEC is based on the actual concentrations given in the original study report (see 3.2.2).

3.6 Derivation of the $QS_{dw, hh}$

According to the WFD-guidance, the quality standard for surface water intended for drinking water abstraction should be based on existing drinking water standards, where available. In the Netherlands, a provisional drinking water limit of 50 μg/L has been established (see section 2.6). Information on removal efficiency may be taken into account, considering a standard treatment consisting of aeration, coagulation, and/or filtration by sand or active carbon. Because melamine is hydrophylic (log Kow -1.37), not readily biodegradable and not volatile, removal efficiency is expected to be low. Therefore, the provisional $QS_{dw, hh}$ is set to 50 μg/L.

3.7 Derivation of the NC and SRC

The Negligible Concentration is derived as the AA-EQS divided by 100. The NC_{fw} is 5.25 μg/L, the NC_{sw} is 0.525 μg/L.

The Serious Risk Concentration for ecosystems is derived as the geometric mean of the chronic toxicity values when the chronic data set covers the three trophic levels of the base set. The ≥-values are included as such, leading to a SRC_{eco} of 231 mg/L. This value is valid for freshwater and marine waters.

4 Discussion and conclusions

In this report, quality standards for melamine in surface water are derived according to the WFD-methodology. Because melamine does not accumulate in fish, direct ecotoxicity is the only relevant route for the generic surface water quality standards for long term and short term exposure (AA-EQS and MAC-EQS). Ecotoxicity data are available for bacteria, protozoans, algae, crustaceans and fish and there is reasonable certainty that the available data are sufficiently representative for the aquatic ecosystem as a whole. In addition to the generic water quality standards, a specific quality standard for surface water for drinking water abstraction is presented ($QS_{dw, hh}$), based on the provisional drinking water limit derived earlier by RIVM. A summary of the derived values is shown in Table 6.

Table 6. Overview of derived water quality standards for melamine.

Type	**Water type**	**Standard**	**Value [µg/L]**
AA-EQS	freshwater	$AA\text{-}EQS_{fw}$	525
	saltwater	$AA\text{-}EQS_{sw}$	52.5
MAC-EQS	freshwater	$MAC\text{-}EQS_{fw, eco}$	6000
	saltwater	$MAC\text{-}EQS_{sw, eco}$	600
QS_{dw}	drinking water intake points	$QS_{dw, hh}$ (provisional)	50
NC	freshwater	NC_{fw}	5.25
	saltwater	NC_{sw}	0.525
SRC	fresh and saltwater	SRC_{eco}	231

The proposed AA-EQS for freshwater and saltwater are similar to the corresponding PNECs as derived in the REACH-dossier. An initial comparison with monitoring data (section 2.4) indicates that the highest measured concentrations of melamine at drinking water intake points are all well below the proposed AA-EQS of 525 µg/L and the provisional $QS_{dw, hh}$ of 50 µg/L for this compound.

The values in Table 6 refer to melamine only. Drinking water companies indicate that structural related compounds such as melem and melam, ammelide and cyanuric acid may be present as well. Because these compounds may have similar effects on humans or even increase toxicity, an additional safety factor was taken into account when deciding on the derogation limit for drinking water intake. As indicated in section 2.6, further information on occurrence and toxicity of structural analogues is needed to derive a scientifically based drinking water limit that covers the human toxicological risks of melamine and related compounds in combination.

Toxicity of related compounds, including metabolites, is generally not included in the derivation of environmental quality standards according to the WFD, nor in the compliance assessment. An exception are some isomers (e.g., xylenes, trichlorobenzenes) for which the EQS refers to the sum of the individual compounds in that group, or substance groups such as dioxins and dioxin-like compounds for which a toxic unit or toxic equivalence approach is followed.

Information on the ecotoxicological relevance of the above mentioned structure analogues of melamine is limited to cyanuric acid. The OECD SIDS evaluation of cyanuric acid indicates that the toxicity for algae, daphnids and fish is comparable to that of melamine (OECD, 1998b). If there is evidence that the presence of melamine and cyanuric acid is linked (e.g., because they are always emitted or found together) this may be a reason to consider if this should be taken into account in EQS-derivation or compliance check. Monitoring data and a thorough evaluation of the ecotoxicity data would be needed to evaluate this aspect. It should be noted, however, that at present there is no formal decision if, when and how combination toxicity should be implemented in surface water quality assessment under the WFD. This is identified as an important issue to be addressed in the WFD-review and in other substance frameworks, such as REACH (Brack et al., 2017; Van Broekhuizen et al., 2016).

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List of terms and abbreviations

AA-EQS	Annual Average Environmental Quality Standard
AF	Assessment Factor
BCF	Bioconcentration factor
BMD10	Benchmark Dose for 10% incidence increase
BMDL10	lower limit of the 90% confidence interval of the BMD10
CAR	Competent Authority Report
CLH	Harmonised Classification and Labelling
CLP	Classification Labelling and Packaging of substances
DAR	Draft Assessment Report
DEA	desethyl atrazine, metabolite of atrazine
DEDIA	desethyl desisopropyl atrazine, metabolite of atrazine
DIA	desisopropyl atrazine, metabolite of atrazine
DT50	dissipation or degradation half-life time
ECCC	Environment and Climate Change Canada
ECHA	European Chemicals Agency
EC_x	Concentration at which x% effect is observed
EFSA	European Food Safety Authority
EQS	Environmental Quality Standard
ILT	Inspectie Leefomgeving en Transport, Human Environment and Transport Inspectorate
Koc	Organic carbon-water partitioning coefficient
Kow	Octanol-water partitioning coefficient
LCx	Concentration at which x% mortality is observed
MAC-EQS	Maximum Acceptable Concentration for ecosystems
$MAC\text{-}QS_{fw,\ eco}$	Maximum Acceptable Concentration for ecosystems in freshwater
$MAC\text{-}QS_{sw,\ eco}$	Maximum Acceptable Concentration for ecosystems in the saltwater compartment
Marine species	Species that are representative for marine and brackish water environments and that are tested in water with salinity > 0.5 ‰.
NC	Negligible Concentration
NC_{fw}	Negligible Concentration in freshwater
NC_{sw}	Negligible Concentration in saltwater
NOEC	No Observed Effect Concentration
pKb	Dissociation constant (base)
OECD SIDS	Screening Information Dataset activity of the Organization for Economic Cooperation and Development
PNEC	Predicted No Effect Concentration
$QS_{biota,\ hh\ food}$	Quality standard for based on human health expressed as concentration in biota
$QS_{biota,\ secpois,\ fw}$	Quality standard for freshwater based on secondary poisoning expressed as concentration in biota
$QS_{biota,\ secpois,\ sw}$	Quality standard for saltwater based on secondary poisoning expressed as concentration in biota
$QS_{dw,\ hh}$	Quality standard for water used for abstraction of drinking water
$QS_{fw,\ eco}$	Quality standard for freshwater based on ecotoxicological data

$QS_{fw,\ secpois}$	Quality standard for freshwater based on secondary poisoning
$QS_{sw,\ eco}$	Quality standard for saltwater based on ecotoxicological data
$QS_{sw,\ secpois}$	Quality standard for saltwater based on secondary poisoning
$QS_{water,\ hh\ food}$	Quality standard for freshwater and saltwater based on consumption of fish and shellfish by humans
REACH	Registration, Evaluation, Authorisation of Chemicals (Regulation (EC) No 1907/2006)
Ri	Reliability Index
RIVM	Rijksinstituut voor Volksgezondheid en Milieu National Institute for Public Health and the Environment
SRC_{eco}	Serious Risk Concentration for ecosystems
TDI	Tolerable Daily Intake
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
WFD	Water Framework Directive (Directive 2000/60/EC)
WHO	World Health Organization

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Appendix 1. Summary of bioconcentration studies

Legend to column headings	
A	analysis method: liquid scintillation counting
Test type	S = static
Purity	refers to specific purity of 14C-labelled melamine
Test water	dtw = dechlorinated tap water
T	temperature
Conc	exposure concentration
Method	calculation method for BCF = ratio of concentration in organism and water
Ri	Reliability index according to Klimisch et al. (1997). Studies with R1 1 or 2 are acceptable (indicated in bold/shaded)
N	Notes

Species	**Proper-ties**	**A**	**Test type**	**Test compound**	**Purity [%]**	**Test water**	**pH**	**T [°C]**	**Conc. [mg/L**	**Uptake [h]**	**Elim [h]**	**BCF [L/kg]**	**Based on**	**Method**	**Ri**	**N**	**Reference**
Pimephales promelas	2.74 (m); 1.52 (f)	LSC	S	^{14}C-melamine	90-97	dtw	7.0	20.1	0.082	96 h		0.48	viscera	Corg/Cw	**2**	1	Lech & Szmania (1984a)
Pimephales promelas	2.5 (m); 1.43 (f)	LSC	S	^{14}C-melamine	90-97	dtw	7.0	20.1-20.2	0.082	96 h	72 h	0.32	viscera	Corg/Cw	**2**	2	
Oncorhynchus mykiss	6.84 g	LSC	S	^{14}C-melamine	90-97	dtw	7.59	13.9	0.089	72 h		0.11	viscera	Corg/Cw	**2**	3	Lech & Szmania (1984b)
Oncorhynchus mykiss	5.57 g	LSC	S	^{14}C-melamine	90-97	dtw	7.59	13.9-14.0	0.091	64 h	72 h	0.11	viscera	Corg/Cw	**2**	4	

Notes

1 uptake experiment; equilibrium not fully reached after 96 h

2 uptake/elimination experiment; for the uptake phase, kinetics were not determined; depuration half-life 11.5 h for viscera and 14.84 h for muscle with depuration constants of 0.06 and 0.0465 respectively

3 uptake experiment; equilibrium reached after 48 h

4 uptake/elimination experiment; for the uptake phase, kinetics were not determined; depuration half-life 8.06 h for viscera and 6.79 h for muscle with depuration constants of 0.0856 and 0.1015 respectively

Appendix 2. Summary of aquatic toxicity studies

Legend to column headings	
A	test water analysed Y(es)/N(o)
Test type	S = static; R = renewal; CF = continuous flow
Purity	refers to purity of active substance or content of active substance in formulation [%]; ag = analytical grade; tg = technical grade
Test water	am = artificial medium; dtw = dechlorinated tap water; nw = natural water; rw = reconstituted water
Temp	temperature
Crit	criterion (EC50, IC50, LC50)
Ri	Reliability index according to Klimisch et al. (1997). Studies with R1 1 or 2 are acceptable (indicated in bold/shaded)
N	Notes

ACUTE

Species	**Species properties**	**A**	**Test type**	**Purity [%]**	**Test water**	**Hardness $CaCO_3$ [mg/L]**	**pH**	**Temp. [°C]**	**Exp. time**	**Crit.**	**Test endpoint**	**Value [mg/L]**	**Ri**	**N**	**Reference**
Bacteria															
Pseudomonas putida		N	S		am		7.2-7.4	22	30 min	EC50	oxygen consumption	>1000	**2**	1	Tillmann (1990)
Protozoa															
Tetrahymena pyriformis	1e4 cells/mL	N	S	ag	am			28	52 h	IC50	relative generation time	780	3	2	Wang et al. (2009)
Tetrahymena pyriformis	1e4 cells/mL	N	S	ag	am			25	52 h	IC50	relative generation time	1854	**2**	3	Wang et al. (2011)
Tetrahymena thermophila	B2086, 2e5 cells/mL	N	S	ag	am			30	20 h	IC50	proliferation	1000	**2**	4	Li et al. (2015)
Algae															

Species	Species properties	A	Test type	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	Temp. [°C]	Exp. time	Crit.	Test endpoint	Value [mg/L]	Ri	N	Reference
Scenedesmus pannonicus	CCAP276/4a, 1e4 cells/mL	N	S		am				67 h	EC50	growth rate	2415	**2**	5	Oldersma & Hanstveit (1982)
Scenedesmus pannonicus	CCAP276/4a, 1e4 cells/mL	N	S		am				90.5 h	EC50	growth rate	2544	**2**	6	
Pseudokirchneriella subcapitata	22662, 1e4 cells/mL	N	S		am			23	72 h	EC50	growth rate	320-1000	3	7	Drozdowski (1988)
Pseudokirchneriella subcapitata		Y	S	99	am	25.7	8.0-8.5	24.2-24.5	72 h	EC50	growth rate	>100	**2**	8	EC (2007)
Crustacea															
Daphnia magna	<24 h old	N	S		am		8.3-8.5	20	48 h	LC50	mortality	2176	**2**	9	Adema (1978)
Daphnia magna	<24 h old	N	S	>99	nw	206-275	8.4-8.7	19-20	48 h	LC50	mortality	>1000	**2**	11	Frazier (1988)
Daphnia magna	<24 h old	N	S	>99	nw	206-275	8.4-8.7	19-20	48 h	EC50	adverse effects	205	**2**	12	
Daphnia magna	<24 h old	Y	S	99	am	284	7.9-8.1	20	48 h	EC50	immobilisation	60	**1**	13	EC (2007)
Pisces															
Leuciscus idus	5.78 cm,2.5 g		S						48 h	LC50	mortality	>500	4	14	ECHA (2018)
Oncorhynchus mykiss	4 cm, 0.58 g	Y	S	99		120	8.0-8.5	12.2-12.9	96 h	LC50	mortality	>120	**1**	15	EC (2007)
Oncorhynchus mykiss	70-74 mm, 3.5-4.1 g	N	R		dtw		6.3-8.0	12.9	96 h	LC50	mortality	>3000	**2**	16	Lech (1985)
Oryzias latipes									48 h	LC50	mortality	>1000	4	17	ECHA (2018)
Poecilia reticulata	4-5 w old	N	S			210	8	22	96 h	LC50	mortality	>4590	3	18	ECHA (2018)
Poecilia reticulata									96 h	LC50	mortality	>4400	4	19	ECHA (2018)

Notes

1 test according to DIN 38412; concentrations 100, 500, 1000 and 10000 mg/L, control and glucose-free control with 10000 mg/L; oxygen consumption in test vessels 104-133% of control; test solutions of 1000 mg/L and lower reported to be clear, therefore, EC50 is set to >1000 mg/L

2 test concentrations 0.05-5 g/L; generation time of the control is the same as reported in Wang et al 2011, and regression lines of cell density versus times are also the same; reported EC50 is obtained from linear regression of concentration versus relative generation time, but visual inspection of the line shows that the relationship is not linear, therefore the recalculated EC50 based on the reported generation times in Wang et al 2011 is considered more accurate

3 test concentrations 0.05-5 g/L; relative generation time is calculated from the doubling time in the treatment as compared to the control; generation time increased with concentration from 5.7 h in the control to 30 h at 5 g/L; author report IC50 of 820 mg/L, based on linear regression of concentration versus relative generation time; EC50 recalculated by evaluator by non-linear regression of inversed generation time as 1851 mg/L

4 test concentrations 1-4 g/L; significant reduction in cell counts at 1 g/L and higher; EC50 checked by evaluator

5 test medium according to NEN 6506; EC50 calculated by evaluator by non-linear regression of growth rate calculated according to OECD 201 with log-transformed reported cell counts; ≈40% effect at highest test concentration 2000 mg/L, EC50 is extrapolated value; control meets current validity criterion of <35% CV in day-to-day growth rate, <7% CV in controls; test in duplicate, whereas current guidelines prescribe triplicates

6 test medium according to NEN 6506; EC50 calculated by evaluator by non-linear regression of growth rate calculated according to OECD 201 with log-transformed reported cell counts; ≈40% effect at highest test concentration 2000 mg/L, EC50 is extrapolated value; control meets current validity criterion of <35% CV in day-to-day growth rate, <7% CV in controls; test in duplicate, whereas current guidelines prescribe triplicates; DAR and REACH dossier report 96 h EC50 of 940 mg/L as given by authors, most likely based on direct cell counts, and calculation method differs from OECD 201.

7 cell counts <10E4 after 24 h in all flasks; no viable cells at 1000 mg/L after 72 h; exponential growth in control not proven: CV for day-to-day growth rate in control replicates 35.7-47% for days 0-3, and 57.5-77.1% for day 0-4; daily growth rate over day 2-3 (1.23/d) and 3-4 (0.44/d) is lower than average according to OECD 201 (1.5-1.7); authors report EbC50 of 196 mg/L, but ErC50 is preferred; based on data for day 0-3, ErC50 is between 320 and 1000 mg/L; test is not considered reliable because of irregular growth (delay over 0-24 h, lower daily growth rate in control as from day 2)

8 data taken from DAR summary; test according to OECD 201; actual concentrations 93-98% of nominal, results expressed as nominal; cell counts not provided

9 test according to NEN 6501, no control mortality, 100% survival at 180-1000 mg/L, 80 and 65% survival at 1800 and 3200 mg/L, respectively; LC50 is extrapolated value; authors report that except in control, condition of the animals was poor at all concentrations

11 test according to FIFRA guidelines, no control mortality, 100% survival at 56 mg/L, 80-95% survival at 100-1000 mg/L; authors report that except in control, animals are affected at all test concentrations

12 test according to FIFRA guidelines; no control mortality, 100% survival at 56 mg/L, 80-95% survival at 100-1000 mg/L; authors report that except in control, animals are affected at all test concentrations, effects include quiescence, surfacing and/or daphnids tending to the bottom of test vessels; EC50 is calculated by evaluator using reported effect percentages, EC50 reported by author is 200 mg/L

13 data taken from DAR summary which contains details on test methods and immobilisation per concentration; test according to OECD 202; actual concentrations 90-104% of nominal, endpoint based on nominal; concentration-related immobilisation of 5-65% at 12.5-100 mg/L, recalculation of EC50 with reported immobilisation data gives same result

14 data taken from REACH dossier (supporting study 2), no details on test methods; no information on test concentrations; Ri4 in REACH dossier

15 data taken from DAR summary which contains details on test methods; test according to OECD 203; actual concentrations 104-111% of nominal, results expressed as nominal; no mortality in any concentration

16 28-days study, 96 h data included as acute study in REACH dossier (weight of evidence study 7); first renewal after 48 h; no mortality within 96 h, mortality at 3000 mg/L only (30%; 1 fish at day 10, 18, 22), no mortality at other concentrations

17 data taken from REACH dossier (supporting study 6), no details on test methods, value taken from Japanese database; no information on test concentrations; Ri2 in REACH dossier because used by the Japanese authorities but too limited information to assign Ri

18 data taken from REACH summary (supporting study 1); to obtain the highest possible concentration in water, an excess of melamine powder was added and stirred at 60 °C for 24 hours, cooled solution was filtered twice to remove crystals, then dilutions were prepared; test substance concentrations reported as 1.14, 2.31 and 4.59 g/L, not clear if/how analysed; no mortality in any concentration; Ri4 in REACH dossier

19 data taken from REACH dossier (supporting study 3), no details on test methods; no information on test concentrations, reported as saturated melamine solution in water (4.4 g/L); Ri4 in REACH dossier

CHRONIC

Species	Species properties	A	Test type	Purity [%]	Test water	Hardness CaCO3 [mg/L]	pH	Temp. [°C]	Exp. time	Crit.	Test endpoint	Value [mg/L]	Ri	N	Reference
Bacteria															
Pseudomonas putida		N	S		am		7.2-7.4	22	30 min	EC10	oxygen consumption	≥1000	**2**	1	Tillmann (1990)
Protozoa															
Tetrahymena pyriformis	1e4 cells/mL	N	S	ag	am			25	52 h	IC10	relative generation time	216	**2**	2	Wang et al. (2011)
Tetrahymena thermophila	B2086, 2e5 cells/mL	N	S	ag	am			30	20 h	EC10	proliferation	250	**2**	3	Li et al. (2015)
Algae															
Scenedesmus pannonicus	CCAP276/4a, 1e4 cells/mL	N	S		am				67 h	EC10	growth rate	573	**2**	4	Oldersma & Hanstveit (1982)
Scenedesmus pannonicus	CCAP276/4a, 1e4 cells/mL	N	S		am				90.5 h	EC10	growth rate	601	**2**	4	
Pseudokirchneriella subcapitata	strain 22662, 1e4 cells/mL	N	S		am			23	72 h	EC10	growth rate	304	3	5	Drozdowski (1988)
Crustacea															
Daphnia magna	<24 h old	N	S		am		8.3-8.5	20	7 d	NOEC	mortality	32	**2**	6	Adema (1978)
Daphnia magna	<24 h old	N	S		am		8.3-8.5	20	21 d	NOEC	reproduction	32	**2**	7	
Daphnia magna	<24 h old	N	S		am		8.3-8.5	20	21 d	EC10	mortality	32	**2**	8	
Daphnia magna	<24 h old	Y	R	99.9	am	239-254	7.5-8.4	20	21 d	NOEC	reproduction	≥ 11	**1**	9	Salinas & Fabian (2015)
Pisces															
Jordanella floridae	embryos, <6 h old	N	S/R		am	210	7.9-8.6	25	35 d	NOEC	hatching, growth, survival	≥ 1000	**2**	10	Adema (1982)
Oncorhynchus mykiss	70-74 mm, 3.5-4.1 g	N	R		dtw		6.3-8.0	12.9	28 d	NOEC	weight, mortality	750	**2**	11	Lech (1985)
Oncorhynchus mykiss	embryo	N				304	8	15		NOEC	mortality	≥ 1000	3	12	ECHA

Species	Species properties	A	Test type	Purity [%]	Test water	Hardness CaCO3 [mg/L]	pH	Temp. [°C]	Exp. time	Crit.	Test endpoint	Value [mg/L]	Ri	N	Reference
Oncorhynchus mykiss	embryo	N				304	8	15		NOEC	malformations	< 125	3		(2018)
Pimephales promelas	embryos, <4 h old	Y	CF	99.9	dtw	105-118	7.8-8.1	25	36 d	NOEC	length, mortality	5.25	**1**	13	Salinas et al. (2015)

Notes

1 test according to DIN 38412; concentrations 100, 500, 1000 and 10000 mg/L, control and glucose-free control with 10000 mg/L; oxygen consumption in test vessels 104-133% of control; test solutions of 1000 mg/L and lower reported to be clear, therefore, EC10 is set to >1000 mg/L

2 test concentrations 0.05-5 g/L; relative generation time is calculated from the doubling time in the treatment as compared to the control; generation time increased with concentration from 5.7 h in the control to 30 h at 5 g/L; author report IC50 of 820 mg/L, based on linear regression of concentration versus relative generation time; EC10 recalculated by evaluator by non-linear regression of inversed generation time as 216 mg/L

3 test concentrations 1-4 g/L; significant reduction in cell counts at 1 g/L and higher; EC10 calculated by evaluator by non-linear regression of cell counts as 0.25 g/L

4 test medium according to NEN 6506; EC10 calculated by evaluator by non-linear regression of growth rate calculated according to OECD 201 with log-transformed reported cell counts; control meets current validity criterion of <35% CV in day-to-day growth rate, <7% CV in controls; test in duplicate, whereas current guidelines prescribe triplicates

5 cell counts <10E4 after 24 h in all flasks; no viable cells at 1000 mg/L after 72 h; exponential growth in control not proven: CV for day-to-day growth rate in control replicates 35.7-47% for days 0-3, and 57.5-77.1% for day 0-4; daily growth rate over day 2-3 (1.23/d) and 3-4 (0.44/d) is lower than average according to OECD 201 (1.5-1.7). EC10 is estimated by evaluator using non-linear regression of growth rate calculated according to OECD 201 with log-transformed reported cell counts for day 0, 48 and 72 h; test is not considered reliable because of irregular growth (delay over 0-24 h, low daily growth rate as from day 2)

6 test according to NEN 6502, concentrations 0-1800 mg/L; 100% mortality of F0 at 56 mg/L after 7 d, 93-100% survival at 0-32 mg/L

7 test according to NEN 6502, concentrations 0-56 mg/L; 100% mortality of F0 at 56 mg/L after 7 d, 93-100% survival at 0-32 mg/L after 7 d, 88-100% survival of remaining animals over day 7-21 d; reproduction 96-108% of control

8 test according to NEN 6502, concentrations 0-56 mg/L; 100% mortality of F0 at 56 mg/L after 7 d, 93-100% survival at 0-32 mg/L after 7 d, 88-100% survival of remaining animals over day 7-21 d, control corrected survival over whole period is 86-105%; REACH dossier gives NOEC 18 mg/L based on mortality at 32 mg/L, however, control survival is 95% and EC10 is estimated as ≈ 32 mg/L. DAR also gives 32 mg/L as NOEC (p. 485).

9 test according to OECD 211; 5 test concentrations 0.625-10 mg/L; overall time weighted mean 105-111% of nominal, results based on mean measured; 1 dead animal at 1.25 and 10 mg/L, otherwise no mortalities; 160 young/female in control, 144-171 at test concentrations, differences not significant; no other effects; note of evaluator: test concentration 5 mg/L contains one obvious outlier (8 young/female vs 126-185 at other replicates), statistics checked after removal from dataset, conclusions remain the same

10 static test until hatching (day 7), thereafter renewal 3 times per week; feeding with Artemia nauplii; average weight after 35 d at 1000 mg/L was 88% of control, difference not significant

11 first renewal after 48 h, thereafter every 5th day; no significant effect on length, significant effect on end weight at 3000 mg/L, difference in length to weight ratio at 1500 and 3000 mg/L significantly different from 0 and 750 mg/L; 30% mortality at 3000 mg/L (1 fish at day 10, 18, 22), no mortality at other concentrations; authors give 1500 mg/L as NOEC, most likely based on differences in end weight and condition factor, but it

is not clear how condition factor is calculated; ECCC (2016) uses 750 mg/L as NOEC, based on weight loss at 1500 and 3000 mg/L; NOEC of 750 mg/L is used here also.

12 data taken from REACH summary; exposure duration not clear; hatching rate 31 and 33% in control, 34, 31, 24 and 19% at 125, 250, 500 and 1000 mg/L; histological malformations 12% in control, 40, 32, 32 and 90% at 125-1000 mg/L; malformed embryos 3% in control, 13, 6, 8 and 31% at 125-1000 mg/L; no statistical analysis; NOEC mortality 1000 mg/L and NOEC hatching 250 mg/L in REACH (Ri 3); ECCC (2016) reports NOEC mortality 1000 mg/L and LOEC < 125 mg/L based on malformations, but acknowledges that statistical evaluation is absent; control hatching (31-33%) does not meet validity criterion of current OECD 210 (75% hatching); relevance of histological malformations unknown; malformations in embryos do not follow clear concentration response relationship.

13 test according to OECD 210; 5 test concentrations 0.625-10 mg/L; mean measured concentrations 94-105% of nominal; result based on mean measured; significant effect on juvenile survival and overall survival at 10 mg/L (94% vs 97% in control, 3.1% decrease), significant decrease in length (mean 2.8 mm vs 2.9 in control, 5.9% decrease); NOEC in REACH summary given as 5.1 mg/L actual, but in report as 5.0 mg/L nominal or 5.25 mg/L actual

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