Scientific Committee on Health, Environmental and Emerging Risks

SCHEER

Toxicological reference values for certain organic chemicals emitted from squishy toys with regard to adopting limit values under the Toy Safety Directive 2009/48/EC '*Chemicals in squishy toys*'

The SCHEER adopted this document via written procedure on 3 June 2021

ABSTRACT

Following a request from the European Commission, the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) reviewed two reports related to the use of squishy toys published by the Danish Environmental Protection Agency (EPA) and the Swedish Chemical Agency (KEMI). The SCHEER also reviewed the available data on the toxicity of eight organic compounds emitted by squishy toys, as indicated in the Terms of Reference, and derived their toxicological reference values taking into account different routes of exposure and the type of effects (e.g. local vs systemic) by using the weight of evidence approach, in accordance with the SCHEER Memorandum on weight of evidence and uncertainties. Based on realistic exposure scenarios where assumptions were made to mimick children sleeping or playing with squishy toys, SCHEER considered the short-term DNEL values of the emitted chemicals, rather than their TDIs or chronic DNEL values, to be sufficiently protective.

The SCHEER is of the opinion that limiting the risk assessment to the inhalation exposure is not adequate to assess the risk for the children playing with squishy toys. Besides inhalation, the oral route of exposure cannot be excluded, since children, in view of the appearance and smell of this type of toys, might bite or suck on the toys with the possibility of substances released into saliva. In addition, children can ingest parts of squishy toys when they put them into the mouth: besides the possibility of suffocation, occurring when the piece is large enough, the possibility exist that substances are released into the gastro-intestinal tract. Therefore, a risk assessment based on the migration data of chemical content in squishy toys should be considered. Regarding dermal exposure, experimentally measured data reported by the above-mentioned Swedish and Danish report for the compounds under assessment indicates no migration to sweat stimulant. For this reason, the dermal route was considered not relevant for these chemicals. However, the SCHEER is of the opinion that dermal exposure may be relevant for other chemicals, especially for fragrances and other chemicals with irritating or sensitising properties.

The SCHEER does not recommend to apply EU-LCI values as toxicological reference values for inhalation exposure to chemicals from toys in general, since they are derived for construction products on the basis of specific exposure scenarios, which may differ from those to be used when assessing health risks for children playing with toys.

For the eight chemicals, the SCHEER identifies, on the basis of a described general procedure, the related emission limits.

Keywords: organic chemicals, squishy toys, Toy Safety Directive 2009/48/EC, chemicals in squishy toys

Opinion to be cited as: SCHEER (Scientific Committee on Health, Environmental and Emerging Risks), Final opinion on toxicological reference values for certain organic chemicals emitted from squishy toys with regard to adopting limit values under the Toy Safety Directive 2009/48/EC, 'Chemicals in squishy toys', 3 June 2021

ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this opinion. The members of the Working Group are:

The SCHEER members:

Renate Krätke	(Chair)
Teresa Borges	
Peter Hoet	
Theo Samaras	
Emanuela Testai	(Rapporteur)
Theo Vermeire	

All Declarations of Working Group members are available at the following webpage:
https://ec.europa.eu/transparency/regexpert/index.cfm

About the Scientific Committees (2016-2021)

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER). The Scientific Committees review and evaluate relevant scientific data and assess potential risks. Each Committee has top independent scientists from all over the world who are committed to work in the public interest.

In addition, the Commission relies upon the work of other Union bodies, such as the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCHEER

This Committee, on request of Commission services, provides Opinions on questions concerning health, environmental and emerging risks. The Committees addresses questions on:

- health and environmental risks related to pollutants in the environmental media and other biological and physical factors in relation to air quality, water, waste and soils.

- complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health, for example antimicrobial resistance, nanotechnologies, medical devices and physical hazards such as noise and electromagnetic fields.

SCHEER members

Roberto Bertollini, Teresa Borges, Wim de Jong, Pim de Voogt, Raquel Duarte-Davidson, Peter Hoet, Rodica Mariana Ion, Renate Krätke, Demosthenes Panagiotakos, Ana Proykova, Theo Samaras, Marian Scott, Emanuela Testai, Theo Vermeire, Marco Vighi, Sergey Zacharov

Contact:

European Commission
DG Health and Food Safety
Directorate C: Public Health
Unit C2: Health information and integration in all policies
L-2920 Luxembourg
SANTE-SCHEER@ec.europa.eu

PDF ISSN 2467-4559 ISBN 978-92-76-54951-2 doi:10.2875/839032 EW-CA-22-003-EN-N

The Opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The Opinions are published by the European Commission in their original language only.

https://health.ec.europa.eu/scientific-committees_en

TABLE OF CONTENTS

1. MANDATE FROM THE EU COMMISSION SERVICES

1.1 Background

The Toy Safety Directive 2009/48/EC requires that chemicals in toys must not jeopardise the health and safety of children when used in the intended or in a foreseeable way, bearing in mind the behaviour of children.[1, 2]

Investigations of squeezable toys made of polymer foams, such as toy animals, different food products, e.g. ice cream, cakes and fruit, or emojies, revealed that these so-called squishy toys can emit chemicals in quantities that may give rise to concern.[3,4] Risk assessments considered that the risk characterisation ratio (RCR) was exceeded in several instances, sometimes more than 100-fold, and that the toys could thus not be considered as safe.

The risk assessments were prepared using diverging toxicological reference values for the chemicals emitted, thus leading to diverging RCRs. This concerned a number of amines, cyclohexanone, xylenes and dichloromethane as summarised in the following table.

Substance		**Toxicological reference values used for risk assessment, µg/m³**		
Name/Abbreviation	**CAS No**	**1**	**2**	**3**
N,N-dimethylaminoethanol (DMAE)	108-01-0	116	1160	1160
N,N-dimethylformamide (DMF)	68-12-2	80	80	270
Triethylenediamine (TEDA)	280-57-9	24	240	240
Bis(2-(dimethyl-amino)ethyl)ether (DMAEE)	3033-62-3	2	20	20
1,1,4,7,7-pentamethyl-diethylenetriamine (PDT)	3030-47-5	28	280	280
Cyclohexanone (CH)	108-94-1	410	9700	410
Xylenes (X)	1330-20-7	125	250	500
Dichloromethane, methylene chloride (DCM)	75-09-2	100	88000	-

1.2 Terms of reference

The SCHEER is asked:

1. To review the available data on the toxicity of the organic compounds in the above table.

2. To advise on a toxicological reference value for each organic compound in the above table based on the most relevant data, taking into account the reasoning for each

[1] Article 10(2) of the Toy Safety Directive 2009/48/EC. OJ L 170, 30.6.20019, p. 1. https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1568041444770&uri=CELEX:02009L0048-20181126
[2] Annex II, Part III, Point 1 of the Toy Safety Directive.
[3] Danish Environmental Protection Agency (2018) Analysis and risk assessment of fragrances and other organic substances in squishy toys. Survey of chemical substances in consumer products no. 165, August 2018. https://www2.mst.dk/Udgiv/publications/2018/08/978-87-93710-64-1.pdf
[4] Swedish Chemicals Agency (2019) Enforcement of squishies. ENFORCEMENT 6/19.

toxicological reference value, possible additive effects and different routes of exposure. To advise whether EU-LCI values can be applied for long-term inhalation, exposure risk for children with an adjusting assessment factor to be determined.

3. To advise on how to derive limit values for these compounds in squishy toys under the Toy Safety Directive 2009/48/EC, if appropriate taking account of the exposure to these compounds from sources other than toys.

2. OPINION

To address the ToR for this opinion, the SCHEER compiled information on the hazard profile of the eight chemicals listed in the mandate above. The SCHEER weighted the evidence of its assessment according to the five levels reported in the Memorandum on the Weight of Evidence and uncertainties (SCHEER, 2018). The SCHEER concluded the following, answering the three questions of the ToR:

1. To review the available data on the toxicity of the organic compounds in the above table.

The SCHEER, before starting the work, agreed with the Commission to expand the mandate to include the evaluation of the approaches and exposure scenarios used in the reports cited in the mandate, namely the Danish Environmental Protection Agency Report (2018) and the Swedish Chemicals Agency Report (2019).

The SCHEER agrees with the inhalation exposure scenario, as described in the Danish Environmental Protection Agency Report (2018) referred to in the mandate, and with the listed assumptions, although a 50% air change (included in the climatic chamber used for the measurement) is not considered as a realistic worst-case, especially during cold seasons and at night.

The SCHEER is of the opinion that a good example of a conservative scenario regarding inhalation would be that of a 3-year-old child living in a single-family house and sleeping in a room with squishy toys for 10 hours and with a squishy in his/her arms. Although some products are marked with a warning, indicating that squishy toys are not intended for small children (<3years), the target age group has not been always clearly indicated on the package and therefore it could be considered that the product is safe for all age groups. Indeed, it can be expected, that small children would use the squishy toys especially due to their appealing appearance and/or due to the sensory experiences they offer.

The emission values used for all the exposure scenario calculations are assumed immediately after unpacking, which simulate the worst-case situation.

The above-mentioned Danish report and the Swedish Chemicals Agency Report (2019), equally referred to in the mandate, considered the dermal route as not relevant, since no migration into sweat was detectable for the eight chemicals under evaluation. Overall, the SCHEER agrees, but note that the detection of any chemicals is strongly dependent on the sensitivity of the analytical method used. The Limit of Quantification (LoQ) of a method with a relatively poor sensitivity could correspond to a non-negligible amount and therefore as a worst case, it could be appropriate to consider the presence of a migrant at the LoQ level. The SCHEER considers that the dermal route is expected to contribute to a low extent to the total exposure of emitted chemicals, nevertheless fragrances and other chemicals with irritating or sensitising properties should be considered regarding dermal exposure.

The SCHEER is of the opinion that the oral route of exposure also needs to be included when assessing the safety of chemicals from squishy toys. Children, especially the smallest ones, might bite or suck on the toy with the possibility of substances released into saliva due to migration. It can be expected that also children younger than 6 years are attracted by squishies and foreseeably use them although they may not be the target group. A direct extrapolation of migration data obtained with sweat simulants is not possible, since the composition of saliva is different, and the sucking action can increase the migration. In addition, related to the SCHER Final Opinion on Estimates of the amount of toy materials

ingested by children (2016), children can ingest parts of squishy toys when they put them into the mouth (especially, but not limited to, those toys which have the shape, smell and taste of food as well as the ones that have various other shapes). Besides the possibility of suffocation, occurring when the piece is large enough, it is necessary to conduct an exposure assessment, starting from migration data in gastric fluid simulants. In the absence of information on migration in saliva or gastric fluids, it can be assumed as a worst case that 100% of the chemical content is bioavailable. In addition, it will be necessary to use the reference values related to oral exposure, for the risk assessment.

For this reason, the SCHEER considers that limiting the risk assessment to the inhalation exposure is not sufficient for evaluating the actual risk for the children playing with squishy toys. Hence, the oral exposure scenario and related assumptions are also described.
The oral exposure scenarios 'sucking and chewing on the toys' and 'ingestion of small pieces' are based on the following assumptions:

- Body weight of 14 kg for a 3-year-old child and of 20 kg for a child over 6 years
- Ingestion of 100 mg/d
- Data on migration into saliva or gastric fluids content
- Data on chemicals content
- Data on oral absorption (or in the absence a 100% absorption as a default value)
- Reference values related to oral exposure.

According to the RIVM Report on Chemicals in toys (2008)[5], the parameters needed for this scenario are:

- concentration in the product [mg/kg]
- initial leaching rate [g/(cm^2 x min)
- weight of the toy [g]
- density of the individual toy [g/cm^3]
- the surface in contact with the mouth [cm^2]
- duration of contact [min]

Regarding the duration of contact, the mouthing time during the day is highly variable in children as described in the experimental studies reported in the above-mentioned RIVM report. It is therefore recommended to use 3 hours as a default for mouthing duration for children up to 3 years of age.

The SCHEER considers that it is in principle possible to combine the two oral exposure scenarios 'sucking and chewing on the toys' and 'ingestion of small pieces' on the same single day. However, it is not realistic that they could both occur daily for a number of consecutive days. The two scenarios were therefore considered separately, and the most conservative one was chosen to set the migration limits. Emission limits were also derived using the most conservative scenarios.

2. To advise on a toxicological reference value for each organic compound in the above table, based on the most relevant data, taking into account the reasoning for each toxicological reference value, possible additive effects and different routes of exposure. To advise whether EU-LCI values can be applied for long-term inhalation exposure risk for children with an adjusting assessment factor to be determined.

[5] https://www.rivm.nl/bibliotheek/rapporten/320003001.pdf, not all parameters were used in SCHEER calculation.

The most relevant literature data for the eight compounds as indicated in the ToR were collected and revised in order to answer to question 2. The toxicological reference values were derived for the eight chemicals included in the ToR, taking into account the relevant routes of exposure (oral and inhalation) and the type of effects (e.g. local vs systemic) by using a WoE approach in accordance with SCHEER (2018).

The results are summarised in Table 1. Considering that the exposure scenarios are limited in time, the use of short-term reference values (rather than the TDI or Chronic DNEL) are considered sufficiently protective. The only exception is the cancer risk value used for dichloromethane as a non-threshold carcinogen with a mutagenic mode of action.

Table 1: Oral and inhalation DNELs, toxicological endpoints and WoE conclusions

Substance			**Toxicological reference values**			**WoE**
Name	**Abbreviation**	**CAS No**	**$DNEL_{inhalation}$** ($\mu g/m^3$)	**$DNEL_{oral}$** ($mg/kg_{BW}/dy$)	**Toxicological endpoint**	
N,N-dimethylamino-ethanol	DMAE	108-01-0	1160		irritancy	strong
				/		
N,N-dimethylformamide	DMF	68-12-2	800		irritancy	strong
			170		hepatic effects	strong
				2.4	hepatic effects	moderate
Triethylene diamine	TEDA	280-57-9	800		irritancy	strong
				1	kidney effects	moderate
Bis(2-(dimethylamino) ethyl)ether	DMAEE	3033-62-3	20		irritancy	strong
				0.29	irritancy	weak
1,1,4,7,7-pentamethyl-diethylenetriamin	PDT	3030-47-5	283		irritancy	moderate
				0.3	body weight loss	strong
Cyclohexanone	CH	108-94-1	716		liver degeneration	moderate /strong
				2.4	decreased weight gain	strong

Xylenes	X	1330-20-7	130		neurotoxicity	moderate
				0.36	behaviour (hyperactivity)	moderate
Dichloromethane, methylene chloride	DCM	75-09-2	-		carcinogenicity/ [6]	moderate to strong
				0.175	liver changes	moderate

Regarding the use of EU-LCI, the SCHEER considers that they are used to assess VOC indoor emissions after 28 days from a single construction product in a laboratory test chamber procedure as defined in the Technical Specification TS 16516 of the horizontal testing method developed by CEN TC 351/WG 2. The EU-LCI Working Group stressed that the EU-LCI values derived are not to be considered as indoor air quality guidelines but are to be used only in the context of material emission testing.

The SCHEER does not recommend applying the EU-LCI values as toxicological reference values for inhalative exposure to chemicals from toys in general. EU-LCI values are derived for construction products on the basis of specific exposure scenarios that may differ from those to be used when assessing health risks for children playing with toys.

Regarding the risk assessment, the SCHEER considers that if inhalation and oral exposure lead to systemic adverse effects in the same organ/tissue, aggregate exposures should be considered. Regarding combined exposure to different chemicals, the SCHEER considers that total exposure to primary amines should be accounted for, since they are similarly acting chemicals. This can be done considering additivity as the default approach: the effects can be estimated directly from the sum of the doses/concentrations, scaled for relative toxicity (dose/concentration addition). It can be preformed by applying any of the methodologies usually applied for dose addition; for example, the hazard index (HI) approach. The hazard index (HI) is the sum of the hazard quotients (HQ), i.e. the ratios between exposure and the reference value (RV) for each component to be evaluated. When the HI is less than 1, the combined risk is considered acceptable; values higher than 1 would indicate a potential health concern. The reciprocal of the HQ can also be used; the cumulative risk index is the reciprocal of the sum of the HQs. The component-based approach, which is described elsewhere in details for mixture risk assessment (SCHER, SCENIHR, SCCS, 2012; EFSA, 2019), can be applied for any other possible combined exposure. The SCHEER does not support a default mixture assessment factor per substance, replacing the approach described above, but considers that a cumulative effect may occur when a child is exposed to several amines in the squishy toys.

3. To advise on how to derive limit values for these compounds in squishy toys under the Toy Safety Directive 2009/48/EC, if appropriate taking account of the exposure to these compounds from sources other than toys.

[6] Due to carcinogenic effects of DCM no DNEL can be derived for inhalation.

The procedure to derive limit values based on the derived DNELs for the chemicals in squishy toys included in the ToR is described, starting from the exposure scenarios for the oral and the inhalation routes. In addition, in order to take into account the exposure of these compounds from sources other than toys, an allocation factor of 10% of the reference value has been considered for systemic effects, as indicated by SCHER in a previous Opinion (SCHER, 2016). For dichloromethane no DNEL could be derived for inhalation because of the carcinogenic effects of this substance classified as CMR cat 2; H351, for which no threshold could be identified. Therefore, the SCHEER does not calculate an emission limit value via inhalation.

No allocation factor has been used in relation to local effects.
All the parameters and the formulas for calculation are reported in Annex 1.

The SCHEER considers three inhalation scenarios for calculating the emission limits:

- Inhalation Scenario 1
  A 3-year-old child sleeping in a room for 10 h, holding one squishy toy in her/his arms
- Inhalation Scenario 2
  A 6-year-old or older child playing in a room with several squishy toys (n= 40)
- Inhalation Scenario 3
  A 3-year-old child sleeping in a room with several squishy toys around (n=40) and holding a squishy toy

In each of the three scenarios, two different values for air change rate (R) were used: 0.35/h, which is the mean value reported for a child's bedroom in a single-family house and 0.51/h, corresponding to the mean value in a multi-family house (Bornehag et al, 2005).

The complete set of results is reported in Table A1 (see Annex 1), whereas Table 2 shows the emission limit values related to the exposure scenarios considered to be the most conservative ones (worst case), that is the inhalation scenario 3 with the lowest air change rate.

Table 2. Emission limits for exposure via inhalation

Substance			Toxicological reference and emission limit values		
Name	Abbreviation	CAS No	$DNEL_{inhalation}$ ($\mu g/m^3$)	Allocation factor %	Emission limit (mg/hr)
N,N-dimethylamino-ethanol	DMAE	108-01-0	1160	100	0.096
N,N-dimethylformamide	DMF	68-12-2	170	10	0.003
Triethylenediamine	TEDA	280-57-9	800	100	0.066
Bis(2-(dimethylamino) ethyl)ether	DMAEE	3033-62-3	20	100	0.002
1,1,4,7,7-pentamethyl-diethylenetriamine	PDT	3030-47-5	283	100	0.023
Cyclohexanone	CH	108-94-1	716	10	0.014
Xylenes	X	1330-20-7	130	10	0.003

Dichloromethane, methylene chloride (DCM) being classified as Carc. Cat 2 H351, the SCHEER does not propose a limit value. According to Directive 2009/48/EC on the safety of toys, substances that are classified as carcinogenic, mutagenic or toxic for reproduction (CMR) of category 1A, 1B or 2 under Regulation (EC) No 1272/2008 shall not be used in toys, in components of toys or in micro-structurally distinct parts of toys.

A derogation to this rule can be allowed, if the substance is inaccessible to children in any form, including inhalation. The SCHEER does not recommend the application of such a derogation in the case of DCM, since the latter is a volatile compound and children can be exposed to it through inhalation when playing with squishy toys as intended or foreseeable.

Regarding the oral scenarios, all the parameters and the formulas for calculation are reported in Annex 1. The migration limits are related to

- Mouthing: A 3-year-old child sucking and chewing on the toys after putting a toy in his/her mouth
- Ingestion: A 3-year-old child swallowing small pieces of the squishy toy after putting a toy in his/her mouth

are shown in Table 3.

Table 3. Migration limits for exposure via the oral route

Substance			Toxicological reference values		Migration limit values	
Name	Abbreviation	CAS No	$DNEL_{oral}$	Allocation factor	Mouthing	Ingestion
			($mg/kg_{BW}/dy$)	%	($\mu g/cm^2/hr$)	(mg/g)
N,N-dimethylamino-ethanol[a]	DMAE	108-01-0	-	-	-	-
N,N-dimethylformamide	DMF	68-12-2	2.4	10	102.7	33.6
Triethylenediamine	TEDA	280-57-9	1	10	46.7	14.0
Bis(2-(dimethylamino) ethyl)ether	DMAEE	3033-62-3	0.29	100	135.3	40.6
1,1,4,7,7-pentamethyl-diethylenetriamine	PDT	3030-47-5	0.3	10	14.0	4.2
Cyclohexanone	CH	108-94-1	2.4	10	112	33.6
Xylenes	X	1330-20-7	0.36	10	16.8	5.0
Dichloromethane, methylene chloride	DCM	75-09-2	0.175	10	8.2	2.5

[a] No DNEL for the oral route is available, a risk assessment for this route of exposure is not possible

3. MINORITY OPINIONS

None

4. DATA AND METHODOLOGY

Before starting the work, the Working Group decided that it was relevant to address also the following issues to be considered in the Opinion:

- not only to evaluate the different toxicological reference values presented in the table in the mandate but also the approaches and exposure scenarios used in the reports cited in the mandate, namely the Danish Environmental Protection Agency report (2018) and the Swedish Chemicals Agency Report (2019),
- to choose the appropriate reference values and assessment factors for every chemical listed and to recommend a procedure for deriving limit values for chemicals from squishy toys,
- to address all relevant exposure routes.

This interpretation of the ToR was discussed and agreed with the Commission services. The WG has conducted its work in accordance with the SCHEER Memorandum on weight of evidence and uncertainties (SCHEER, 2018).

4.1. Data

In order to select data and assessment factors useful for deriving appropriate health-based values for the chemical listed in the ToR, the Opinions or the evaluation reports of other International/European Agencies, Scientific Committees and Institutions have been consulted and considered as the main source for information. Some original papers, when available as scientific journal publications, were retrieved and analysed.

In addition, the Danish Environmental Protection Agency report (2018) and the Swedish Chemicals Agency Report (2019) on squishy toys were considered as sources of information.

The obtained level of information was considered sufficiently robust and updated, therefore an extensive literature review was not deemed necessary and was not performed.

4.2. Methodology

The methodology used to acquire, process and integrate the data as reported above was to consult the opinions or the evaluation reports of other International/European Agencies, Scientific Committees and Institutions and whenever necessary, the cited original papers when available as scientific journal publications were retrieved and analysed.

The specific criteria (quantity, quality, strength, relevance, etc.) used for critically selecting and evaluating data and scientific information and for attributing a weight to the various lines of evidence in order to determine the existence of risks, and characterise them and to draw conclusions, were those indicated in the SCHEER Memorandum on WoE (SCHEER, 2018). The WoE considerations are listed as a narrative text at the end of each chemical sub-chapter (in Section 6) and summarised in a table format in the Opinion (Section 3).

5. ASSESSMENT

5.1. Exposure to chemicals from squishy toys

The two reports (the Danish Environmental Protection Agency report (2018) and the Swedish Chemicals Agency Report (2019)) present the risk assessment related to the use of squishy toys.

5.1.1 Description of the Danish report

The study carried out for the Danish Environmental Protection Agency in 2018 on 43 different squishies (2-3 of each product) includes a detailed description of type of squishy toys with indication of countries of production and typical sales points, making them available on the Danish market. The report also specifies the most frequent uses (e.g. playing or collecting) by the target population (children of different ages) in order to build real-life exposure scenarios. Regarding the chemical composition, squishies are expected to be made of polyurethane foam (PUR) and since they often have a specific smell, it was concluded that this implies they may potentially contain a number of hazardous chemicals.

Eight squishies were selected for a screening of chemical content and emission to provide an overview of substances to be analysed in the main study. Thirty-five out of 100 substances found in each of the eight squishies were identified with great certainty and are likely coming from the polymer material the squishies are made of (among which dimethylaminoethanol, dimethylformamide, triethyl phosphate, used as a catalyst in the polymerization process).

Volatile organic compounds, carbonyls, and fragrances were identified in the emission analyses of individual samples from the same eight selected squishies.

At the end of the screening study, a number of substances including those with very high emissions or high content levels were selected together with other substances based on the concerns they raise in terms of hazard classification.

The emission to air from all the squishies of the selected substances were detected after 1 hour and after 3 days in a 113 L climatic chamber according to standardised ISO methods for emission from materials at 23°C, 50% relative humidity and an air change of ½ times per hour (0.5 h^{-1}).

Relevant results indicated that samples have totally different emission profiles, with no clear correlation with respect to the content, so that no conclusions can be drawn on the possibility to predict emission based on the initial content. Measured values were used to perform a risk assessment based on identified different exposure scenarios. The report also describes the migration study for the selected chemicals from the squishies into artificial sweat. However, migration was below the detection limit (< LOD).

Exposure scenarios in the Danish Report

Worst-case scenario:
It has been assumed that a 3-year-old child sleeps for 10 hours with a squishy in her/his arms: in these conditions, the child may breathe relatively concentrated vapours and the dermal contact time was assumed to be 10 hours.

Inhalation exposure:
Assuming for a 3 year–old child:

- a body weight of 14 kg as the average of the body weight indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg) based on the 25th percentile
- an inhalation rate during rest/sleep: 0.18 m^3/hour (again mediating data indicated by ECHA and RIVM)

the inhalation exposure is calculated as follows:

- Inhalation (mg/kg /d) = measured chamber concentration mg/m^3 x 0.18 m^3/hour x 10 hours/d/14 kg
- Inhalation (local exposure eyes/respiratory tract, mg/m^3) = measured chamber concentration mg/m^3

Dermal exposure:

Regarding **dermal contact**, the surface area of two palms in contact with the squishy was assumed to be 150 cm^2 (50% of the value obtained by mediating the indications given by RIVM for the two groups of age). Dermal contact and possible migration by sucking was

considered together, since it is assumed that the entire migrating amount from the squishy toy is transferred to both hands, from where the substance is absorbed through skin or may be absorbed by sucking the hands.

The exposure was calculated as follows:

- Skin exposure + sucking = migration/cm^2 during 10 hours x 150 cm^2/14 kg

Since no migration in sweat was detectable for some chemicals that tested as volatile, the dermal route was considered by the Danish report as not relevant.

The Authors also identify a **typical inhalation exposure scenario** for children over 6 years of age, assuming that the child stays in the room for 15 hours with up to 40 squishies collected in the room (exposed mainly by inhalation) including 2 hours of direct skin contact, playing with the squishies daily.

Inhalation exposure:
Assumptions for a child over 6 years of age are:

- Body weight of 20 kg as the average indicated by RIVM and ECHA for two age groups: 3-6 and 6-11 years old
- Child's Room, volume: 17.4 m^3 (corresponding to a floor space of 7 m^2), as established by the Danish EPA
- Air change: 0.5 times per hour (corresponding to 8.7 m^3 per hour)
- Child's inhalation volume per day: 12.5 m^3 (the average of values indicated by RIVM for two age groups: 3- and 6-11 years old)
- Surface area of two palms in contact with the squishy: 230 cm^2

Values obtained with a single squishy toy in the small climatic chamber (with ventilation rate of 0.5 times per hour) with volume of 0.113 m^3 have been scaled up to a "standard" child's room of 17.4 m^3 (with ventilation rate of 0.5 times per hour as well).

- Inhalation (mg/kg bw/d) = calculated room concentration mg/m^3 x 12,5 m^3/day/20 kg
- Inhalation (local exposure eyes/respiratory tract, mg/m^3) = calculated room concentration mg/m^3

Dermal exposure:
In the typical exposure scenario, the dermal exposure was assumed to be:

- Skin exposure + sucking (mg/kg/d) = migration/cm^2 during 2 hours x 214 cm^2/20 kg

The palm surface in the calculation was 214 cm^2 instead of 230 cm^2, as indicated in the assumptions.
Since no migration in sweat was detectable, the dermal route was considered by the Author's reports as not relevant.

Risk assessment in the Danish Report

The report identified a RCR (exposure/$DNEL_{inhalation}$) >1 for seven of the emitted substances both in the typical scenario and the worst case scenario for systemic effects, but local effects (e.g. irritation) are considered as well.

5.1.2 Description of the Swedish report

The methodology described and followed was quite similar to the one described in the Danish report and is therefore not reported in detail here, only relevant differences have been highlighted.

The Report from KEMI described a study in which the total content and emissions to air of the seven chemical substances identified by the Danish EPA as posing a risk for eye and respiratory irritation in children were measured in 21 samples of squishy toys. The measurements (µg/m^3) were made in the emission chamber at one hour and 72 hours after the toys were removed from their packaging and placed in the chamber. The test condition inside the chamber conditions were reported as follows:

Chamber volume	not reported
Temperature	23 ± 0.5 °C
Relative humidity	50 ± 2% RF
Air change	0.68 times/hour
Unit specific airflow	0.021 m^3/unit hour
Air velocity at sample plot	0.1 – 0.3 m/s

Experimental conditions resemble the ones used in the Danish study as described above, with two exceptions: the volume of the chamber was not properly indicated (a volume of 0.00 m^3 was reported) and the air change in the chamber was 0.68 vs 0.5 times per hour used by the Danish EPA.
The other differences are related to the exposure scenarios.

Exposure scenarios in the Swedish Report

Two different exposure scenarios for children were used, similar to the ones described above for the report by the Danish EPA:

- Worst-case scenario: A child holding a squishy close to the eyes and airways. The concentration in the test chamber was used as a proxy for the concentration a child might inhale when in close contact with the squishy during sleeping or hugging a squishy. The inhalation rate, which was used in the Danish exposure calculation, was not included in the Swedish exposure scenario.

- Typical Exposure Scenario: A child playing in a room where 42 different squishies are present (instead of 40 as in the Danish Report). The reference room has a floor area of 7 m^2, a volume of 17.4 m^3 and an air change in the room of 0.5 h^{-1}, exactly as the one considered in the Danish EPA Report, but again the inhalation rate was not included.

5.1.3 Considerations of the SCHEER related to the Reports

Comments regarding exposure scenarios for inhalation

The methodology used and described in both reports for the identification and quantification

of emitted chemicals from squishy toys is considered acceptable.

The SCHEER agrees that the chosen scenarios are realistic, as is the consideration of 3-year-old children, for the following reasons:

The target age group has not been always clearly indicated on the squishy toys label. Therefore, it may be expected that the product is safe for all age groups unless the products are marked with a warning, indicating that squishy toys are not intended for small children. However, it can be expected that small children would also use the squishy toys, especially due to their appealing appearance and/or due to the sensory experiences they offer.

The assumptions for inhalation exposure scenarios and calculation as described in the Danish Report are endorsed by the SCHEER, since the inhalation rate is included in the calculation, differently from the Swedish approach and this is relevant especially for systemic exposure.

The only SCHEER comment has to do with the 50% air change (which, as a standard value, is included in the climatic chamber used for the measurement and then also used as the estimate for a typical child's room). The SCHEER did not consider it as the worst case, especially during cold seasons and at night. This consideration is supported by literature data (Bornehag *et al*., 2005; Strom-Tejsen, 2016, and for this reason, SCHEER used also a lower air change rate (see Annex 1) to calculate emission limits.

Overall, the inhalation exposure scenarios are considered sufficiently conservative to protect children's health, because the emissions used for all the calculations are measured immediately after unpacking the toys, whereas squishies that have been in use for a while generally emit lower concentrations.

Using different measurement times for emission provides information on the extent of emission and a rough idea of emission patterns during the first days after unpacking. Emission is generally reduced over time, with amines released from the material more slowly when compared to VOCs. The diffusion rate of a substance varies depending on the physico-chemical features of the molecule and the nature of interaction of the material in which it is present. Chemicals localised inside the polymer material are expected to be slow-emitting substances, while substances added after termination of the foam formation (e.g. fragrance, colourants in solvent) are not trapped inside in the polymer matrix and are, therefore, able to emit faster.

However, having only two time points cannot give an indication about the kinetics of emission. This information can be very useful in terms of recommending measures already adopted for other toys for reducing risks due to inhalation by knowing the curve of decay of the emission, as a risk mitigation measure it could be recommended on the label to air out toys for a certain period of time after unpacking and before use.

Comments regarding dermal exposure scenarios

The SCHEER agrees that for 7 of the 8 chemicals tested the dermal exposure is not relevant, due to the absence of experimentally measured migration in sweat simulants. However, the SCHEER noted that the detection of any chemicals is strongly dependent on the sensitivity of the analytical method used. The LoQ of methods that are not highly sensitive could correspond to a non-negligible amount and therefore, as a worst case, it could be appropriate to consider the presence of migrant chemicals at the LoQ level.

Overall, the SCHEER considers the dermal route as not relevant for the 8 chemicals evaluated in this specific study, However, fragrances and other chemicals with irritating or sensitising properties should be considered regarding dermal exposure.

Comments regarding oral exposure scenarios

In the risk assessments performed, only the inhalation and dermal routes are taken into account. This limitation is shared by the two studies, although it is mentioned by Kemi that: *Squishies should not be given to small children who might bite or suck on the toy, as there is a risk that small pieces of the squishy get dislodged and caught in their throat, which might lead to suffocation*.

The SCHEER is of the opinion that the oral route of exposure needs to be included when assessing the safety of chemicals from squishy toys. Indeed, children, especially the ones younger than 6 years old, might bite, chew or suck on the toy with the possibility of a release of substances into saliva due to migration. It can be expected that children younger than 6 years are also attracted by squishies and use them, even if they are not the target group.

A direct extrapolation of migration data obtained with sweat simulants is not possible, since the composition of saliva is different, and the sucking action can increase the migration. In addition, related to the SCHER Final Opinion on Estimates of the amount of toy materials ingested by children (2016), children can ingest parts of squishy toys when they put them into the mouth and bite (especially, but not limited to, those toys which have the shape, smell and taste of food as well as the ones which have various other shapes). Besides the possibility of suffocation, occurring when the piece is large enough, it is necessary to conduct a risk assessment, starting from migration data in gastric fluids simulants. In the absence of information on migration in saliva or gastric fluids it can be assumed as a worst case that 100% of the chemical content is bioavailable; in addition, it will be necessary to use the reference values related to oral exposure.

For this reason, the SCHEER considers that limiting the risk assessment to the inhalation exposure is not adequate for evaluating the actual risk for the children playing with squishy toys.

The oral exposure scenario for the **ingestion of small pieces** or **sucking and chewing** on the toys is proposed to be:

- Body weight of 14 kg for a 3-year-old child and of 20 kg for a child over 6 years
- Ingestion of 100 mg/d
- Data on migration in saliva or in gastric fluids
- Data on chemicals content in the small piece ingested
- Data on oral absorption (or in the absence a 100% default value)
- Reference values related to oral exposure

According to the RIVM Report on Chemicals in toys (2008)[7], the parameters needed for this scenario are:

- concentration in the product [mg/kg]
- initial leaching rate [g/(cm^2 x min)
- weight of the toy[g]

[7] https://www.rivm.nl/bibliotheek/rapporten/320003001.pdf not all parameters were used in SCHEER calculation.

- density of the individual toy [g/cm^3]
- the surface in contact with the mouth [cm^2]
- duration of contact [min]

Regarding the duration of contact, the mouthing time during the day is highly variable in children as described in experimental studies reported in the above-mentioned RIVM report, therefore it is recommended to use 3 hours as a default for mouthing duration for children up to 3 years age.

SCHEER considerations on risk assessment

Regarding the evaluation of risks described in the two reports, the SCHEER agrees with the differentiation between the risks associated to local effects and to systemic exposure. Considering that exposure scenarios are limited in time and definitely shorter than the life span, the SCHEER considers the use of short-term reference values (rather than the TDI or a chronic DNEL) as sufficiently protective. The identification of the reference values is described in the next sections, chemical by chemical.

Risk assessment for combined exposure

Regarding combined exposure to different chemicals, the SCHEER considers that total exposure to primary amines should be accounted for, since they are similarly acting chemicals. This can be done considering additivity as the default approach: the effects can be estimated directly from the sum of the doses/concentrations, scaled for relative toxicity (dose/concentration addition).

This can be done by applying any of the methodologies usually applied for dose addition; for example the hazard index (HI) approach[8], which is described elsewhere in detail for mixture risk assessment (SCHER, SCENIHR, SCCS, 2012; EFSA 2019) and can be applied for any other possible combined exposure. The SCHEER does not support the use of an additional default mixture assessment factor per substance.

5.1.4 SCHEER considerations on the use of EU-LCI

Regarding the use of EU-LCI, the SCHEER considers that the values are derived using a compilation of epidemiological or toxicological data from risk assessments published by established international and national committees and/or other relevant studies. EU-LCIs are thus based on reported toxicity data and expert judgment and represent concentration levels that are considered not likely to cause adverse effects over the longer term considering a model room as a reference for the exposure scenario.

EU-LCI values are used to assess VOC indoor emissions after 28 days from a single building product during a laboratory test chamber procedure as defined in the Technical Specification TS 16516 of the horizontal testing method developed by CEN TC 351/WG 2.

[8] The hazard index (HI) is the sum of the hazard quotients (HQ), i.e. the ratios between exposure and the reference value (RV) for each component to be evaluated. When the HI is less than 1, the combined risk is considered acceptable; values higher than 1 would indicate potential health concern to be considered. The reciprocal of the HQ can also be used; the cumulative risk index is the reciprocal of the sum of the HQs.

The EU-LCI Working Group stressed that the EU-LCI values derived are not to be considered as indoor air quality guidelines but are to be used only in the context of material emission testing.

The SCHEER does not recommend applying the EU-LCI values as toxicological reference values for inhalation exposure to chemicals from toys in general. EU-LCI values are derived for building products on the base of specific exposure scenarios that may differ from those to be used when assessing health risks for children playing with toys. Children are a vulnerable group and specific assessment factors may have to be applied on a case-by-case basis, especially for children under the age of three.

5.1.5 SCHEER Approach for derivation of emission/migration limits

When reviewing the overall database for the substances of the ToR in this Opinion, the SCHEER noticed that it was not always possible to derive a BMD, which would be preferable, since full dose-response data were often lacking. Therefore, the SCHEER based the DNELs used on the N(L)OAELs as points of departure.

For systemic effects, 10% of the DNEL should be allocated to exposure from toys, as indicated by SCHER in a previous Opinion (SCHER, 2016), while no allocation factor is considered for local effects.

In the case of dichloromethane, being a volatile substance classified as CMR cat 2; H351, no DNEL can be derived for inhalation and therefore, the SCHEER does not calculate an emission limit value for the inhalation.

In the risk assessment, the routes have been considered separately since different toxicological endpoints are involved for oral and inhalation exposure to the chemical under evaluation.

The SCHEER considers that, in principle, it is possible to combine the two oral exposure scenarios "sucking and chewing on the toys" and "ingestion of small pieces" on the same single day. However, it is not realistic that they may occur simultaneously for a number of consecutive days. The two scenarios were therefore considered and verified separately, and then the most conservative one was chosen to establish the migration limits. Emission limits have been derived on the basis of the most conservative scenarios as well.

For the exposure via inhalation, three different scenarios have been considered by the SCHEER, which may reflect realistic situations, as follows:

Inhalation

Scenario 1: "3-year-old child sleeping with a squishy toy in her/his arms"

A 3-year-old child sleeping with a squishy toy in her/his arms. It is assumed that all the mass of the emitted substance stays within the breathing zone of the child, who inhales it. Body weight averages used are those indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg). The other parameters used as well as the equation for deriving the maximum allowed emission are reported in detail in Annex 1.

Scenario 2: "6-year old child playing in a room with several squishy toys"

A 6-year old child playing in a room with several squishy toys (n=40).

Body weight averages used are those indicated by ECHA and RIVM for 3-6-year-olds and for 6-11-year-olds. The average inhalation rate was that indicated by RIVM for two age groups: 3-6 and 6-11 year-old children. It is assumed, that the child plays/stays in the room all day. According to Bornehag et al. (2005), the air change rate of 0.35 corresponds to a child's bedroom in a single-family house and 0.51 is the mean value in a multi-family house. This value for the number of toys is not unrealistic, considering the fact that the squishy toys are sold at electronic shops in packages of 10-40 items.

The other parameters used, as well as the equation for deriving the maximum allowed emission, are reported in detail in Annex 1.

Scenario 3: "3-year-old child sleeping in a room with several squishy toys around and holding a squishy toy"

Scenario 3 is that of a 3-year-old child sleeping in a room with 40 squishy toys around and holding a squishy toy. It is assumed that one toy is in the breathing zone of the child and the rest (N_u) are inside the room. Body weight averages used are those indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg). The average inhalation rate is that indicated by ECHA and RIVM. According to Bornehag et al. (2005), the air change rate of 0.35 corresponds to a child's bedroom in a single-family house and 0.51 is the mean value in a multi-family house. This value for the number of toys is not unrealistic, considering the fact that the squishy toys are sold at electronic shops in packages of 10-40 items.

The other parameters used, as well as the equation for deriving the maximum allowed emission, are reported in detail in Annex 1.

For the three scenarios, the SCHEER calculated the emission limit values summarised in table A1 in Annex 1. For scenario 2 and 3, high and low air change rates have been addressed. The most conservative approach is scenario 3, i.e. a 3 year old child sleeping in a multi-family house with squishy toys in their bedroom and one toy in their arms. The corresponding results are reported in Table 4.

Table 4. Emission limits for exposure via inhalation

Substance			Toxicological reference and emission limit values		
Name	Abbreviation	CAS No	$DNEL_{inhalation}$ ($\mu g/m^3$)	Allocation factor %	Emission limit (mg/hr)
N,N-dimethylamino-ethanol	DMAE	108-01-0	1160	100	0.096
N,N-dimethylformamide	DMF	68-12-2	170	10	0.003
Triethylenediamine	TEDA	280-57-9	800	100	0.066
Bis(2-(dimethylamino) ethyl)ether	DMAEE	3033-62-3	20	100	0.002
1,1,4,7,7-pentamethyl-diethylenetriamine	PDT	3030-47-5	283	100	0.023
Cyclohexanone	CH	108-94-1	716	10	0.014
Xylenes	X	1330-20-7	130	10	0.003

For Dichloromethane, methylene chloride (DCM), which has been classified as Carc. Cat 2 H351, the SCHEER does not propose a limit value. According to Directive 2009/48/EC on the safety of toys (TSD) substances that are classified as carcinogenic, mutagenic or toxic for reproduction (CMR) of category 1A, 1B or 2 under Regulation (EC) No 1272/2008 shall not be used in toys, in components of toys or in micro-structurally distinct parts of toys. A derogation to this rule can be allowed, if the substance is inaccessible to children in any form, including inhalation. The SCHEER does not recommend the application of such a derogation in the case of DCM, since DCM is a volatile compound and children can be exposed to it through inhalation when playing with squishy toys as intended or foreseeable.

Dermal exposure

The SCHEER did not calculate limit values for dermal exposure, as no migration to sweat simulant was measured for these substances.

Oral Exposure

For oral exposure the following scenarios have been developed:

Scenario 1: "3-year old child mouthing a squishy toy"

A 3-year old child mouthing a squishy toy.
The average body weight is as indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg). Values for surface and mouthing time are taken from RIVM (2008). The following is assumed: 100% bioavailability (meaning that in the absence of migration data in saliva, 100% is able to leach and 100% is then absorbed), frequency for mouthing is once per day, the concentration of the substance in the toy is constant and uniform.

The other parameters, used as well as the equation for deriving the maximum allowed emission, are reported in detail in Annex 1.

Scenario 2: "3-year old child swallowing a piece of the squishy toy"

A 3-year old child swallowing a piece of the squishy toy.
The average body weight is that indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg). The amount of material ingested is estimated from RIVM (2008). The following is assumed: 100% bioavailability (meaning that, in the absence of migration data in gastric juice, 100% is able to leach, corresponding to the content of the chemical in 100 mg, and 100% is then absorbed), the concentration of the substance in the toy is constant and uniformly distributed.

The other parameters used, as well as the equation for deriving the maximum allowed emission, are reported in detail in Annex 1.

The limit values obtained are shown in Table 5. The worst case is always the one determined by ingestion of small pieces.

Table 5. Migration limits for exposure via the oral route

Substance			Toxicological reference values		Migration limit values	
Name	Abbreviation	CAS No	$DNEL_{oral}$	Allocation factor	Mouthing	Ingestion
			($mg/kg_{BW}/dy$)	%	($\mu g/cm^2/hr$)	(mg/g)
N,N-dimethylamino-ethanol	DMAE	108-01-0	-	-	-	-
N,N-dimethylformamide	DMF	68-12-2	2.4	10	112.0	33,6
Triethylenediamine	TEDA	280-57-9	1	10	46.7	14.0
Bis(2-(dimethylamino) ethyl)ether	DMAEE	3033-62-3	0.29	100	135.3	40.6
1,1,4,7,7-pentamethyl-diethylenetriamine	PDT	3030-47-5	0.3	10	14.0	4.2
Cyclohexanone	CH	108-94-1	2.4	10	112.0	33.6
Xylenes	X	1330-20-7	0.36	10	16.8	5.0
Dichloromethane, methylene chloride	DCM	75-09-2	0.175	10	8.2	2.5

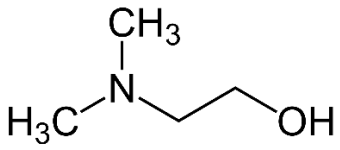
5.2. Hazard characterization of chemicals

5.2.1 Dimethylaminoethanol (DMAE)

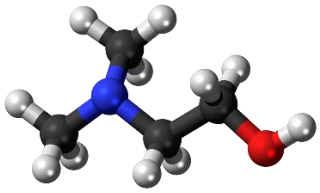
5.2.1.1 Physicochemical information

- IUPAC name: 2-(dimethylamino)ethanol
- CAS number: 108-01-0
- Molecular formula: $C_4H_{11}NO$
- Molecular weight 89.14 g/mol
- Physical state: liquid
- Water solubility: miscible
- log P_{ow}: -0.55
- Vapour pressure: 612 Pa
- Density: 0.887 g/cm^3
- Boiling point: 134.0 °C
- Melting/freezing point: -59.0 °C

- Conversion factor: 1 ppm = 3.65 mg/m^3
- 2D Structure:



- 3D Structure:



Uses of the substance (OECD, SIDS)

DMAE is used as a precursor in the production of flexible and rigid polyurethane foams and polyurethane lacquers and as a dispersant and neutralising agent in paints and surface coatings. Acrylate and methacrylate esters of DMAE are used in the synthesis of polyelectrolytes for use as water flocculants. DMAE is further used as a chemical raw material in the manufacture of ion exchange resins and pharmaceuticals, and as a component of corrosion inhibitor formulations.

5.2.1.2 Human Health Hazard Assessment

Toxicological information

Toxicokinetics, metabolism, and distribution

According to the ECHA dossier, DMAE is well absorbed via the digestive tract and rapidly transported to the liver where much of it is metabolized. Based on the available studies, no definitive conclusion can be drawn regarding the percentage absorption of DMAE via oral, dermal and inhalation exposure routes. Thus, based on physicochemical properties of DMAE, the moderate logP of -0.55, the low molecular weight of 89.1 and the high water solubility and absorption rate of 100% via oral, dermal and inhalation exposure are assumed in a worst- case scenario.

Acute toxicity, skin and eye irritation

The LC50 value (95% confidence limits) for rats was 1641 (862 to 3,125) ppm, equivalent to 5990 mg/m^3. Signs of toxicity included signs of nasal and ocular irritation, respiratory difficulties, loss of coordination, decreased motor activity and body weight loss (Klonne et al., 1987).
The well-known irritancy potential to the upper airway of saturated aliphatic and alicyclic amines is related to the lipophilicity of the substance. The greater the degree of lipophilicity, the greater the likelihood of significant irritation. Water soluble amines like DMAE are likely to be cleared in the nose (Gagnaire et al. 1993).

Sensitisation

No full study report available. DMAE was reported to be tested for skin sensitisation potential in a Buehler test with guinea pigs. The test substance did not cause sensitisation after challenge exposure (ECHA registration dossier).

Repeated dose toxicity

One repeated dose inhalation study of good quality was available (Klonne et al., 1987). The derivation of the TRV should be based on this study.
Klonne et al. exposed groups of 20 male and 20 female rats, 6 h/d, 5d/wk, for 13 weeks to target concentrations of 0, 8, 24 and 76 ppm, equivalent to 29, 88, and 277 mg/m^3, respectively (conversion factor used by Danish EPA and in the REACH dossier: 1 ppm = 3.65 mg/m^3). One-half of all rats per group were sacrificed after at least 2 days of exposure during the 14th week of the study; the remaining rats were sacrificed after 5 complete weeks of recovery. The study is equivalent to OECD test guideline 408.
Statistically lower body weight gains were observed at 76 ppm. The only exposure-related adverse effects are summarised in Table x. The effects are related to the ocular and upper respiratory tract irritancy of DMAE. Systemic effects were not found.

Table 1: Exposure related adverse effects in rats after repeated exposure to DMAE (Klonne et al., 1987)

Dose ppm	Sex	n	Rhinitis	Squamous metaplasia	Degeneration respiratory epithelium	Atrophy Olfactory epithelium	Microcysts respiratory epithelium
0	m	10	0	0	0	0	0
8	m	10	0	0	0	0	0
24	m	10	2	0	0	0	0
76	m	10	0	9	8	10	10
0	f	10	0	0	0	0	0
8	f	10	0	0	0	0	0
24	f	10	2	0	0	0	0
76	f	10	7	4	7	3	3

The incidence and severity of these lesions were decreased at the end of the recovery period. Additionally, 4/10 males at 76 ppm had laryngitis and two of these rats also showed tracheitis. Transient corneal opacity occurred in the 24 and 76ppm groups at the end of the daily exposure, beginning approximately 2-3 weeks after initiation of exposures. The opacity regressed during nocturnal non-exposure hours. Vacuolisation of the corneal epithelium was observed in 3/10 female rats at the end of the 76 ppm exposure, but not after recovery.

Genetic toxicity

DMAE was not genotoxic in a suite of tests including the Salmonella reverse gene mutation test, the CHO HGPRT forward gene mutation test, a sister chromatid exchange test in

cultured CHO cells, and an *in vivo* peripheral blood micronucleus test in Swiss–Webster mice (Leung and Ballantyne, 1997).

Carcinogenicity

Lifetime administration of DMAE in drinking water to two sublines of female mice carrying a lifelong germinal mammary tumour virus did not have a significant effect on longevity. DMAE did not induce an increase of any type of neoplasm or a change in the age of onset of neoplasms in either strain (Stenbäck, et al., 1988).

Reproductive toxicity

Developmental toxicity

Pregnant Fischer 344 rats were exposed whole body to DMAE vapour for 6 h/d on gestational days 6-15 at mean analytically measured concentrations of 10.4, 29.8 and 100 ppm. Dams were sacrificed on gestational day 21. There was no maternal mortality in any exposed groups. Maternal toxicity observed in the 100 ppm group included reduced body weight during and after exposures, reduced weight gain during exposure and ocular changes (darkened, cloudy and hazy eyes, slight corneal vascularization and fixed, dilated pupils). Ocular effects were also noted in the other two exposure groups; the effects were quite marked at 29.8 ppm, but only minimal and transient at 10 ppm. There were no effects of treatment on any gestational parameter. Fetal body weights per litter were statistically significantly increased at 100 ppm relative to controls. There were no increases in the incidences of total malformations and no evidence of consistent fetal toxicity. The authors concluded that the no-observed-adverse-effect level is around 10 ppm (36.5 mg/m^3) for maternal toxicity and at or above 100 ppm for embryofetal toxicity and teratogenicity (Leung et al., 1996).

5.2.1.3 Human health hazard characterization

Selection of the Point of Departure

Inhalation
The NOAEC is 29 mg/m^3 (8 ppm) based on the concentration-related respiratory irritation and the ocular changes observed from 88 mg/m^3 (24 ppm). The NOAEL for respiratory irritation and systemic effects is 24 ppm (88 mg/m^3). Transient corneal opacity occurred in the 24 and 76ppm groups at the end of the daily exposure, beginning approximately 2-3 weeks after initiation of exposures. The opacity regressed during nocturnal non-exposure hours. This is consistent with the mechanism of amine vapour toxicity on the eye where vision returns to normal a short time after exposure ceases and permanent effects do not occur. Vapour concentration of the causative amine is a major factor in the development of glaucopsia, and a concentration-effect relationship is usually evident. Therefore, the NOAEL is considered to be 8 ppm (29 mg/m^3).

Oral

An oral DNEL cannot be derived since oral data are lacking and route-to-route extrapolation is not possible for local effects.

Derivation of the Toxicological Reference Value

Inhalation
The PoD of 29 mg/m^3 for rats in the 13-week study should be corrected for:

1. Uncertainty due to differences in exposure duration: the adverse effects observed are related to irritation of the mucous membranes of the eyes and respiratory tract. This irritation is related to the concentration and not to the dose. Therefore, SCHEER does not apply time scaling for differences in exposure duration and frequency. Likewise, SCHEER also does not apply an assessment factor for extrapolation from semi-chronic to chronic. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: For local effects, scaling for metabolic differences is not required. As applied in the DK report, the remaining toxicodynamic differences between the average rat and the average human can be accounted for by a factor of 2.5 (AF1).
2. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (ECHA), as applied in the DK report (AF2).
3. Uncertainty due to weaknesses in the database. Since the key study available is equivalent to OECD 408, is of good quality and well reported in peer-reviewed literature, no correction factor is needed.

The DNEL for DMAE will therefore be 29/(AF1xAF2) mg/m^3= 1160 µg/m^3, based on the NOAEC.
The DNEL will be applicable to both short- and long-term exposure.

WoE Considerations

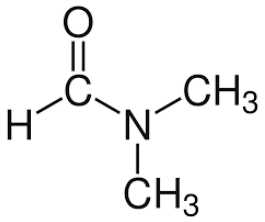
The key study of Klonne et al. is considered to be of high quality. It is equivalent to the OECD test guideline 408 and uses a relevant route of exposure to address the risk of children playing with squishy toys. The critical effect irritancy, as the most sensitive endpoint, is corroborated by other available lines of evidence substantiating a toxicological MoA common to primary amines (Gagnaire et al., 1993). Therefore, the consistency among the available lines of evidence can be considered as good and, overall, the WoE can be concluded to be strong. An oral DNEL cannot be derived since oral data are lacking and route-to-route extrapolation is not possible for local effects.

5.2.2 N,N-dimethylformamide (DMF)

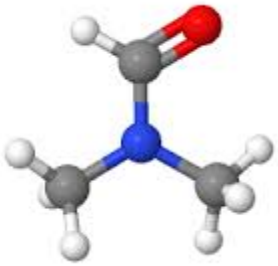
5.2.2.1 Physicochemical information

- IUPAC name: N,N-dimethylformamide
- CAS number: 68-12-2
- Molecular formula: C_3H_7NO
- Molecular weight: 73.09 g/mol
- Physical state: liquid
- Water solubility: 1000 g/L
- log K_{ow}: -1.01
- Vapour pressure: 377 Pa
- Density: 0.944 g/cm^3
- Boiling point: 152°C
- Melting/freezing point: -61°C

- Conversion factor: 1 ppm = 3.04 mg/m^3
- 2D Structure:



- 3D Structure:



Uses of the substance

DMF has been termed the universal organic solvent and is widely used around the world in many applications in the chemical industry. According to ECHA, DMF has been registered with a total tonnage band of 10000-100000 tonnes per year (partly produced in Europe and partly imported). It also has a registered use as intermediate only (ECHA dissemination database of registered substances).
Producers of DMF outside Europe included China and the other major users, Korea, Japan, and USA (in 2001). In Asia, the production volume was 100,000 to 500,000 tonnes per year and in North America it was 50,000 to 100,000 tonnes per year (OECD, 2001).

This substance is used

- for the production of synthetic/artificial leather of polyurethane polymers (China accounting for >60% of the world's use)
- as a solvent, reagent and catalyst for synthesis in organic chemistry (including the pharmaceutical and agrochemicals industries)
- as a cleaning solvent in the leather and artificial leather industries
- for the manufacture of printed circuit boards
- in oil and gas/petrochemical sector, primarily for the separation of gas streams

Individuals are most likely to be exposed to DMF in the workplace. In the ECHA files, there is no declared use for consumers (non-professional uses). Indeed, since it is declared to be mainly used as a solvent in organic synthesis, DMF is not supposed to be a component of the final product. Nevertheless, residues may still remain, especially in textile and plastics (e.g polyurethane polymers) and consumer exposure cannot be excluded.

The industrial release of DMF into the air is considerably larger than releases to other environmental media and therefore critical in determining background exposure to DMF. Due to its good water solubility, it is expected that atmospheric DMF can be transported from air into surface water or soil pore water during rain events (Health Canada, 2001; WHO, 2001). According to its Kow, DMF is unlikely to transfer to sediments, biota, or back to the atmosphere, and once released into surface water, remains in its dissolved form (WHO, 2001). However, there is a lack of robust data for the general population exposure via the environment.

5.2.2.2. Human Health Hazard Assessment

Toxicological information

The substance is proposed to be identified as a substance meeting the criteria of Article 57 (c) of Regulation (EC) 1907/2006 (REACH), owing to its classification as toxic for reproduction category 1B (ECHA, ANNEX XV – Identification of Dimethylformamide (DMF) as SVHC). According to the harmonised classification and labelling approved by the European Union and reported by ECHA (2016), this substance may damage the unborn child, is harmful in contact with skin, causes serious eye irritation and is harmful if inhaled.

Toxicokinetics, metabolism, and distribution

Available data indicate that DMF is readily absorbed following oral, dermal, and inhalation exposure in both humans and animals. Percutaneous absorption is indicated in the ECHA database to be 100%.

DMF is metabolized primarily in the liver mediated by P450 (CYP2E1) to N-(hydroxymethyl)-N-methylformamide (HMMF), N-methylformamide (MMF) and formaldehyde and is relatively rapidly excreted (within 24h) as metabolites in urine, primarily as HMMF (indicated by EPA as a biomarker for DMF exposure). Further cytochrome P450-mediated oxidation of NMF and/or HMMF results in the formation of formamide.

Beside the hydroxylation of the *N*-methyl groups, an additional metabolic pathway has been described for DMF, associated with the oxidation of the formyl moiety and following conjugation with glutathione, leading to the formation S-methylcarbamoylglutathione (SMG), methyl isocyanate, a reactive species associated with hepatotoxicity. The GSH conjugate and its sequel adducts lead to excretion of N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC), which can be postulated as being responsible for developmental toxic effects. The metabolic pathway leading to AMCC seems to be higher in humans than in rodents (Mraz, 1989) and after oral exposure compared to inhalation.

Studies on 10 human volunteers exposed to 10, 30, or 60 mg DMF/m^3 for 8-h exposures or 5 daily exposures of 30 mg/m^3 (Mráz & Nohová, 1992a, 1992b): urine was collected for 5 days and analysed for DMF, HMMF, HMF, and AMCC. In addition, 3 volunteers ingested 20 mg AMCC dissolved in water, and urinary metabolites were determined for a period of 8 h after exposure. After single inhalation exposure to 30 mg/m^3, the elimination in the urine was 0.3% parent compound, 22.3% HMMF, 13.2% HMF, and 13.4% AMCC. The half-times of excretion for these various metabolites were approximately 2, 4, 7, and 23 h, respectively. In contrast, AMCC was rapidly eliminated after ingestion of AMCC, with a half-time of 1 h. When the 10 volunteers were exposed for 5 days, elimination 16 h following the fifth exposure was approximately 14% HMMF, 32% HMF, and 54% AMCC.

Acute toxicity

Following oral, dermal, inhalation, or parenteral administration, the acute toxicity of DMF in a number of species is low.

- LD50 3010 mg/kg bw, oral route, rat
- LC50 5.85 g/m^3, inhalation route, rat
- LD50 >5000 mg/kg bw, dermal route, rabbit

Skin and Eye irritation

Adverse effects were observed for eyes (irritating; Xi), but not for skin. An epidemiological study on workers has reported symptoms of eye and respiratory tract irritation at 22 mg/m^3 (Cirla et al, 1984).

Sensitisation

No adverse effect was observed (not sensitising) for skin, as well as for the respiratory tract (not sensitising).

Repeated dose toxicity

Systemic effects were observed for 28 days exposure (oral route) of rats with a NOAEL of 238 mg/kg bw/day, based on reduced body weight and hepatic effects at higher doses.

For the inhalation route, hepatic effects (increased liver weight with minimal to mild hepatocellular hypertrophy and clinical chemistry alteration at all doses) were observed in a chronic study rats with a LOAEC of 80 mg/m³ (6h/dy, 5dy/wk, 2 years) (Malley et al., 1994). These findings were confirmed by the same authors in a parallel study on mice (same doses, 18 months of treatment) where, in addition, no effect on estrous cycles in female mice was evidenced or reported. A variety of other repeated inhalation studies also support the LOAEC (NTP, 1992; Lynch et al., 2003 and Senoh et al., 2003; IARC, 2018). Concluding for the systemic effects the reference values are:

- NOAEL = 238 mg/kg bw/day (oral route, rat, 28 days)
- LOAEC = 80 mg/m³ (Inhalation route 6h/dy, 5dy/wk, 2years rat)

Chronic occupational exposure to DMF by inhalation has resulted in effects on the liver and digestive disturbances in workers (US-EPA, 2000), consistent with that observed in experimental animals, in addition to irritation to the eyes and in the respiratory tract as reported by workers.

The health effects associated with occupational exposure to DMF were studied by Cirla et al. (1984), Catenacci et al. (1984) and results of these studies are consistent with those of a more recent and carefully conducted study by Fiorito et al. (1997). The LOAEL determined in Cirla et al. (1984) enrolling 100 workers exposed to a mean concentration of 22 mg/m^3 DMF (range of 8 to 58 mg/m^3, determined with personal air sampler) for an average of 5 years (range of 1 to 15 years) was used by US-EPA for the RfC derivation. The LOAEL was derived considering digestive disturbances and evidence suggestive of mild liver

abnormalities as well as irritation to the eyes and the respiratory tract; the adjusted LOAEL was equal to 7.9 mg/m^3 DMF (rounded to 8 mg/m^3).

Genetic toxicity

According to ECHA, DMF does not induce significant chromosome aberrations or gene mutations in various test systems *in vitro* and *in vivo*. For this reason, classification as genotoxic is not warranted according to CLP Regulations No 1272/2008 (ECHA, 2016). The weight of evidence for genotoxicity is overwhelmingly negative, based on extensive investigation in *in vitro* assays, particularly for gene mutation, and a more limited database *in vivo*. However, the IARC most updated evaluation reported that there is moderate evidence that DMF is genotoxic, on the basis of some equivocal results in workers, whereas the results of studies of genotoxicity in various experimental systems *in vivo* and *in vitro* were mostly negative or inconclusive (IARC, 2018).

Carcinogenicity

DMF was not oncogenic (no increases in the incidence of tumours following chronic inhalation exposure) in the adequately conducted bioassays on rats and mice - Malley et al. study (1994) from which the LOAEC was also derived following inhalation exposure (WHO, 2001, HC, 2001). There is no convincing, consistent evidence of increases in tumours at any site associated with exposure to DMF in the occupational environment (WHO, 2001). DMF is not genotoxic in the standard genotoxicity testing (WHO, 2001, HC, 2001). On the basis of this data base, ECHA concluded that no classification is warranted, according to CLP (ECHA, 2016).

Also US-EPA has not classified dimethylformamide with respect to its carcinogenicity: DMF is not classifiable as to its carcinogenicity to humans (Group 3).

The IARC most updated evaluation reported that there is limited evidence in humans for the carcinogenicity of DMF, but the evidence in experimental animals is considered sufficient, therefore as an overall evaluation DMF is classified by IARC as probably carcinogenic to humans (Group 2A) (IARC, 2018).

No adequate oral data is available, but this is not thought to be a relevant route of exposure.

Reproductive toxicity

Available studies (WHO, 2001; Heath Canada, 2001) indicate that DMF can have effects on fertility as well as developmental toxicity with the following reference values (identical for the two endpoints):

- NOAEL = 219 mg/kg bw/day (oral route, mice, drinking water) Effects: alteration of the estrous cycle and other reproductive effects in F1 and F2 mid and high dose (in these groups all the animal have also some hepatic effects)
- NOAEC = 150 mg/m^3 (Inhalation route, rabbit, the lowest dose tested). Maternal toxicity was seen at 0.45 mg/L and 1.36 mg/L (mid and high dose) and clear signs of embryo-/fetotoxicity including indications of teratogenicity were seen at the highest concentration tested
- NOAEL = 200 mg/kg bw/day (oral route, rabbit). The NOAEL was the same, also for maternal toxicity.

The classification as Repr.1B is warranted according to the criteria of CLP Regulations No 1272/2008 (ECHA, 2016).

Only one study is available on the reproductive effects of dimethylformamide in humans, reporting an increased rate of spontaneous abortion among pregnant women occupationally exposed to DMF. However, since they were exposed to other chemicals potentially acting on the same end-point, the relationship with DMF could not be established (US-EPA, 2000).

5.2.2.3. Hazard characterization

There were no compound-related lesions noted in the nose or respiratory tract for any exposure concentration in either rats and mice during the long-term inhalation study (Malley et al., 1994). Therefore, no reference values for the local inhalation effects are derived from animal studies. ECHA also did not derive any DNEL for local effects.

The critical target organ for systemic effects has been identified in the liver in both humans and experimental animals exposed to DMF. The effects on reproduction and development has reference values higher than those identified for the hepatic damages.

Available data indicate that there may be variations between experimental animals and humans in the proportion of DMF metabolized by the putatively toxic pathway, with the resulting implication that humans may be more sensitive to the effects of DMF.

For this reason, the US-EPA (2000) used as PoD the LOAEL =22 mg/m³ adjusted to continuous exposure (7.9 mg/m³) obtained in the epidemiological study on workers by Cirla et al (1984), based on irritation to the eyes and the respiratory tract as well as digestive disturbances and minimal hepatic changes suggestive of liver abnormalities in humans, to which an uncertainty factor of 10 is used for protection of sensitive human subpopulations, and an additional factor of 30 is used to account for use of a LOAEL on the incomplete data base (lack of reproductive toxicity data, and the less than chronic duration of exposure). The Reference Concentration (RfC) for DMF is 0.03 mg/m^3.

Using the same PoD but a different uncertainty factor, the WHO (2001) derived a RfC=0.1 mg/m^3); the same approach has been followed by Health Canada (2001).

ECHA followed a different procedure described in the website[9] using as PoD the NOAEC identified in the chronic study in rats (80 mg/m³) (Malley et al., 1994), adjusted to account for continuous inhalation (80 mg/ m³ x (6/24) = 20 mg/m³), then divided for an assessment factor of 5 (for workers) to account for intraspecies differences, since the interspecies differences were ignored due to the inhalation route: DNEL = (20 mg/m³) / 10 = 4.0 mg/m³. Considering that for the exposure scenarios related to squishy toys we are referring to children, an assessment factor =10 (the one ECHA uses for the general population) would have been considered more appropriate. In addition, toxicokinetic differences between experimental animal species and human may, in part, contribute to the observed species differences in toxicity. The difference in the formation of the putatively toxic metabolite AMCC, which were 2-4 fold higher levels in urine in humans compared to rodents, is of relevance. It is therefore not justified to use data derived from animal studies when human data is available (although animal studies can be used as supporting

[9] https://echa.europa.eu/registration-dossier/-/registered-dossier/15093/7/6/1

information). In the Opinion of the Committee for Risk Assessment the Committee for Socio-economic Analysis[10] restrictions have been adopted for DMF and a worker-based harmonised DNEL for long-term inhalation exposure of 6 mg/m^3 was derived, based on recent human data.

The Danish EPA report analysed for this Opinion used the adjusted LOAEC coming from the Cirla et al. (1984) epidemiological study referring to eye and respiratory tract irritation of 8 mg/m^3 , to which an AF =100 was applied to account for intraspecies variability (10) for use of a LOAEC (10). Consequently, the RfCs in this case = 0.08 mg/m^3.

It has to be highlighted that DMF is classified as a category 1B reproductive toxic substance, which means that it is limited to a 0.3% concentration by weight in accessible parts of any toy.

The SCHEER derivation of Health Based Reference values is described in the following.

Derivation of the Toxicological Reference Value carried out by SCHEER

Inhalation (Local effects):

The PoD in humans was the LOAEC =22 mg/m³ obtained in the epidemiological study on workers by Cirla et al (1984), based on irritation to the eyes and the respiratory tract:

1. Using the LOAEC instead of a NOAEC: A factor of 3 is used, according to ECHA guidance
2. Uncertainty due to differences in exposure duration: irritation of the mucous membranes of the eyes and of the respiratory tract are the adverse effects observed. Irritation is related to the concentration and not to the dose. Therefore, no time scaling for differences in exposure duration and frequency is required.
3. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: since data were obtained in humans, no additional factor was added.
4. Intraspecies variability: the variability between the average human and sensitive humans (including children) can be accounted for by a factor of 10.
5. Uncertainty due to weaknesses in the database. Since the study results are consistent with other studies in humans and with data obtained in animal studies as well, no correction factor is needed.

The final result is: 22 mg/m^3 / (3x10) =0.7 mg/m^3

Inhalation (Systemic effects):

The PoD in humans was again the LOAEL =22 mg/m³ obtained in the epidemiological study on workers by Cirla et al (1984), based on digestive disturbances and minimal hepatic changes suggestive of liver abnormalities:

1. Using the LOAEC instead of a NOAEC: A factor of 3 is used, according to ECHA guidance
2. Uncertainty due to differences in exposure duration: the 8/24 (hours) and 5/7 (days) factors were used to adjust to continuous exposure giving rise to 5.2 mg/m³

[10] https://echa.europa.eu/documents/10162/b6644298-54a4-052a-9bbc-6824966d151e

3. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: since data were obtained in humans, no additional factor was added.
4. Intraspecies variability: the variability between the average human and sensitive humans (including children) can be accounted for by a factor of 10.
5. Uncertainty due to weaknesses in the database. Since the study results are consistent with other studies in humans and with data obtained in animal as well, no correction factor is needed.

The final result is: 5.2 mg/m^3 / (3x10) =0.17 mg/m^3

WoE Considerations

The key study by Cirla et al. in humans is considered to be of good quality, but most importantly results are consistent with those of other similar studies (Catenacci et al., 1984 and Fiorito et al., 1997). The critical effect, irritancy, as the most sensitive endpoint, as well as hepatic effects, are corroborated by other available lines of evidence observed in a number of inhalation toxicity studies in rodents (both rats and mice), one of which after submitting the rodents to chronic exposure, was conducted with methods overlapping with the OECD guidelines. Therefore, the consistency among the available lines of evidence can be considered as good and, overall, the WoE can be concluded to be strong.
The oral exposure should be considered, since DMF can be released in the g.i. tract following sucking activity as well as through the ingestion of small parts of the squishies.

Oral (Systemic effects):

The PoD is the NOAEL = 238 mg/kg bw/day for rats in the 28-day study based on reduced body weight and hepatic effects may be corrected for:

1. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: a factor of 10 is used as default.
2. Intraspecies variability: the variability between the average human and sensitive humans (including children) can be accounted for by a factor of 10.
3. Uncertainty due to weaknesses in the database. No additional factor is deemed necessary to cover the short duration of the study

The final result is: 238 mg/kg bw/10x10= 2.4 mg/kg bw per day

WoE Considerations

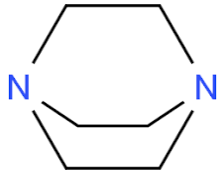
Regarding the oral route, only one short-term study (rat, 28 days) is available. However, considering that the hepatic effects were identified as the critical ones similarly to the inhalation systemic toxicity, the strength of the evidence is considered moderate.

5.2.3. Triethylenediamine (TEDA)

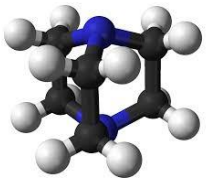
5.2.3.1. Physicochemical information

- IUPAC Name: 1-cyclooctyl-1,4-diazocane
- CAS Number: 280-57-9
- Molecular formula: $C_6H_{12}N_2$
- Molecular weight: 112.17 g/mol
- Physical state: white crystalline solid

- Water solubility: 610 g/L
- log P_{ow}: -0.49
- Vapour pressure: 43 Pa
- Density: 1.14 g/cm^3
- Boiling point: 162.3 °C
- Melting/freezing point: -77 °C
- Conversion factor: 1 ppm= 4.59 mg/m^3
- 2D Structure:



- 3D Structure



Uses of the substance (ECHA)

Triethylendiamine (1,4-diazabicyclo[2.2.2]octane) is used primarily as a catalyst from alcohol and isocyanate functionalized monomers and pre-polymers in the production of polyurethane foam. Approximately 90% of the substance produced is used for this purpose.

This substance is used in adhesives and sealants and coating products. Even though polyurethane foam is used in a wide variety of consumer products, the 1,4-Diazabicyclo[2.2.2]octane that is remaining in the foam after the crushing process appears to be strongly bound into the foam. Attempts to remove it from foam by heating or solvent extraction have not been successful. Therefore, based on the noted difficulty in removing 1,4-Diazabicyclo[2.2.2]octane from the foam, consumer exposure is expected to be low.

5.2.3.2. Human Health Hazard Assessment

Toxicological information

Toxicokinetics, metabolism, and distribution

There are no available toxicokinetic studies or literature data to allow the assessment of the ADME properties of 1,4-diazabicyclo[2.2.2]octane For simplicity 100% absorption for the oral and the inhalative route for animals and humans is assumed (ECHA Guidance R.8: Characterisation of dose [concentration]-response for human health, 2012).

Acute toxicity, skin and eye irritation

The acute oral LD50 range is of 700 - 2260 mg/kg bw in rats, while the dermal LD50 in rabbits is >2000 mg/kg bw (SIDS, 2005). The acute inhalation LC50 in rats is >20.2 mg/L nominal concentration (20% solution) (1 hour) or greater than the saturated vapour concentration (8 hour). In oral studies at non-lethal doses, transient depression and poor grooming were observed. At lethal doses, severe depression and ataxia rapidly progressed to coma and death within a few hours. In the dermal studies, severe erythema that disappeared within a few days was the only finding of note. In the inhalation studies, mild transient irritation of the eyes and mucous membranes and slight depression were the only notable findings. Pharmacologic effects, particularly on blood pressure, have been observed in cats and dogs when 1,4-diazabicyclo[2.2.2]octane was administered intravenously.

Skin and eye irritation studies in rabbits indicate that it is moderately irritating to the skin and severely irritating to the eye.

Sensitisation

Triethylendiamine is not a skin sensitiser in guinea pig testing (SIDS, 2005). In humans, glaucopsia (blue haze or halovision) has been reported at some foam manufacturing facilities and has been attributed to the presence of high concentrations of tertiary amines in the air.

Repeated dose toxicity

Inhalation route

Rats were exposed via inhalation to aerosolized 1,4-diazabicyclo[2.2.2]octane 6 hours/day, 5 days/week for four weeks (20 exposures) at nominal concentrations of 0, 0.0058, 0.063 and 0.62 mg/L (analytical concentrations were 0, <0.011, 0.06 and 0.41 mg/L/6h/day) (SIDS, 2005). The lowest dose was below the analytical limit of detection (0.011 mg/L). The control animals were exposed to the vehicle (distilled water) only. One female in the high-dose group died on day 5. The high-dose animals exhibited necrotic dermatitis of the ears, nose and eyes. Food consumption and body weight gain were decreased in the high-dose group. Histopathology revealed moderate chronic laryngitis in the mid- and high-dose groups (both sexes). The frequency of this finding was dose-related (7 out of 10 high-dose and 3 out of 10 mid-dose). The female that died had severe acute necrotizing laryngitis. No compound-related effects were seen at the lowest dose level. Absolute and relative testes weights and relative adrenal weights (males) were statistically significantly increased at study termination; however, microscopic examination of these organs did not reveal any treatment-related effects. Since the lowest dose level could not be measured analytically, a NOAEC cannot be ascertained. However, the LOAEC for this study was 0.06 mg/L/6h/day and is based on local toxicity at the site of contact, namely, the upper respiratory tract (moderate chronic laryngitis).
Since no systemic toxicological effects were observed, the No Observed Adverse Effect Concentration (NOAEC) for systemic toxicity was 0.41 mg/L/6h/day, which was the highest dose tested.

Oral route

In a combined repeated-dose/reproductive/developmental toxicity screening test (OECD 422), the test substance was administered orally for 28 days to three groups of Sprague-

Dawley rats, once daily, at dosage levels of 100, 300, and 1000 mg/kg/day at a dose volume of 5 ml/kg. A concurrent control group received the vehicle, deionized water, on a comparable regimen at 5 ml/kg. to 1,4-diazabicyclo[2.2.2]octane at dose levels of 0, 100, 300 and 1000 mg/kg bw/day.

Oral administration of 1,4-diazabicyclo[2.2.2]octane resulted in parental (F0) systemic toxicity in both males and females at a dose level of 1000 mg/kg bw/day. This was evidenced by changes in clinical condition of the animals, reduced body weight and food consumption, reduced motor activity (females only), increased serum alkaline phosphatase concentrations (females only), increased liver weights (females only) and microscopic changes (inflammatory and/or proliferative lesions) in the kidneys and/or urinary bladder. With the exception of lesions in the kidneys and urinary bladder of a single 1000 mg/kg bw/day group female, none of the above findings persisted to the end of the 14-day recovery period. F0 systemic toxicity in the 300-mg/kg bw/day group was limited to chronic inflammation of the kidneys in the males. Based on the data obtained, the NOAEL for F0 parental systemic toxicity was considered to be 100 mg/kg/day.

Mating and fertility indices were not affected by the test substance administration. Reproductive and F1 neonatal toxicity were exhibited at 1000 mg/kg bw/day by increased resorptions, decreased live litter size, decreased postnatal pup survival and decreased pup body weights. No indications of neonatal toxicity were observed at 100 and 300 mg/kg bw/day. Based on the data obtained, the NOAELs (no-observed-adverse-effect-level) for F0 reproductive toxicity and F1 neonatal toxicity were 300 mg/kg bw/day. The NOAEL for F0 male and female systemic toxicity and reproductive toxicity were 100 and 300 mg/kg bw/day, respectively.

Mutagenicity

1,4-Diazabicyclo[2.2.2]octane is not mutagenic in bacteria and was not clastogenic in an *in vivo* mouse micronucleus study (SIDS, 2005).

Carcinogenicity

No available data for 1,4-Diazabicyclo[2.2.2]octane.

5.2.3.3. Hazard characterization

Selection of the Point of Departure

PoD for the inhalation DNEL derivation (local toxicity):

NOAEC could not be ascertained analytically, therefore it is used a LOAEC of 0.06 mg/L/6h/day from a sub-acute inhalation rat study (20 exposures, 5d/w) based on local effects observed at the site of contact, namely, the upper respiratory tract.

The PoD for rats in the inhalation 28d study should be corrected for:

1. Uncertainty due to differences in exposure duration: the adverse effects observed are related to irritation of the mucous membranes of the eyes and respiratory tract. This irritation is related to the concentration and not to the dose. Therefore, no time scaling for differences in exposure duration and frequency are required.

2. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: For local effects, scaling for metabolic differences is not required. Remaining toxicodynamic differences between the average rat and the average human can be accounted for by a factor of 2.5 (AF 1)
3. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (AF 2).
4. Extrapolation from LOAEC to NOAEC accounted for by a factor of 3 (AF3).

LOAEC: 0.06 mg/L > 60 mg/m^3
DNEL = LOAEC/(AF1 x AF2 x AF3)
AF1 (interspecies animals to human) = 2.5 (for local irritation effects)
AF2 (intraspecies, difference in human sensitivity) = 10
AF3 (extrapolation LOAEC to NOAEC) = 3
$DNEL_{inhalation}$ = 60 mg/m^3/(2.5 x 10 x 3) = 0.8 mg/m^3 (800 µg/m^3)

WoE considerations

The rat study (1987) used to derive the $DNEL_{inhalation}$ for local effects was performed according to OECD Guidelines 412 (sub-acute inhalation toxicity 28-day study) and classified in the REACH registration dossier with reliability 1 (reliable without restriction). This experimental study was also considered as relevant in the human health hazard characterization in SIDS, 2005. Overall, SCHEER considers that there is strong WoE that irritancy is the critical adverse effect corroborating the investigated toxicological mode of action of this group of chemicals, the amines, based on local effects.

PoD for the oral DNEL derivation (systemic toxicity):
The oral DNEL for systemic effects was derived from the sub-acute NOAEL of 100mg/kg bw/d in the combined repeated dose/reproductive/developmental toxicity screening test (OECD 422) and based on the kidney inflammatory effects observed in males rats at the immediate higher dose level (300 mg/kg bw/d),

The PoD of 100mg/kg bw/d from this study should be corrected for:

1. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: scaling for metabolic and toxicokinetic differences between the average rat and the average human can be accounted for by a factor of 10 (AF1).
2. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (AF2).

NOAEL: 100mg/kg bw/d
DNEL = NOAEL/(AF1xAF2)
AF1 (interspecies animals to human) = 10 (for systemic effects)
AF2 (intraspecies, difference in human sensitivity) = 10
$DNEL_{oral}$ = 100mg/kg bw/d / (10x10) = 1 mg/kg bw/d

WoE considerations

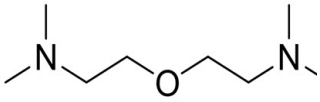
The rat study (2000) used to derive the $DNEL_{oral}$ for systemic effects is a GLP study performed according to OECD Guidelines 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) and classified with reliability 1 (reliable without restrictions) in the REACH registration dossier. It is also used as a key study in the 2005 SIDS report for screening reproductive toxicity evaluation. Although the study is of good quality and adequate, the kidney inflammatory effects observed in males

are based on one available line of evidence and therefore SCHEER considers the overall WoE for systemic toxicity for this type of effects as moderate.

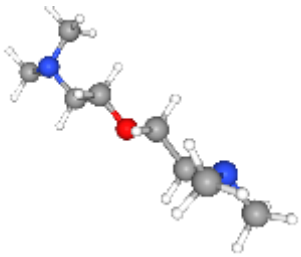
5.2.4 Bis(2-(dimethylamino)ethyl)ether (DMAEE)

5.2.4.1. Physicochemical information

- IUPAC name: 2-[2-(dimethylamino)ethoxy] -N,N-dimethylethanamine
- CAS number: 3033-62-3
- Molecular formula: $C_8H_{20}N_2O$
- Molecular weight 160.26 g/mol
- Physical state: liquid
- Water solubility: miscible
- log P_{ow}: -0.54
- Vapour pressure: 100 Pa
- Density: 0.848 g/cm^3
- Boiling point: 188 °C
- Melting/freezing point: < -70 °C
- 2D Structure:



- 3D Structure:



Uses of the substance

Between 1 000 – 10 000 tonnes per year of this substance is manufactured and/or imported in the European Economic Area (ECHA, 2019).

This substance is used by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing. This substance is used in the following products: adhesives and sealants, coating products and fillers, putties, plasters, modelling clay (e.g. binding agent in paints and coatings or adhesives) (ECHA, 2019).

According to **REACH Annex XVII, Entry No. 3**[11], the substance or a mixture of it shall not be used in:

- ornamental articles intended to produce light or colour effects by means of different phases, for example in ornamental lamps and ashtrays,
- tricks and jokes,
- games for one or more participants, or any article intended to be used as such, even with ornamental aspects.

5.2.4.2. Human Health Hazard Assessment

Toxicological information

Toxicokinetics, metabolism, and distribution
Animal studies indicate the test substance is rapidly absorbed following dermal, i.v. or inhalation exposure, and eliminated, unchanged, primarily in the urine. Based on the i.v. studies, the elimination half-life for the test substance is ~14 - 18 hours in rats (2 mg/kg and 200 mg/kg administered dose, respectively) and ~26 - 40 hours in the rabbit (1 mg/kg and 100 mg/kg administered dose, respectively).

Absorption values:
Dermal: 64.5 %
Inhalation: 60 %

Acute toxicity, skin and eye irritation

The LD50 of the test substance was found to be 677 mg/kg with 95% confidence limits of 636-722 mg/kg when administered once orally via gastric intubation to fasted male and female albino rats (key studies).
The LC50 of the test substance for acute inhalation exposure (approximately 4 hours) in male and female rats observed for a period of 15 days obtained in this study was estimated to be greater than 2.204 mg/L (key studies).
Percutaneous LD50 = 0.406 mL/kg (males) and 0.633 mL/kg (females) for the 4-hr contact with undiluted sample group; Percutaneous LD50 = 0.373 mL/kg (males) and 0.367 mL/kg (females) for the 24-hr contact with undiluted sample group; Percutaneous LD50 = 2.14 mL/kg (of dilution, males) and 2.83 mL/kg (of dilution, females) for the 24-hr contact with 20% aqueous dilution group. The dermal LD50 value was converted using the density of the test substance: 0.848 g/mL. For males 0.373 mL/kg = 316 mg/kg; for females 0.367 mL/kg = 311 mg/kg (key studies).

The substance is considered to be corrosive to skin based on skin irritation studies conducted in rabbits that reported severe irritation (erythema) and corrosion following a 60-minute exposure.

[11] Liquid substances or mixtures which are regarded as dangerous in accordance with Directive 1999/45/EC or are fulfilling the criteria for any of the following hazard classes or categories set out in Annex I to Regulation (EC) No 1272/2008: (a) hazard classes 2.1 to 2.4, 2.6 and 2.7, 2.8 types A and B, 2.9, 2.10, 2.12, 2.13 categories 1 and 2, 2.14 categories 1 and 2, 2.15 types A to F; (b) hazard classes 3.1 to 3.6, 3.7 adverse effects on sexual function and fertility or on development, 3.8 effects other than narcotic effects, 3.9 and 3.10;(c) hazard class 4.1; (d) hazard class 5.1.

It is considered to be corrosive to the eyes based on an eye irritation study conducted in rabbits indicating that the substance is extremely irritating and the effects were not reversible within 72 hours.

Sensitisation

The substance was not sensitising in a standard guinea pig sensitisation study.

Repeated dose toxicity

In a 7-day dose range finding study in rats, where nominal doses of 0 – 320 mg/kg bw were administered with the diet, a NOEL for systemic oral toxicity was established at 150 mg/kg/day. A LOAEL for local effects was established at 220 mg/kg/day (ECHA, 2019, supporting study).

Rats (15/sex/group) were exposed by inhalation at 0, 0.22, 1.25 or 5.8 ppm (1.51 mg/m³, 8.2 mg/m³, 38 mg/m³, respectively) 6 hrs/day, 5 days/wk for 14 weeks. A 6-week recovery period was also included. Signs of ocular and respiratory irritation included swollen periocular tissue at all exposures and periocular and perinasal encrustation, cloudy eyes, and keratitis at 5.8 ppm. Colour changes or opacity of the eyes were observed in one male and six females from the 5.8 ppm group but were not present after 6 weeks of recovery. Microscopic lesions involving the eyes, nostrils, skin of the ears and eyelids, larynx, trachea, and lungs (bronchi and bronchioles) were seen at the highest exposure. The size and number of vacuoles in the mucosal epithelium increased with the duration of exposure. Decreased body weight was observed at the highest exposure. Urinalysis showed slight decreases in creatinine, sodium, potassium, and chloride at 5.8 ppm for both sexes. Changes in hematology and clinical chemistry were also noted at the highest concentration. Significant increases in male adrenal and testes weights relative to both body and brain weights were observed at 5.8 ppm, but no accompanying changes in histopathology were seen. Effects observed at the end of the recovery period included swollen periocular tissue (1.25 and 5.8 ppm) and microscopic lesions of the nasal cavity (all exposure groups). The LOAEC (for local effects) was determined to be 1.51 mg/m³ due to various signs of irritation of the eye and respiratory tract at all concentrations. The NOAEC for systemic effects was 8.2 mg/m³ (ECHA, 2019, OECD, 2012).

CMR properties

Genetic toxicity

DMAEE has shown no evidence of mutagenicity *in vitro*, in the Ames bacterial test and the mammalian cell HGPRT assay. A sister chromatid exchange assay with CHO cells gave equivocal results, while there was no evidence of genotoxicity in an *in vivo* mammalian erythrocyte micronucleus test (OECD, 2012).

Carcinogenicity

No data were available for the carcinogenicity of DMAEE.

Reproductive toxicity

Repeated inhalation exposure (14 weeks) of DMAEE by rats at concentrations of 0, 0.0014, 0.008 or 0.036 mg/L resulted in increased relative testes weights but no histopathological changes. There were no reproductive organ effects in female animals. Effects on reproductive organs were not observed in a 90-d repeated dose dermal study with rabbits. In a prenatal developmental toxicity study, pregnant rabbits were exposed to DMAEE at ca. 0, 2.4, 12 or 24 mg/kg bw/day in water via the dermal route for 6 hrs/day from gestation days 6 through 18. The NOAEL for maternal systemic and local toxicity was ca. 2.4 mg/kg bw/day based on renal lesions and severe skin effects, respectively, at higher doses. The NOAEL for developmental toxicity was ca. 12 mg/kg bw/day based on decreased mean litter weight at 24 mg/kg bw/day (OECD, 2012).

5.2.4.3 Hazard characterisation

Selection of the Point of Departure

Inhalation:
A NOAEC could not be determined for irritancy under the conditions of the study cited above. The LOAEC is 0.22 ppm (1.51 mg/m^3). The NOAEC for systemic effects after inhalation was considered to be 1.25 ppm (8.2 mg/m^3). The local effects are seen as most relevant and the LOAEC of 1.51 mg/m^3 is used as PoD.

Oral:
The NOEL for systemic toxicity of 150 mg/kg/day and the LOAEL for local effects of 220 mg/kg/day were derived from a range finding study. The LOAEL for local effects is used for the derivation of a health-based reference value.

Derivation of the Toxicological Reference Value

Inhalation:

The PoD for rats in the 14-week study may be corrected for:

1. Using the LOAEC instead of a NOAEC: A factor of 3 is used.
2. Uncertainty due to differences in exposure duration: The adverse effects observed are related to irritation of the mucous membranes of the eyes and respiratory tract. This irritation is related to concentration and not the dose. Therefore, no time scaling for differences in exposure duration and frequency is required.
3. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: For local effects, scaling for metabolic differences is not required. Remaining toxicodynamic differences between the average rat and the average human can be accounted for by a factor of 2.5.
4. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (ECHA).
5. Uncertainty due to weaknesses in the database. Since the study available is according to OECD 413 and is well reported, no correction factor is needed.

⇨ 1.51 mg/m^3 /(3x2.5x10) =0.02 mg/m^3

WoE considerations

The evidence is considered to be strong. The key study is of high quality, equivalent to OECD Guideline 413, performed under GLP, and addresses a relevant route of exposure regarding children playing with squishy toys. The critical effect, irritancy, as the most sensitive endpoint is corroborated by other available lines of evidence substantiating a toxicological MoA common to primary amines.

Oral:
The PoD for rats in the range finding study may be corrected for:

1. Using the LOAEL instead of a NOAEL: A factor of 3 is used.
2. Uncertainty due to differences in exposure duration: The adverse effects observed are related to irritation of the mucous membranes of the eyes and respiratory tract. This irritation is related to the concentration and not to the dose. Therefore, no time scaling for differences in exposure duration and frequency is required.
3. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: For local effects scaling for metabolic differences is not required. Remaining toxicodynamic differences between the average rat and the average human can be accounted for by a factor of 2.5.
4. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (ECHA).
5. Uncertainty due to weaknesses in the database. An additional factor of 10 is applied.
   => 220 mg/kg bw/(3x2.5x10x10) = 0.29 mg/kg bw

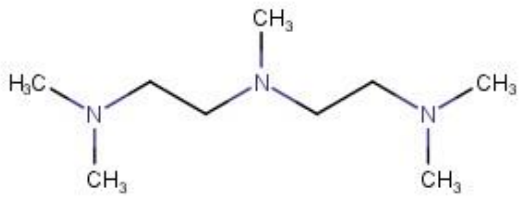
WoE considerations

The evidence is considered to be weak. The description of this range-finding study is poor and no guidance was followed. Supporting data are missing.

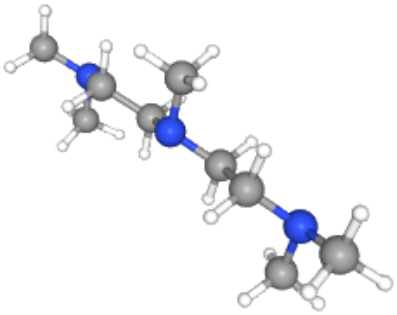
5.2.5 1,1,4,7,7-Pentamethyldiethylenetriamine (PDT)

5.2.5.1. Physicochemical information

- IUPAC name: (2-{[2-(dimethylamino)ethyl](methyl) amino}ethyl)dimethylamine
- CAS number: 3030-47-5
- Molecular formula: $C_9H_{23}N_3$
- Molecular weight: 173.3 g/mol
- Physical state: liquid
- Water solubility: 1000 g/L
- log K_{ow}: -2.1
- Vapour pressure: 27 Pa
- Density: 0.829 g/cm^3
- Boiling point: 201.84 °C
- Melting point: -20 °C
- Conversion factor: 1 ppm = 7.08 mg/m^3
- 2D Structure:



- 3D Structure:



Uses of the substance (ECHA)

ECHA has no public registered data
- indicating whether or in which chemical products the substance might be used,
- on the routes by which this substance is most likely to be released to the environment.

This substance is used
- in polymers, adhesives and sealants and coating products,
- in building and construction work and mining.
- for the manufacture of machinery and vehicles, plastic products and furniture,
- in the following activities or processes at workplace: transfer of chemicals, roller or brushing applications, non-industrial spraying, mixing in open batch processes, batch processing in synthesis or formulation with opportunity for exposure, closed batch processing in synthesis or formulation, laboratory work, the low-energy manipulation of substances bound in materials or articles, closed processes with no likelihood of exposure and high-energy work-up of substances bound in materials or articles (e.g. hot rolling/forming, grinding, mechanical cutting, drilling or sanding).

Other environment release of this substance is likely to occur from indoor use and outdoor use, resulting in inclusion into or onto a material (e.g. binding agent in paints and coatings or adhesives).

5.2.5.2. Human Health Hazard Assessment

Toxicological information

Toxicokinetics, metabolism, and distribution

According to the ECHA dossier[12], the information about toxicokinetics is very poor. No concrete study related to the toxicokinetic behaviour of the substance can be found through a literature search in free or commercial sources. Based on the fact that there are no available toxicokinetic studies and hence no specific data on the oral, dermal and inhalation absorption rates for this substance, the absorption rates used in the DNEL derivations can be the default values based on the ECHA Guidance, i.e., the oral and dermal absorption in humans becomes equal to the oral absorption in rats and the 50% absorption by oral route in rats equals to 100% absorption for inhalation in humans.

Acute toxicity, skin and eye irritation

The LD50 value for rats and oral administration was 1330 mg/kg bw (95% confidence interval: 663 to 2653 mg/kg bw). This value resulted from an 8-daystudy follow-up after administration, with both male and female rats and 5 animals per sex and per dose (doses: 3200, 1600, 800 and 200 µL/kg bw) and corresponds to the dose of 1600 µL/kg bw. At this dose, bloody crusted snouts and dyspnoea were observed, whereas all surviving animals remainedwithout symptoms. On the contrary, at the highest dose, toxicity effects included increased or irregular respiration, slight apathy and eye secretion.

Dermal toxicity was tested on the back of rabbits (the area of exposure was about 10% of the body surface and the duration of exposure 24 hours). Two doses (200 and 1000 mg/kg bw) were investigated for 5 animals per sex and per dose. The LD50 value could not be derived because skin necrosis was observed in all animals for both doses, although, in the highest dose there was also a significant mortality rate (4/5 males, 3/5 females) in the follow-up period (14 days). Other toxicity effects were a decrease of body weight for both doses and enlarged and pale kidneys or pale livers for the highest dose.

The LC50 value for rats was 290 ppm, which is equivalent to 2055.5 mg/m^3. The study was performed with 5 animals per sex and per dose (doses: 69, 164, 230 or 366 ppm) for an exposure of 6 h. Rats exposed to the highest dose died immediately following exposure or were found dead on test day 2. All rats exposed to the three lower doses survived the 14 day follow-up period. However, there was a decrease in mean body weights from pre-exposure values in all three groups during the first week post-exposure. Concentration dependent in-life observations of eye squint, corneal cloudiness, laboured breathing and porphyrin staining of the external nares and eyes indicative of eye and nasal irritation were also observed.

Studies equivalent or similar to the OECD Guideline 404 (Acute Dermal Irritation/Corrosion) and the OECD Guideline 405 (Acute Eye Irritation / Corrosion) involving two rabbits, each have resulted in the conclusion that the substance is irritating to the skin (category 1B) and the eye (category 1, i.e. irreversible effects on the eye).

Sensitisation

The substance was tested for skin sensitisation with a Buehler test on guinea pigs according to the OECD Guideline 406 (Skin Sensitisation) and it was found that it did not cause sensitisation after a challenge exposure. No data are available for respiratory sensitisation.

[12] Registration dossiers published by ECHA are submitted by registrants and are not necessarily evaluated by competent authorities.

Repeated dose toxicity

Repeated dose toxicity was studied for oral administration and inhalation.

The 90 days sub-chronic experimental study for repeated oral administration was performed with Sprague-Dawley rats according to the OECD Guideline 408. Three doses (10, 30, 100 mg/kg bw/day) were investigated. Statistically significant reductions in body weight ($p \leq 0.05$) were noted on test days 78 and 90 for the male rats at the highest dose. A marginal and statistically not significant reduction in body weight was noted for the female animals in the highest dose group. The statistically significant reduction noted for the kidney weights of the male rats in the highest dose group was considered as a secondary effect of the reduced body weight of the male animals. No changes in behaviour or the external appearance were noted for the male animals, whereas for the female animals, changes in the form of piloerection, ptosis, a reduced motility and breathing sounds were noted for 2 of 10 animals in the highest-dose group. The observations disappeared after a few days and were not considered as adverse. Finally, only one death out of 10 male animals in the highest dose group was attributed to the substance administered. As a result of the study, a NOAEL of 30 mg/kg bw/day for both male and female animals was deduced.

The short-term (sub-acute) experimental study of the substance toxicity via inhalation was similar to OECD Guideline 412 (Subacute Inhalation Toxicity: 28-Day Study) with the restriction that the exposure lasted 14 days, 6 hours/day, 5 days/week. There were 5 animals per sex and per dose in every group. Four doses were investigated (0, 3, 12, 48 ppm corresponding to concentrations of 0, 21, 85, 340 mg/m^3). An exposure-related time-dose decrease of body weight for both male and female rats was statistically identified for the 12 and 48 ppm exposure groups. Rats exposed to 12 ppm had bilateral cloudy corneas. The 48 ppm exposure group was necropsied on test day 8 because of their poor physical condition and one female rat from this exposure group was found dead on test day 7. Gross pathologic changes were bilateral cloudy corneas, decreased fat in the abdominal cavity, crusts on the external nares and ear pinnae, and bilateral chromodacryorrhea. The morphologic appearance of the histopathologic changes was primarily characterized as vacuolar degeneration of the epithelium lining the airways and covering the cornea or skin (superficial layers of the epidermis). However, even at the highest concentration tested, there was no indication of damage to organs or tissues that were not directly exposed to the substance vapours. As a result, the LOAEC was determined at 3 ppm (21.26 mg/m^3).

Genetic toxicity

No adverse effect was observed for three *in vitro* tests for mutagenicity (bacterial reverse mutation test (Ames), chromosome aberration test, mouse lymphoma assay). However, based on the available data, the substance cannot be classified as genotoxic.

Carcinogenicity

No data are available.

Reproductive and developmental toxicity

An experimental study according to OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) was performed with Wistar rats orally receiving 10, 30 and 300 mg/kg bw/day of Pentamethyldiethylenetriamine (12 animals in each group per sex and per dose).

With respect to reproduction, the male ability to produce sperm that can fertilise eggs and the female ability to achieve pregnancy was not significantly changed. Therefore, no statistically significant intergroup differences were recorded, although the total number of live pups and mean number of pups per litter at the dose level 300 mg/kg bw/day was markedly decreased in comparison with the control. The total number of live pups and mean number of pups per litter at the dose levels 30 mg/kg bw/day was similar or higher than control. The presence of stillborn pups was recorded only at the highest dose level, at which also the mortality of pups in the lactation period was recorded.

Mean body weights of litters at the dose level of 100 mg/kg bw/day were slightly decreased compared to control but markedly decreased at the dose level of 300 mg/kg bw/day. Mean weights of pups recorded at the first check of litter after parturition in treated groups decreased more than in the control group. Mean body weight increment of pup (from the first check of litter after parturition to the fourth day of lactation) was similar in the treated and control groups. The authors concluded that the NOAEL for reproduction was higher than 300 mg/kg bw/day, and for the development of pups was 100 mg/kg bw/day.

In another study performed according to OECD Guideline 414 (Prenatal Developmental Toxicity Study), Sprague-Dawley rats were administered the substance at three dose levels (20, 60, 120 mg/kg bw/day) from the 6th to the 20th day of pregnancy. Each group involved 25 animals per dose to obtain at least 20 pups for evaluation. At 120 mg/kg bw/day, signs of maternal toxicity were noted in the form of a reduced body weight and a transiently reduced food consumption. The reproductive parameters (number of implantation sites, number of resorptions and number of fetuses) were not influenced at any dose level. Moreover, no dead fetuses, no malformations, variations or retardations were noted. The authors set the NOAEL at 60 mg/kg bw/day for maternal toxicity, a value which is higher than the 30 mg/kg bw/day resulting from the repeated dose toxicity obtained above. They also deduced a NOAEL higher than 120 mg/kg bw/day for fetal development, which does not contradict the developmental study above.

5.2.5.3. Hazard characterization

Selection of the Point of Departure

Inhalation

As PoD the LOAEC of 21.26 mg/m^3 (3 ppm) can be used from the short-term (sub-acute) study of 14 days exposure. This value is based on the fact that already at 12 ppm (immediately higher concentration) there was damage in the epithelium of the airways and the cornea (local effects).

Oral

As PoD the NOAEL of 30 mg/kg bw/day can be used from the sub-chronic 90-day study with repeated oral exposure. This value is based on the fact that only at the highest dose (100 mg/kg bw/day) there was statistically significant reduction in body weight of the animals.

Derivation of the Toxicological Reference Value

Inhalation

The PoD for rats should be corrected for:

1. Uncertainty due to differences in exposure duration: the adverse effects observed are related to irritation of the epithelium lining the eyes and respiratory tract. This

irritation is related to concentration and not the dose. Therefore, no time scaling for differences in exposure duration and frequency are required.
2. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: For local effects scaling for metabolic differences is not required. Remaining toxicodynamic differences between the average rat and the average human can be accounted for by an assessment factor of 2.5 (AF1).
3. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by an assessment factor of 10 (AF2).
4. Uncertainty due to weaknesses in the database. Since the key study available is similar to OECD Guideline 412, it is of good quality and no correction factor is needed.
5. Extrapolation from LOAEC to NOAEC can be accounted for by introducing an assessment factor of 3 (AF3).

Therefore,

LOAEC: 21.26 mg/m^3
DNEL = LOAEC/(AF1 × AF2 × AF3)
AF1 (interspecies animals to human) = 2.5
AF2 (intraspecies, difference in human sensitivity) = 10
AF3 (extrapolation LOAEC to NOAEC) = 3
DNEL = 21.26 mg/m^3/(2.5 × 10 × 3) = 0.283 mg/m^3 (283 µg/m^3)

and the DNEL for local effects of Pentamethyldiethylenetriamine would be 283 µg/m^3.

WoE considerations

The rat study (1988) used to derive the DNEL for local effects due to inhalation of the substance is a GLP study performed in a manner that is equivalent or similar to OECD Guideline 412 (Subacute Inhalation Toxicity: 28-Day Study). This study is of good quality and the observed histopathologic changes were indicative of nonselective localized irritation to tissues at risk by exposure to a sufficient vapour concentration of the substance, as expected. However, SCHEER considers the WoE for local effects as moderate, because it is based on a single study, even if these effects observed were treatment-related.

Oral

The PoD for rats should be corrected for:

1. Uncertainty due to differences in exposure duration: the adverse systemic effects in rats were observed in a repeated sub-chronic study; therefore, no correction factor is needed.
2. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: For systemic effects, the allometric factor for differences in metabolic rate is 4 from rats to humans and the remaining toxicodynamic differences between the average rat and the average human can be accounted for by a factor of 2.5. Therefore, the interspecies assessment factor can be considered as 4 × 2.5 = 10 (AF1).
3. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by an assessment factor of 10 (AF2).
4. Uncertainty due to weaknesses in the database. Since the key study was conducted according to OECD Guideline 408, it was of good quality and no correction factor is needed.

Therefore,

NOAEL: 30 mg/kg bw/day
DNEL = NOAEL/(AF1 × AF2)
AF1 (interspecies animals to human) = 10
AF2 (intraspecies, difference in human sensitivity) = 10
DNEL = 30 mg/kg bw/day/(10 × 10) = 0.3 mg/kg bw/day (300 µg/kg bw/day)

and the DNEL for systemic effects of Pentamethyldiethylenetriamine would be 300 µg/kg bw/day.

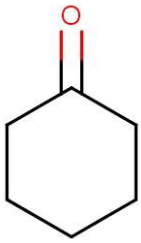
WoE considerations

The rat study (2016) used to derive the DNEL for systemic effects via the oral route of exposure is a GLP study performed according to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) and according to EU Method B.26 (Sub-Chronic Oral Toxicity Test: Repeated Dose 90-Day Oral Toxicity Study in Rodents). It is classified with reliability 1 (reliable without restrictions) in the REACH registration dossier. The study is of good quality and adequate. Its results are supported by the reported body weight loss of rats exposed to high vapuor concentrations via inhalation in the study of local effects. Therefore, SCHEER considers the WoE for systemic toxicity as strong.

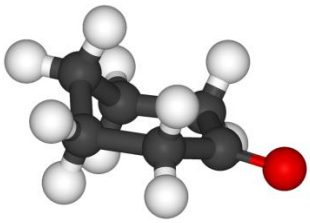
5.2.6 Cyclohexanone (CH)

5.2.6.1. Physicochemical information

- IUPAC name: cyclohexanone
- CAS number: 108-94-1
- Molecular formula: $C_6H_{10}O$
- Molecular weight: 98.14 g/mol
- Physical state: liquid
- Water solubility: 86 g/L
- log P_{ow}: 0.86
- Vapour pressure: 700 Pa
- Density: 0.948 g/cm^3
- Boiling point: 154.3 °C
- Melting/freezing point: -31 °C
- Conversion factor: 1 ppm = 4.01 mg/m^3
- 2D Structure:



- 3D Structure:



Uses of the substance (ECHA)

This substance is manufactured and/or imported in the European Economic Area in 1 000 000 - 10 000 000 tonnes per year.

Consumer Uses

This substance is used in the following products: coating products, inks and toners, adhesives and sealants, biocides (e.g. disinfectants, pest control products), plant protection products and polymers. Cyclohexanone emission has been reported from different polymer-based toys, other than squishy toys, such as plopper figurines (Even et al., 2019) and costume masks (Even et al., 2021).

Other release to the environment of this substance is likely to occur from: outdoor use and indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners).

Professional Use

Cyclohexanone is used for the manufacture of machinery and vehicles and furniture.

The substance is used in: adhesives and sealants, coating products, inks and toners, plant protection products, fillers, putties, plasters, modelling clay, laboratory chemicals and biocides (e.g. disinfectants, pest control products).

The substance is used in the following areas: building and construction work, printing and recorded media reproduction and agriculture, forestry and fishing.

5.2.6.2. Human Health Hazard Assessment

Toxicological information
Toxicokinetics, metabolism, and distribution (ATSDR, 2009; US-EPA, 2010, MAK 2010)

- Cyclohexanone is absorbed via lung and skin, no data was identified on gastrointestinal absorption.

- Human percutaneous absorption was evaluated through immersion of a hand in cyclohexanone. The permeation rate (of a hand) of cyclohexanone is calculated, with a

mean of 0.056 (0.037 - 0.969) mg / cm² h. Via inhalation, cyclohexanone was rapidly absorpted, cyclohexanone is absorbed rapidly via the lungs. A steady state developped at an experimental exposure of 25, 50 or 100 ml cyclohexanone/m^3 (dose independent), and the retention stayed constant over 1, 3 and 5 hours – retention rate was calculated as 58%. Uptake via inhalation is marked as the main route.

- Cyclohexane is metabolised (cyclohexanol, 1,2-cyclohexanediol and 1,4-cyclohexanediol) and excreted via the urine after glucuronidation.
- At an air exposure of 207 mg/m^3, the metabolic yields of cyclohexanol, 1,2- and 1,4 - cyclohexanediol were 1%, 39% and 18%, respectively. The elimination half-times (t½) of the 1,2- and 1,4-diols, respectively, were 16 h and 18 h. Upon repeated exposure to cyclohexane (5 consecutive days), a maximum urinary excretion rates of the metabolites cyclohexanediols was found on days 2 & 3.

TOXICITY

Acute Effects

The registration dossier available at the ECHA[13] dissemination database reports an LD50 for acute oral toxicity in the rat that is between 1890 (test with 2 - 50% aqueous emulsion with traganth) and 2650 mg/kg (test with solution in olive oil). Clinical signs were prone and lateral position and narcosis. Pathology showed no abnormal findings. An LC50 value of > 6.2 mg/l/4 hours for rat (both sexes) was found in an acute inhalation study. No mortality was reported, clinical signs were watery eye and nose secretion, intermittent and accelerated breathing, apathetic, narcosis and scrubby fur – all these symptoms remained until day of sacrifice. These data would not indicate a classification for this exposure route.

Other studies:

Smyth et al. (1969) found an acute oral LD50 value of 1620 mg/kg for male rats (gastric intubation).

The oral LD50 for cyclohexane in rats ranges from 8.0 to 39 mL/kg (both greater than 5 g/kg), depending upon the age of the animals. The oral LD50 for mice is 1.3 g/kg; the minimum lethal oral dose in rabbits is 5.5-6.0 g/kg; and the dermal LD50 in rabbits is >180 g/kg (Longacre 1987).

Skin irritation

Application of 1.55 g/day of cyclohexane to the skin for 2 days produced minimal irritation. In humans, it is irritating to the eyes at 300 ppm; undiluted cyclohexane is also irritating to the skin (Longacre 1987).

Oral Exposure

Subchronic and chronic Studies

Lijinsky and Kovatch (1986) conducted 2-year drinking water studies in rats and mice. In the rat study, groups of 52 male and 52 female F344 rats were treated with cyclohexanone (96% purity) in acidified drinking water at 0, 3300, or 6500 ppm for 2 years. In the study, an interim evaluation after 25 weeks of exposure was included (only 5 animals per group). The main observation in the 25-week study was a 10% decrease in weight gain compared to controls in the high dose group (6500ppm). The second highest dose was therefore considered a NOAEL (4700ppm) Lijinsky and Kovatch (1986) reported that high-dose rats exhibited significant decreases in weight gain compared to controls. Based on the weight curves reported by the authors, high-dose rats of both sexes experienced an estimated

[13] https://echa.europa.eu/registration-dossier/-/registered-dossier/15388/7/3/1

body-weight deficit of >30% (in comparison to controls) at study termination. No change in weight gain was noted in the lower dose group (3300ppm of 462 mg/kg). No treatment-related nonneoplastic lesions were observed among either treatment group.

In the companion mice (B6C3F1) study, 41 or 47 mice were dosed per group. In this study, also an interim evaluation was included, after 13 weeks of exposure (10 animals per group). The highest dose (47000ppm) induced a lethal response, some doses induced coagulative liver necrosis, and hyperplasia of the thymus. A NOAEL of 13,000 ppm and 25,000 ppm was established for males and females respectively. Body weights of high-dose mice of both sexes were decreased by approximately 15−20% compared to controls during most of the study. Body weights were only slightly depressed among mid-dose female mice and were comparable to controls among low-dose mice of both sexes. Lymphoid hyperplasia and lymphocytic infiltrates were common in lymph nodes, spleen, salivary gland, kidneys, pancreas, lungs, and meninges of the brain and spinal cord of most control and treated female mice in this study. The lymphatic lesions in control and treated females were considered a potentially confounding observation, and effect levels for females were not defined. For male mice, the low dose of 1530 mg/kg-day (6500 ppm) was considered without adverse effects.

Inhalation Exposure

Subchronic Studies

Rabbits (sex not specified) were exposed by inhalation to 0, 190, 309, 773, 1414, or 3082 ppm [converted to 0, 763, 1241, 3103, 5677, or 12,373 mg/m^3] cyclohexanone for 6 hours/days, 5 days/week, for 10 weeks (all other groups) (Treon et al., 1943). At the highest concentration, 2/4 rabbits died, and clinical signs such as narcosis, laboured breathing, loss of coordination, weight loss, and hypothermia were observed after 3 weeks of exposure. Rabbits exposed to 309-ppm (1241-mg/m^3) cyclohexanone only exhibited very slight conjunctival congestion, and no clinical signs or effects on body weight were observed among rabbits exposed at the lowest concentration. Lower exposures (309 mg/kg and lower) did not induce lesions (US EPA, 2010). No significant hematological changes were observed at any concentration of the 10-week exposure protocol. Two months after the end of exposure, pathology revealed "barely demonstrable" degenerative changes in liver and kidneys.

A recent study (Lim et al 2018) exposed 10 male and 10 female rats and mice per group to cyclohexanone vapors at 0, 100, 250, and 625 ppm for 6 h per day, 5 d per week, for 13 weeks. Cyclohexanone-exposed F344 male rats showed increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, increased liver weight, and bile duct hyperplasia at 250 and 625 ppm cyclohexanone. Female rats showed increased ALT levels and bile duct hyperplasia at 625 ppm cyclohexanone, and increased blood urea nitrogen (BUN) and tubular basophilia in the renal cortex in the males exposed to 625 ppm cyclohexanone. B6C3F1 mice exposed to cyclohexanone showed no obvious exposure-related effects. The NOAEC was determined to be 100 ppm in F344 rats and >625 ppm in B6C3F1 mice.

Carcinogenicity

The International Agency for Research on Cancer (IARC, 1999, 1989) classified the carcinogenicity of cyclohexanone in Group 3 (*Not Classifiable As to Human Carcinogenicity*) based on lack of human cancer data and inadequate evidence of carcinogenicity in animals.

5.2.6.3 Hazard characterization

Selection of the Point of Departure for the inhalation:

A study of Lim et al (2018) exposing rats (more sensitive compared to mice) to cyclohexanone vapors, shows a NOAEC of 100 ppm in a 13 week study.

DNEL derivation for inhalation: the PoD should be corrected for:

1. The PoD is corrected for the exposure regime in the toxicity study being 6 hours per day, 5 days per week, resulting in a correction factor of (5/7) × (6/24) (AF1).
2. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: The liver and bile effect are observed after respiratory absorption. Therefore both toxicodynamics and toxicokinetic uncertainty should be taken into account - a factor of 10 (2.5x4) (AF 2)
3. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (AF 3).

NOAEC: 100 ppm OR 401 mg/m³

DNEL = NOAEC (AF1) / (AF2 x AF3)

AF1 (correction for non-contineous exposure) (5/7) × (6/24)

AF2 (interspecies animals to human) = 10

AF3 (intraspecies, difference in human sensitivity) = 10

$DNEL_{inhalation}$ = 100 ppm ((5/7)x(6/24))/ (10 x 10) = 0.179 ppm = 0.716 mg/m³

WoE considerations
The Lim et al (2018) study was conducted in a correct way, shows a clear difference between rats and mice. The dosing groups were sized in a sufficient way and the endpoints were clearly described. The study is close in line with the Treon et al (1943) study. The **evidence** is therefore considered **moderate to strong**.

Point of Departure for the oral intake

Lijinsky and Kovatch (1986) conducted 2-year drinking water studies in rats and mice, including an evaluation after 13 weeks exposure in mice and after 25 weeks of exposure in rats (groups of 5 animals). The highest dose in rats (1010 mg/kg/d) showed significant decreases in body weight gain compared to controls. No change in weight gain was noted in the lower dose group (**731 mg/kg/d**). No treatment-related non-neoplastic lesions were observed among either treatment group in the 2-year study.

DNEL derivation for oral intake: the PoD should be corrected for:

1. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: scaling for metabolic and toxicokinetic differences between the average rat and the average human can be accounted for by a factor of 10 (AF1).
2. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (AF2).

3. An additional factor of 3 is considered since only 5 rats were included per dosing group.

NOAEL: 731mg/kg bw/d

DNEL = NOAEL/(AF1xAF2xAF3)

AF1 (interspecies animals to human) = 10 (for systemic effects)

AF2 (intraspecies, difference in human sensitivity) = 10

AF3 (low confidence) = 3

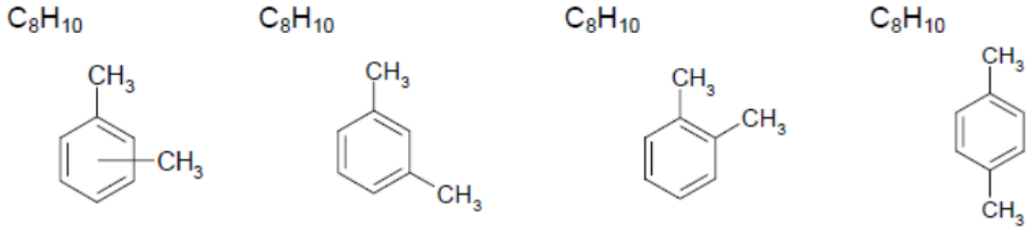
$DNEL_{oral}$ = 731mg/kg bw/d/(10x10x3) = 2.44 mg/kg bw/d

WoE considerations
The Lijinsky and Kovatch, 1986 is a good/correct study considering the 2-year exposure (carcinogenesis) including both rat and mice of both sexes and including enough animals per group (**strong**). The shorter-term study, 13 and 25 weeks for respectively mice and rats, only includes 5 animals per sex which is considered as moderate/weak, therefore data from the 2-year study (**strong**) was included.

5.2.7 Xylenes (X)

5.2.7.1. Physicochemical information

- IUPAC name: mixture of 1,2-xylene; 1,3-xylene; 1,4-xylene
- CAS number: 1330-20-7
- Molecular formula: C_8H_{10} (each isomer)
- Molecular weight: 106.17 g/mol
- Physical state: liquid
- Water solubility: 106 mg/L
- log P_{ow}: 3.16
- Vapour pressure: 1065 Pa
- Density: 0.864 g/cm^3
- Boiling point: 138.5 °C
- Melting/freezing point: -47.4 °C
- Conversion factor: 1 ppm = 4.37 mg/m^3
- 2D Structure:



General appearance

It is a colourless, flammable liquid with a sweet odour. Xylene evaporates and burns easily. Xylene does not mix well with water; however, it does mix with alcohol and many other chemicals.

There are three forms of xylene in which the methyl groups vary on the benzene ring: meta-xylene, ortho-xylene, and para-xylene (m-, o-, and p-xylene) (see figure above). Xylene is primarily a synthetic chemical. Chemical industries produce xylene from petroleum. Xylene also occurs naturally in petroleum and coal tar and is formed during forest fires (and smoking), to a small extent.

Most people begin to smell xylene in air at 0.08–3.7 parts of xylene per million parts of air (ppm) and in water at 0.53–1.1 ppm.

Uses of the substance (ECHA)

This substance is manufactured and/or imported in the European Economic Area at a volume of 1 000 - 10 000 tonnes per year.

This substance is used in the following consumer products: lubricants and greases, anti-freeze products, biocides (e.g. disinfectants, pest control products), polishes and waxes and adhesives and sealants.

Recently it has been reported that xylenes emitted from polymer-based toys such as plopper figurines (Even et al., 2019) and costume masks (Even et al., 2021).

For professional use:

- Xylenes are applied in: fuels, coating products, fillers, putties, plasters, modelling clay and plant protection products.
- Xylenes is used in the following areas: building and construction work, agriculture, forestry and fishing, health services and scientific research and development.
- This substance is used for the manufacture of chemicals and fabricated metal products.

5.2.7.2. Human Health Hazard Assessment

Toxicological information

Toxicokinetics, metabolism, and distribution

Xylenes, because of their lipophilic properties, are rapidly absorbed by all routes of exposure, rapidly distributed throughout the body, and, if not metabolized, quickly eliminated in exhaled air. In humans, absorption has been estimated as >50% through the lungs following inhalation exposure and <50% through the gastrointestinal system. (In humans exposed by inhalation, up to 2% of the absorbed dose may be absorbed through the skin.)

The major pathway for metabolism involves mixed function oxidases in the liver, resulting mainly in the formation of isomers of methylhippuric acid that are eliminated in the urine and are used as an index of exposure for occupational monitoring.

The target organs and adverse health effects of xylenes are similar across species. Toxicokinetic studies have been performed in humans, rats, mice, rabbits, and monkeys. There is reasonable correlation between the end points examined in these studies. The metabolism of *m*-and *p*-xylenes is similar in rats and humans. However, a difference in the metabolism of *o*-xylene in rats and in humans exists. Whereas *o*-xylene is almost exclusively metabolized to *o*-methylhippuric acid in humans, 10–56% of *o*-xylene is also conjugated by glucuronide and glutathione in rats. Toxic metabolic intermediates of xylene such as benzaldehyde found in rats have not been found in humans.

No sex-related difference in excretion in men and women occupationally exposed to xylenes has been reported: sex-related differences in the toxicokinetics of xylene have been identified in animals.

No data are available regarding the effects of exposure to xylenes in children, but it is expected that children would experience the same effects as exposed adults. The lipophilic properties of xylenes suggest that the absorption and distribution in children are likely to be similar to those of adults (ATSDR, 2007).

TOXICITY

Acute lethal toxicity
The registration dossier available at the ECHA[14] dissemination database reported an oral LD50 of 3 523 mg/kg bw in rat and an inhalation LC50 of 6700 ppm in rat.

Acute toxicity: irritation
Air exposure at 50 ppm in human subjects: xylenes produce irritant effects on the eyes, skin, and mucous membranes; impaired respiratory function; and mild central nervous system effects, including headache and dizziness (ATSDR, 2007).
In rats, dermal exposure to m-xylene, o-xylene, or mixed xylenes at 2.3 mg/kg resulted in skin irritation (erythema and edema) and more serious effects (eschar formation in some animals and epidermal thickening) at topical doses of ≥114 mg/kg. Rat skin that developed moderate erythema after treatment with *m*-or *o*-xylene exhibited increases in transepidermal water loss and increases in pro-inflammatory cytokines (interleukin 1-alpha and tumor necrosis factor-alpha – TNF-α).

Sensitisation

No full study report available, but Basketter et al (1996) showed the sensitisation potential of mixed xylenes when tested in the LLNA. They applied 100% of mixed xylenes (topical treatment of mice) and reported an SI = 3.1. This result was somewhat questioned by the same authors in 1999 (Basketter et al, 1999), as they conclude that a SI of 3.5 3.5 would lead to greater specificity in the interpretation of LLNA results.
It is also important to mention that despite the widespread use of mixed xylenes, no human cases have been identified.

[14] https://echa.europa.eu/registration-dossier/-/registered-dossier/15448/7/3/2

Repeated dose toxicity

ORAL

Hepatic effects in laboratory animals exposed orally at ≥750 mg/kg/day or by inhalation at ≥300 ppm include increases in liver weight, serum enzyme levels, and cytochrome P-450 levels, but no histopathological changes were reported.

Wolfe (1988) studied subchronic toxicity in groups of 50 male and 50 female Fischer 344 rats and 50 male and 50 female B6C3F1 mice. These animals were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 9.1% o-xylene, 17.0% ethylbenzene) in corn oil by gavage at doses of 0, 250, or 500 mg/kg- Body weight gains over the entire study period decreased ($p <= 0.05$) in mid- and high-dose males (89% and 75% of controls', respectively) and high-dose females (85% of controls') rats. A thorough histologic examination revealed no other abnormal findings in these rats. In male and female B6C3F1 mice, hyperactivity was noted immediately after oral gavage dosing 5 days/week with 1,000 mg/kg (710 mg/kg/day duration adjusted) with mixed xylene, beginning at week 4 of the 103-week NTP (1986). Hyperactivity was not observed at 500 mg/kg (360 mg/kg/day, duration-adjusted) LOAEL (710 mg/kg/day, adjusted for intermittent exposure), and a NOAEL of 500 mg/kg (360 mg/kg/day, duration-adjusted).

Inhalation

Cardiovascular effects (increased thickness of coronary microvessels) were observed in rats exposed to 230 ppm mixed xylenes 6 hours/day, 5 days/week for 4 weeks (Morvai et al. 1987). Hepatic effects (increased liver weight) were observed at a LOAEL of 600 ppm in rats discontinuously exposed to mixed xylenes for 4 weeks (Toftgard et al. 1981). In rats exposed gestationally to mixed xylenes or o-xylene, a LOAEL of 500 ppm was identified for decreased fetal body weights in the absence of maternal toxicity (Bio/dynamics 1983). LOAELs for adult body weight effects were 1,000 ppm or higher (Tatrai et al. 1981). Increased deaths among squirrel monkeys and rats were noted following discontinuous intermediate-duration exposure to 780 ppm o-xylene (Jenkins et al. 1970), but no systemic effects were noted in rats or dogs exposed to 810 ppm mixed xylenes (Carpenter et al. 1975a).

Korsak et al. (1992) rats exposed to m-xylene alone exhibited statistically significantly decreased rotarod performance and decreased spontaneous activity, as measured 24 hours after termination of the exposures, when compared with controls. The percentages of failures in the rotarod test were roughly 60% in rats exposed to 1000 ppm for 3 months, 35% in rats exposed to 100 ppm for 6 months, and 0% for controls at either time period. The mean spontaneous motor activity in rats exposed to 100 ppm for 6 months was about 400 movements per hour, compared with about 800 movements per hour for controls. Similar results were noted in Korsak et al. (1994) exposing rats to m-xylene. In a neurobehavioral assay, a LOAEL of 50 ppm was identified for reduced mean latency of the paw-lick response (indicative of increased sensitivity to pain) in rats exposed to m-xylene for 3 months (Korsak et al. 1994).

Chronic occupational exposure of workers to an unspecified concentration of vapours of mixed xylene has also been associated with laboured breathing and impaired pulmonary function (Hipolito 1980; Roberts et al. 1988). A significant ($p<0.01$) increase in the prevalence of nose and throat irritation was reported by workers chronically exposed to mixed xylene vapours at a geometric mean TWA concentration of 14 ppm (Uchida et al. 1993).

Neurotoxicity

The neurotoxicity of xylenes has been examined in short- and long-term inhalation studies

in humans and animals and in acute oral studies in animals (interference of metabolized xylene with neuronal membranes.)
Mild central nervous system effects (subjective symptoms of intoxication, headache, fatigue, and dizziness) have been observed following acute exposure of humans to m-xylene at 50 ppm and chronic-duration occupational exposure to mixed xylene at 14 ppm.
Results of experimental studies with humans indicate that acute inhalation exposure to 100 ppm mixed xylene Korsak et al. (1992) or 200 ppm m-xylene Wolfe (1988 causes impaired short-term memory, impaired reaction time, performance decrements in numerical ability, and alterations in equilibrium and body balance.

Carcinogenicity
There is no definitive evidence for carcinogenic effects of xylene in humans. Epidemiological studies looking for associations with xylene exposure and specific cancers either reported no cases or a limited number of cases exposed to xylene and/or reported concurrent exposure to multiple solvents. Two-year cancer bioassays in rats and mice exposed by oral gavage provided no evidence for carcinogenicity of mixed xylene. The Department of Health and Human Services (DHHS) has not classified xylene as to its carcinogenicity. Both IARC and EPA have determined that xylene is not classifiable as to its carcinogenicity in humans, due to inadequate evidence for the carcinogenicity of xylenes in humans and animals.

Development toxicty
In general, developmental studies in animals reported adverse fetal effects only at concentrations that caused maternal toxicity.
No reproductive effects were found in rats following inhalation of 500 ppm xylene before mating and during gestation and lactation. Histopathological examination following intermediate and chronic oral bioassays revealed no adverse effects on the reproductive organs of rats and mice dosed with mixed xylene 5 days/week at 800 and 1,000 mg/kg/day, respectively.

5.2.7.3 Hazard characterization

PoD for the inhalation and DNEL derivation:

The 3-month exposure study of Korsak et al. (1994) derived a LOAEL of 50 ppm for m-xylene in rats for neurologic effects.

The PoD for rats for inhalation should be corrected for:

1. The POD is corrected for the exposure regime in the toxicity study being 6 hours per day, 5 days per week, resulting in a correction factor of (5/7) × (6/24) (AF1).
2. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: The neurologic effects are systematic effect due to respiratory absorption. Therefore both toxicodynamics and toxicokinetic uncertainty should be taken into account - a factor of 10 is applied (2.5x4) (AF2)
3. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (AF3).
4. A LOAEC was derived from the study; a factor of 3 is reccommended for deriving a NOAEL from the experimental LOAEC. (AF4)

LOAEL: 50 ppm OR 218.5 mg/m³

DNEL = NOAEC (AF1)/(AF2 x AF3 x AF4)

AF1 (correction for non-contineous exposure) (5/7) × (6/24)

AF2 (interspecies animals to human) = 10

AF3 (intraspecies, difference in human sensitivity) = 10

AF4 (LOAEL to NOAEL) = 3

$DNEL_{inhalation}$ = 50 ppm ((5/7) × (6/24)) / (10 x 10 x 3) = 0.020 ppm = 0.130 mg/m³

WoE Considerations

In line with the consistency among the different studies in animals and humans, inhalation and neurotoxicity can be considered as an important route and target in xylene toxicity. The key studies of Korsak (1992 and 1994) were well conducted The study of the critical effect (neurotoxicity) in the Korzak 1994 was based on the findings of 1992 and it provides the lowest LOAEC/NOAEC, but no sensitive testing for neurological endpoints was applied, therefore the WoE is considered **moderate.** The study of Uchida et al. 1993 reported an increase in the prevalence of nose and throat irritation by workers at chronical exposure to mixed xylene vapours at a geometric mean TWA concentration of 14 ppm, although this is considered to be a valid observation, the studies of Korsak were considered to be more robust and controlled.

PoD for oral exposure and DNEL derivation

No study focussing on neurologic endpoints after exposure to mixed xylenes for intermediate multiple exposure could be identified. One study, in male and female B6C3F1 mice, noted hyperactivity immediately after oral gavage dosing 5 days/week with 1,000 mg/kg (710 mg/kg/day duration adjusted) with mixed xylene beginning at week 4 of the 103-week NTP (1986). Hyperactivity was not observed at 500 mg/kg (360 mg/kg/day, duration-adjusted) LOAEL (710 mg/kg/day, adjusted for intermittent exposure), and a NOAEL of 500 mg/kg (360 mg/kg/day, duration-adjusted)

1. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences- both toxicodynamics and toxicokinetic uncertainty should be taken into account - a factor of 10 is applied (2.5x4) (AF 1)
2. Intraspecies variability: the variability between the average human and sensitive humans can be accounted for by a factor of 10 (AF 2).
3. A modifying factor of 10 for the lack of testing for sensitive neurological endpoints was applied (AF3)

LOAEL: 360 mg/kg bw/d

DNEL = NOAEC/(AF1 x AF2 x AF3)

AF1 (interspecies animals to human) = 10

AF2 (intraspecies, difference in human sensitivity) = 10

AF3 (LOAEL to NOAEL and no specific endpoints) = 10

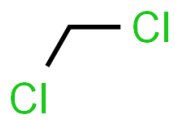
$DNEL_{oral}$ = 360 mg/kg bw/d / (10 x 10 x 10) = = 0.36 mg/kg bw/d

WoE oral
In the oral toxicity studies, no specific neurotoxicity endpoints have been evaluated. One study (NTS 1986) is a high quality, well conducted study in both mice and rats. In male and female B6C3F1 mice, noted hyperactivity immediately after oral gavage dosing 5 days/week with 1,000 mg/kg (710 mg/kg/day duration adjusted) with mixed xylene beginning at week 4 of the 103-week NTP (1986). Hyperactivity was not observed at 500 mg/kg (360 mg/kg/day, duration-adjusted) LOAEL (710 mg/kg/day, adjusted for intermittent exposure), and a NOAEL of 500 mg/kg (360 mg/kg/day, duration-adjusted). The NTS-study (1986) is considered a good study but without refined endpoints for neurotoxicity and the WoE is therefore considered **moderate**.

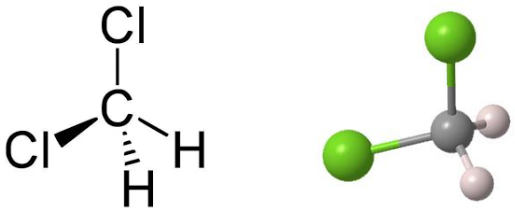
5.2.8 Dichloromethane, methylene chloride (DCM)

5.2.8.1. Physicochemical information

- IUPAC name: dichloromethane
- CAS number: 75-09-2
- Molecular formula: CH_2Cl_2
- Molecular weight: 84.93 g/mol
- Physical state: volatile liquid
- Water solubility: 13.2 g/L
- log P_{ow}: 1.25
- Vapour pressure: 47.4 kPa
- Density: 1.33 g/cm^3
- Boiling point: 40 °C
- Melting/freezing point: - 97 °C
- Conversion factor 1 ppm = 3.47 mg/m^3
- 2D Structure:



- 3D Structure:



Uses of the substance

Due to its physical properties, it is a widely used industrial solvent with a worldwide production of several hundred thousand tonnes/year. The white paper on dichloromethane (DCM) by the Halogenated Solvents Industry Alliance in March 2008 (40) lists its uses as: paint removal (wood and metal); formulated product (adhesives, foam production, aerosols); pharmaceutical manufacture (solvent for reactions, re-crystallisations and extractions, carrier for tablet coatings); chemical processing (manufacture of polycarbonate resin and cellulose triacetate, solvent welding of plastics,
In the REACH registration dossier, this substance is used as adhesives and sealants, biocides (e.g. disinfectants, pest control products), coating products, plant protection products, washing & cleaning products and cosmetics and personal care products.

Since 6 December 2011, a REACH restriction (entry 59, Annex XVII) has been applicable to the use of dichloromethane in paint strippers in a concentration equal to or greater than 0,1% by weight, establishing that products are not allowed to be placed on the market for supply to the general public.

EPA risk evaluation for DCM (2020) determined (among other uses) unreasonable risk for "Industrial and commercial use in toys, playground and sporting equipment" based on cancer and non-cancer effects (CNS and liver).

Hazard classification and Labelling

The European Union Harmonised Classification and Labelling as Carc. 2 H351 has been assigned to dichloromethane- index number: 602-004-00-3, Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation). In addition, a CLH proposal to reclassify this substance as carc. 1B H350 is intended to be submitted to ECHA by the end of 2021.

5.2.8.2 Human Health Hazard Assessment

Toxicological information

The hazard assessment performed is mainly based on animal and human data from the evaluations by IPCS/WHO (1996); SCCS (2012, 2015) and EPA (2011, 2020).

Toxicokinetics, metabolism, and distribution

Dichloromethane (DCM) is rapidly and extensively absorbed from the lungs into the systemic circulation (uptake in humans 70-75%) and is well absorbed from the gastrointestinal tract of animals (uptake 97%).
Liquid DCM can be absorbed via the skin (absorption rate in mice 6.6 mg/cm^2/h). However, due to its high volatility, this route of exposure is of less significance than other routes of exposure under non-occlusive conditions. Dermal absorption of DCM vapour in rats is not significant. DCM is distributed to many organs, including liver, kidney, lungs, brain, muscle and adipose tissue, after respiratory and oral exposure.
DCM is quite rapidly excreted after oral exposure, mostly via the lungs in the exhaled air. It can cross the blood-brain barrier and be transferred across the placenta, and small amounts can be excreted in urine or in milk. At high doses, most of the absorbed DCM is exhaled

unchanged. The remainder is metabolized to carbon monoxide, carbon dioxide and inorganic chloride, whereby two routes of oxidative metabolism have been identified, one mediated by cytochrome P450 (predominantly in humans) and the other by glutathione-S-transferase (especially in mice).

Acute toxicity, Skin and eye irritation

The available animal LD50 values (oral, 2000 mg/kg bw, rat; dermal, 2000mg/kg bw/d, rat) and the calculated 4-h inhalation LC50 of 49 mg/L air (mouse) indicate that the acute toxicity of dichloromethane is low.
In man, due to its volatile properties, inhalation is the primary route of exposure, where it can cause slight irritation to the upper respiratory tract with signs of mild depression of the central nervous system (CNS) such as dizziness, nausea, inability to concentrate and reduced coordination. Exposure to high concentrations may result in unconsciousness, pulmonary oedema, respiratory failure and death; hyperbaric oxygen therapy has been used to treat acute intoxication. Neurotoxicity is the main effect of an acute inhalation dose of dichloromethane in humans. Dependent on dichloromethane concentration and exposure time, carbon monoxide is formed by oxidative metabolism being an agent that depresses CNS by forming an adduct with haemoglobin (carboxyhaemoglobin, COHb) (SCCS, 2012).

Animal studies indicated that neat DCM is a skin and eye irritant. In humans, dichloromethane was shown to be corrosive to the eye and respiratory tract (Zarrabeitia et al., 2001). Dichloromethane is classed as a moderate to severe irritant and can cause second and third degree burns if contact is prolonged. Repeated low-level skin contact may result in dermatitis (redness and irritation). Eye contact with dichloromethane vapour may cause mild to severe irritation depending on the concentration while the liquid may cause temporary damage to the cornea.

Skin sensitisation

No adverse effects were observed in a (LLNA) skin sensitisation study (not sensitising).

Repeated dose toxicity

Systemic toxicity

Oral

IPCS WHO (1996) reviewed a few oral studies. In rats, oral administration of dichloromethane in drinking water (125 mg/L for 13 weeks) did not result in any adverse effects (the concentration in drinking water was equivalent to 17.5 mg/kg bw/day, assuming a rat body weight of 350 g and an intake of 0.049 litres water/day). When dichloromethane was administered in the drinking water to rats and mice for 3 months, slightly decreased body weights and histopathological changes in liver were noted in both species from a concentration equivalent to approximately 607 and 226 mg/kg bw/day for rats and mice, respectively.) Overall the observed effects in rats and mice by oral route showed that the liver is the target organ.
The 2-year NOAEL for oral toxicity was 6 mg/kg bw/day in rats (Serota et al, 1986a,b), based on increased incidence of foci/areas of cellular alteration and fatty changes in the liver (OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies).

Dermal

Dichloromethane can be absorbed across the skin in man and animals. Studies on the permeability of rat and mouse skin to dichloromethane *in vitro* and *in vivo* have shown transdermal fluxes ranging from 2.7-6.6 mg/cm²/h (the corresponding value in man is 2.4mg/cm^2/h (Ursin et al., 1995). Studies where dichloromethane was applied to human skin *in vitro* show rapid absorption and skin exposure could potentially make a significant contribution to the total exposure to dichloromethane. Dermal absorption depends on the type of skin and surface area and the duration of exposure. Due to its capacity for absorption by the dermal route, SCOEL has given dichloromethane a 'skin' notation (SCOEL, 2009).

Inhalation

The 2-year NOAEC for non-cancer inhalation toxic effects in rats was 200 ppm (695 mg/m^3) based on histopathological changes in the liver (Nitschke et al, 1988). Overall the observed effects in rats by inhalation route showed that the liver is the non-cancer target organ.
The EPA report (2011) includes additional well-conducted chronic inhalation studies that consistently identified the liver as the most sensitive non-cancer target organ in rats (NTP, 1986; Burek et al., 1984).
No evidence of adverse effects on health has been found at the workplace following occupational exposure concentrations of about 100 ppm DCM (353 mg/m^3) over several years. Therefore, 100 ppm (353 mg/m^3) was considered by SCOEL to be a clear no-observed-adverse-effect concentration (NOAEC) for repeated dose toxicity of dichloromethane in these settings. This concentration was used to set a 8 h TWA OEL value, being the STEL (15mn) value of 200 ppm (706mg/m^3) to prevent acute neurotoxic effects (SCOEL 2009).
Peterson (1978) (cited in ATSDR, 2000) showed that for exposure to 50 ppm (178mg/m^3) of dichloromethane for 5 weeks, the primary effects are neurological.

Genetic Toxicity

IPCS/WHO (1996) and IARC (1999), reviewed numerous mutagenicity and genotoxicity tests performed on bacteria, fungi and cultured mammalian cells as well as a number of *in vivo* studies on mice and rats. The results of the studies with dichloromethane (methylene chloride) have been summarised by SCOEL as follows: "Methylene chloride is consistently mutagenic in microorganisms. Weaker and less consistent responses are seen in mammalian systems. Methylene chloride induced sister chromatid exchanges, chromosome breakage and chromosome loss *in vitro* in human cells. *In-vitro* results in rodent cells were inconclusive or negative. Methylene chloride induced DNA single-strand breaks in mammalian cell cultures, but inconclusive or negative effects were reported for induction of gene mutations. It did not induce unscheduled DNA synthesis either *in vivo* in rodents or in human fibroblast cultures. It was genotoxic in fungi but not in Drosophila in the sex-linked recessive lethal assay."
In general dichloromethane induces gene mutations in bacteria and it is clastogenic *in vitro* at high concentrations. From the large number of tests performed, it can be concluded that dichloromethane is not clastogenic *in vivo* via several routes of exposure and there were

also no indication of gene mutations (via UDS testing). In addition, DCM was tested negative in a OECD Guideline 474 study (Mammalian Erythrocyte Micronucleus Test).

Carcinogenicity

Although a number of studies are available, there are still uncertainites regarding the evaluation of DCM carcinogenity and its relevance for humans. Some of the evaluation carried out over time have been reported in the following.
IPCS/WHO (1996) and IARC (1999) reviewed several inhalation studies performed in rats, mice and hamsters. The results of the studies can be summarised as follows:
Dichloromethane showed clear evidence of carcinogenicity in mice, causing both alveolar/bronchiolar neoplasms and hepatocellular neoplasms, following exposure to high concentrations (>7100 mg/m^3 6 hours/day, 5 days/week for 26 weeks and maintained for a further 78 weeks).
In rats, an increased incidence of benign mammary tumours has been reported for female rats (three studies) and for male rats (one study). In contrast, hamsters showed no evidence of carcinogenic effects related to exposure to dichloromethane (up to 12 400 mg/m^3 6 hours/day, 5 days/week for 2 years).
IARC and IPCS/WHO reviewed a few oral studies performed in rats and mice. No clear evidence of a carcinogenic effect was observed (up to 250 mg/kg bw/ day for 2 years in drinking water; or up to 500 mg/kg bw/day for 64 weeks by gavage in olive oil.
IARC (1999) concluded that there is sufficient evidence in experimental animals for the carcinogenicity of dichloromethane. In the evaluation it was pointed out that mechanistic studies have established a link between glutathione S-transferase-mediated metabolism of dichloromethane and its genotoxicity and carcinogenicity in mice. The glutathione S-transferase (GST) responsible for the metabolism of dichloromethane is expressed to significantly greater extents in mouse tissues than in rat, hamster or human tissues and thus, the available data suggest a plausible mechanism for the development of liver and lung tumours occurring in mice which is assumed to be of less importance in rats and hamsters.

MAK (2016) concluded that following long-term inhalation exposure to dichloromethane concentrations of 1000 ppm, benign mammary tumours occurred in rats, and liver and lung tumours occured in mice. In humans, the dichloromethane metabolising GST levels are even lower than in rats and hamsters and, to date, carcinogenic effects in humans could not be demonstrated. Dichloromethane is classified for carcinogenicity according to CLP and has a CLH as Carc. 2;H351 Suspected of causing cancer however the relevance to humans of the genotoxic mode of action observed in rodents (rats and mice) has not yet been fully demonstrated (SCCS, 2012, 2015).

ANSES (2017) reviewed critically the 8h TWA OEL (SCOEL, 2009), supplementing it with a biomonitoring evaluation (urinary DCM Concentrations) and published the collective expert appraisal report on dichloromethane in which it recommended an 8h-OEL of 50 ppm, i.e. 178 mg.m^{-3}. The aim of this recommendation was to prevent possible effects in the workplace resulting in the overproduction of carbon monoxide (CO) in the body and genotoxicity. In humans, the GST metabolic pathway that produces carcinogenic metabolites was shown to be activated between 100 and 200 ppm.

EPA (2011, 2020) performed an extensive toxicologic evaluation of dichloromethane and used data for liver and lung tumors in male and female B6C3F1 mice following exposure to airborne dichloromethane to develop inhalation unit risks (Mennear et al., 1988; NTP, 1986). The liver tumor dose response data were also the basis of an oral slope factor

derived by route-to-route extrapolation using the PBPK models to compare with an oral slope factor based on liver tumor data in mice exposed to dichloromethane in drinking water (Serota et al., 1986b). In the NTP (1986) study, significant increases in incidence of liver and lung adenomas and carcinomas were observed in both sexes of B6C3F1 mice exposed 6 hours/day, 5 days/week for 2 years. EPA (2020) concluded that there is evidence that the metabolites of dichloromethane produced via the GST pathway are primarily responsible for dichloromethane carcinogenicity in mouse liver. However, EPA also highlighted the lack of data pertaining to clearance rates of these active carcinogenic metabolite(s) in mice and humans. In addition, there are remaining uncertainties on identification of a threshold for the activation/non-activation of the GST metabolism in humans at low concentrations (<10-30 ppm). Therefore, EPA considered that the carcinogenic effects seen in the chronic inhalation mice studies are relevant to humans and derived an inhalation Unit Risk of 1 µg/m^3 dichloromethane. The value is based on allometrically-scaled tissue-specific GST metabolism rate, whose dose metric was obtained by multiplying the human internal dose tumour risk factor by the human average daily internal dose.

Reproductive Toxicity

In a OECD Guideline 416 (Two-Generation Reproduction Toxicity Study, 1983), DCM was tested by inhalation at the highest dose (1500 ppm = ca. 5300 mg/m^3) approximately equal to a limit dose of 1000 mg/kg bw/d (assuming a respiratory rate of the rat of 0.2 L/min, a body weight of 250 g (defaults listed in the REACh guidance) and correcting for a 5 days/week exposure instead of 7 days/week). Oestrus cycle, sperm parameters, organ weights, implantation sites, and histopathological data were not collected, but were not routinely required under OECD TG 416 as conducted at the time. Exposure of rats to concentrations as high as 1500 ppm methylene chloride (ca. 5300 mg/m^3), which has been shown in a 2-year study to produce treatment-related liver effects and increased incidence of benign mammary tumors, did not affect any of the reproductive parameters examined. NOAEL was considered above the highest dose tested: > ca. 5300 mg/m^3

5.2.8.3 Hazard characterization

Systemic effects

Inhalation

No DNEL is derived based on mutagenic effects after inhalation.

WoE considerations:
Based on the studies available, and the remaining uncertainites regarding the evaluation of DCM carcinogenity and its relevance for humans, the weight of evidence for cancer risk is considered to be moderate to strong.

PoD for the oral DNEL derivation

The PoD is the NOAEL of 17.5 mg/kg bw/day obtained from the 13-week rat study which may be corrected for:
1. Uncertainty due to interspecies toxicokinetic and toxicodynamic differences: a factor of 10 (AF1) is used as default.

2. Intraspecies variability: the variability between the average human and sensitive humans (including children) can be accounted for by a factor of 10 (AF2).

DNEL= NOAEL/(AF1xAF2)
$DNEL_{oral}$= 17.5 mg/kg bw/day / (10X10) = 0.175 mg/kg bw/day

WoE considerations

Several rat and mice studies used to set the $DNEL_{oral}$ for dichloromethane were reviewed by IPCS (1996), showing that the liver is the target organ. Based on this outcome, SCHEER considers that there is strong evidence in relation to this systemic effect.

Local toxicity

PoD for the DNEL derivation

Dichloromethane was shown to have irritant properties to skin, eye and respiratory tract, but a dose-response relationship could not be ascertained from the available studies to identify a dose descriptor (N(L)OAEL), enabling the DNEL derivation for local effects.

6. REFERENCES

All publications that are used and/or cited in the scientific output should be reported. In the case of systematic literature reviews, it may be appropriate to list the references either in a technical report or in an annex to the scientific output.

ATSDR (Agency for Toxic Substances and Disease Registry) (2000). Toxicological profile for methylene chloride. Atlanta, GA: Agency for Toxic Substances and Disease Registry. 567 p

ATSDR (Agency for Toxic Substances and Disease Registry) (2007). Toxicological profile for xilene U.S. Department of Health and Human Services, Public Health Service. Available online at http://www.atsdr.cdc.gov/

ATSDR (Agency for Toxic Substances and Disease Registry) (2009). Toxicological profile information sheet. U.S. Department of Health and Human Services, Public Health Service. Available online at http://www.atsdr.cdc.gov/toxpro2.html

ANSES (2017) Request n° 2012-SA-0261- BLV dichloromethane - Collective expert appraisal: summary and conclusions.

Basketter D.A., Gerberick G.F., Kimber I., Loveless S.E. (1996). The local lymph node assay: A viable alternative to currently accepted skin sensitization tests. Food and Chemical Toxicology, 34(10):985–997.

Basketter D.A., Lea L.J., Dickens A., Briggs D., Pate I., Dearman R.J., Kimber I. (1999). A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. J Appl Toxicol, 19(4):261–266.

Bornehag C.G., Sundell J., Hägerhed-Engman L. Sigsgaard T. (2005). Association between ventilation rates in 390 Swedish homes and allergic symptoms in children. Indoor Air, 15:275–280.

Carpenter C.P., Kinkead E.R., Geary D.J., et al. (1975). Petroleum hydrocarbon toxicity studies: V. Animal and human response to vapors of mixed xylenes. Toxicol Appl Pharmacol, 33:543-558.

Catenacci G., Grampella D., Terzi R., Sala A., Pollini G. (1984). Hepatic function in subjects exposed to environmental concentrations of DMF lower than the actually proposed TLV. Giornale Italiano di Medicina del Lavoro, 6:157–158.

Cirla A.M., Pisati G., Invernizzi E. and Torricelli P. (1984). Epidemiological study on workers exposed to low dimethylformamide concentrations. G. Ital. Med. Lav, 6:149–156.

ECHA (2016). N,N-dimethylformamide. Substance description. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.000.617

ECHA (2019) N,N,N',N'-tetramethyl-2,2'-oxybis(ethylamine). Substance description. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.019.292

EFSA (2019) Guidance on harmonised methodologies for human health, animal health an ecological risk assessment of combined exposure to multiple chemicals https://www.efsa.europa.eu/en/efsajournal/pub/5634

EPA (2011) TOXICOLOGICAL REVIEW OF DICHLOROMETHANE (METHYLENE CHLORIDE) (CAS No. 75-09-2) In Support of Summary Information on the Integrated Risk Information System (IRIS) https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0070tr.pdf

EPA (2020) Risk Evaluation for Methylene Chloride (Dichloromethane, DCM) CASRN:75-09-2 https://www.epa.gov/sites/production/files/202006/documents/1_mecl_risk_evaluation_final.pdf

Fiorito A., Larese F., Molinari S., Zanin T. (1997). Liver function alterations in synthetic leather workers exposed to dimethylformamide. Am J Ind Med, 32(3):255–60.

Gagnaire F., Azim S., Simon P., Cossec B., Bonnet P., de Ceaurriz J. (1993). Sensory and pulmonary irritation of aliphatic amines in mice: A structure–activity relationship study. Journal of Applied Toxicology, 13(2), pp.129–135.
Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/jat.2550130210

Health Canada (2001). Priority Substances List Assessment Report:N,N-Dimethylformamide under the Canadian Environmental Protection Act, 1999 Minister of Public Works and Government Services, pp.1-78.

IARC (1999). Monographs on the evaluation of carcinogenic risks to humans Dichloromethane 71 Pt 1 251-315.

Hipolito R.N. (1980). Xylene poisoning in laboratory workers: Case reports and discussion. Lab Med, 11:593-595.

IARC (2018) Monographs on the evaluation of carcinogenic risks to humans. Some industrial chemicals N,N-Dimethylformamide Vo. 115, 118-162.

Jenkins L.J., Jones R.A., Siegel J. (1970). Long-term inhalation screening studies of benzene, toluene, o-xylene, and cumene on experimental animals. Toxicol Appl Pharmacol, 16:818-823.

Klonne R.K., Dodd D.E., Pritts I.M., Nachreiner D.J., Fowler E.H., Troup C.M., Homan E.R., Ballantyne B. (1987). Dimethylethanolamine: acute, 2-week, and 13-week inhalation toxicity studies in rats. Toxicological Sciences, 9(3):512–521.

Korsak Z., Sokal J.A., Gorny R. (1992). Toxic effects of combined exposure to toluene and m-xylene in animals. III. Subchronic inhalation study. Pol J Occup Med Environ Health, 5(1):27-33.

Korsak Z., Wisniewska-Knypl J., Swiercz R. (1994). Toxic effects of subchronic combined exposure to n-butyl alcohol and m-xylene in rats. Int J Occup Med Environ Health, 7:155-166.

Lijinsky W., Kovatch R.M. (1986). Chronic toxicity study of cyclohexanone in rats and mice (NCI study). J Natl Cancer Inst, 77:941−949.

Leung H.-W, Tyl R.W., Ballantyne B., Klonne D.R. (1996). Developmental toxicity study in Fischer 344 rats by whole-body exposure to N,N-Dimethylethanolamine vapor. Journal of applied Toxicology, 16(6): 533-538.

Leung H.-W., Tyl R.W., Ballantyne (1997). Evaluation of the genotoxic potential of alkylalkanolamines. Mutation Research, 393:7–15.

Longacre S.L. (1987). Cyclohexane. In: Ethel Browning's Toxicity and Metabolism of Industrial Solvents, 2nd ed. Snyder R, Ed. Elsevier, Amsterdam, pp. 225-235.

Lynch D.W., Placke M.E., Persing R.L., Ryan M.J. (2003). Thirteen-week inhalation toxicity of N,N-dimethylformamide in F344/N rats and B6C3F1 mice. Toxicol Sci, 72(2):347–58.

Malley L.A., Slone T.W. Jr, Van Pelt C., Elliott G.S., Ross P.E., Stadler J.C., Kennedy G.L. Jr (1994). Chronic toxicity/oncogenicity of dimethylformamide in rats and mice following inhalation exposure. Fundamental and applied toxicology, 23:268–279.

Mennear J.H., McConnell E.E., Huff J.F., Renne R.A., Giddens E. (1988) Inhalation toxicity and carcinogenesis studies of methylene chloride (dichloromethane) in F344/N rats and B6C3F1 mice. Ann NY Acad Sci, 534:343-51.

Morvai V., Ungvary G., Herrmann H.J., et al. (1987). Effects of quantitative undernourishment, ethanol and xylene on coronary microvessels of rats. Acta Morphol Hung, 35:199-206.

Mráz J., Cross H., Gescher A., Threadgill M.D., Flek J. (1989). Differences between rodents and humans in the metabolic toxification of N,N-dimethylformamide. Toxicology and applied pharmacology, 98:507–516.

Mráz J. and Nohová H. (1992a). Percutaneous absorption of N,N-Dimethylformamide in humans. International archives of occupational health, 64:79–83.

Mráz J. and Nohová H. (1992b). Absorption, metabolism and elimination of N,N-dimethylformamide in humans. International archives of occupational health, 64:85–92.

Nitschke K.D., Burek J.D., Bell T.J., Kociba R.J., Rampy L.W., McKenna M.J., (1988). Methylene chloride: A 2-year inhalation toxicity and oncogenicity study in rats. Fundam Appl Toxicol, 11(1):48-59. http://dx.doi.org/10.1016/0272-0590 (88)90269-2

NTP (1992) NTP technical report on toxicity studies of N,N-Dimethylformamide (CAS No. 68-12-2) administered by inhalation to F344/N rats and B6C3F1 mice. Research Triangle Park, NC, National Institutes of Health, National Toxicology Program, 44 pp. (Toxicity Report Series No. 22; NIH Publication No. 93-3345; NTIS Publication No. PB93-131936).

OECD (2001) SIDS International Chemical Assessment Report for SIAM 13–Dimethyl-formamide.

OECD (2012) SIDS INITIAL ASSESSMENT PROFILE, CoCAM2,17-19 April 2012
https://hpvchemicals.oecd.org/ui/handler.axdd=2b0d4680-ca07-46c5-a52f-4a3315a426cd

Roberts F.P., Lucas E.G., Marsden C.D., et al. (1988). Near-pure xylene causing reversible neuropsychiatric disturbance [Letter]. Lancet, 2(8605):273.

SCHER (2016). Final Opinion on Estimates of the amount of toy materials ingested by children. Available online: https://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_170.pdf

SCHEER (2018). Memorandum on weight of evidence and uncertainties Revision 2018. Available online: https://ec.europa.eu/health/sites/health/files/scientific_committees/scheer/docs/scheer_o_014.pdf

SCHER, SCENIHR, SCCS (2012). Toxicity and Assessment of Chemical Mixtures.Available online:https://ec.europa.eu/health/sites/health/files/scientific_committees/environmental_risks/docs/scher_o_155.pdf

SCCS, 2012 - OPINION on Dichloromethane SCCS/1408/11. Available online: https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_118.pdf

SCOEL (2009). SCOEL/SUM/130 Recommendation from the Scientific Committee on Occupational Exposure limits for methylene chloride (dichloromethane). European Commission, pp. 38.

Senoh H., Katagiri T., Arito H., Nishizawa T., Nagano K., Yamamoto S., Matsushima T. (2003). Toxicity due to 2- and 13-wk inhalation exposures of rats and mice to N,N-Dimethylformamide. J Occup Health, 45(6):365–75.

SIDS/SIAM 21, 18-20 October 2005 - 1,4-Diazabicyclo[2.2.2]octane

Smyth H.F., Carpenter C.P., Weil C.S., Pozzani U.C., Striegel J.A., Nycum J.S. (1969). Range-Finding Toxicity Data: List VII American Ind. Hyg. Ass. J, 30, 470–476.

Stenbäck F., Weisburger J.H., Williams G.M. (1988). Effect of lifetime, administration of dimethylaminoethanol on longevity, aging changes, and cryptogenic neoplasms in C3H mice. Mechanisms of Ageing and Development, 42:129-138.

Strøm-Tejsen P., Zukowska D., Wargocki P., Wyon D. P. (2016). The effects of bedroom air quality on sleep and next-day performance. Indoor Air, 26:679–686.

Swedish Chemicals Agency (KEMI). Enforcement of squishies. ENFORCEMENT 6/19. Stockholm 2019. Article number: 511 328. Available online: https://www.kemi.se/download/18.164ad6b3172927a9289477/1591628343988/enforcement-6-19-squishies.pdf

Tatrai E., Ungvary G., Cseh I.R., et al. (1981). The effects of long-term inhalation of ortho-xylene on the liver. Ind Enviv Xenobiotica, Proceedings of International Conference, Prague, Czechoslovakia, May 2730, 1980. New York, NY: Springer-Verlag, pp. 293-300.

The Danish Environmental Protection Agency. Analysis and risk assessment of fragrances and other organic substances in squishy toys. Survey of chemical substances in consumer

products no. 165, August 2018. ISBN: 978-87-93710-64-1. Available online: https://www2.mst.dk/Udgiv/publications/2018/08/978-87-93710-64-1.pdf

Toftgard R., Nilsen O.G. (1981). Induction of cytochrome P-450 in rat liver after inhalation of aromatic organic solvents. In: Industrial and environmental xenobiotics: Metabolism and pharmacokinetics of organic chemicals and metals: Proceedings of International Conference, Prague, Czechoslovakia, May 27-30, 1980. New York, NY: Springer-Verlag, pp. 307-317.

The MAK-Collection Part IV: BAT Value Documentations, Vol. 5. DFG, Deutsche Forschungsgemeinschaft© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 978-3-527-32614-3.

Uchida Y., Nakatsuka H., Ukai H., Watanabe T., Liu Y.T., Huang M.Y., et al. (1993). Symptoms and signs in workers exposed predominantly to xylenes. Int. Arch. Occup. Environ. Health, 64:597-605.

Ursin C., Hansen C.M., Van Dyk J.W., Jensen P.O., Christensen I.J., Ebbehoej J. (1995). Permeability of commercial solvents through living human skin. Am Industr Hyg Assoc J, 56(7):651-660.

U.S. Environmental Protection Agency (2000). Integrated Risk Information System (IRIS) on N,N-Dimethylformamide. National Center for Environmental Assessment, Office of Research and Development, Washington, DC. 1999. Updated in 2000.

US-EPA (2010) Provisional Peer-Reviewed Toxicity Values for Cyclohexanone (CASRN 108-94-1) EPA/690/R-10/009F Final 9-15-2010.

World Health Organization (2001). Concise International Chemical Assessment Document 31: N,N- Dimethylformamide. First draft prepared by G. Long and M.E. Meek, Environmental Health Directorate, Health Canada, and M. Lewis, Commercial Chemicals Evaluation Branch, Environment Canada. WHO Geneva, pp. 1-61.

Zarrabeitia M.T., Ortega C., Altuzarra E., Martinez M.A., Mazarrasa O., Calvet R. (2001). Accidental dichloromethane fatality: a case report J Forensic Sci 46(3):726-7.

7. LIST OF ABBREVIATIONS

ADME	Absorption, Distribution, Metabolismus und Elimination
AF	Assessment Factor
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	Benchmark Dose
bw	body weight
CLP	Classification, Labelling and Packaging of substances
DNA	Deoxyribonucleic acid
DMEL	Derived Minimum Effect Level
DNEL	Derived No-Effect Level
ECHA	European Chemical Agency
EPA	Environmental Protection Agency
HI	Hazard Index
HQ	Hazard Quotient
IARC	International Agency for Research on Cancer
IPCS	WHO International Programme on Chemical Safety
ISO	International Organization for Standardization
KEMI	Kemikalieinspektionen
LC	Lethal Concentration
LCI	Lowest Concentration of Interest
LLNA	Local Lymph Node Assay
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
logP	ogarithmic partition coefficent
LOQ	Limit of Quantification
MAK	Maximale Arbeitsplatzkonzentration
NOAEL	No Observed Adverse Effect Level
NOAEC	No Observed Adverse Effect Concentration
OECD	Organisation for Economic Co-operation and Development
PoD	Point of Departure

PUR	Polyuretan Foam
RCR	Risk Characterisation Ratio
RFC	Reference Concentration
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
SCOEL	Scientific Committee on Occupational Exposure Limits
SIDS	OECD Screening Information Dataset
TDI	Tolerable Daily Intake
ToR	Terms of Reference
TWA-OEL	Time-Weighted-Average Occupational Exposure Limit
UDS	Unscheduled DNA Synthesis
VOC	Volatile Organic Compounds
WG	Working Group
WHO	World Health Organization
WoE	Weight of Evidence

ANNEXES

APPENDIX A: Calculation of emission and migration/content limits

The current Appendix includes the various exposure scenarios considered for calculating emission and migration limits reported in the Opinion. All the assumptions made for the calculations are included in the corresponding scenarios. There are three inhalation scenarios for calculating the emission limits and two oral scenarios for calculating migration limits.

Inhalation Scenario 1
A 3-year-old child sleeping in a room and holding one squishy toy in her/his arms

Child			Notes
Age	3	yr	
BW	14	kg	Average of the body weight indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg)
Inhalation rate	0.18	m^3/hr	Average of the values indicated by ECHA and RIVM
Exposure (sleep) time	10	hr/dy	
Intermittent Exposure Factor (IEF)	2.40		When systemic effects arise from inhalation this factor corrects for exposure duration, because DNEL values correspond to continuous (24hr) exposure
Number of toys (units) (N_u)	1		

Maximum allowed emission per toy unit (E_u)	
$$E_u \left[\frac{\text{mg}}{\text{hr}}\right] \leq \frac{IEF \times DNEL \left[\frac{\text{mg}}{\text{m}^3}\right] \times \dot{V}_{\text{sleep}} \left[\frac{\text{m}^3}{\text{hr}}\right]}{N_u}$$	It is assumed that all the mass of the emitted substance stays within the breathing zone of the child, who inhales it.

Inhalation Scenario 2
A 6-year or older child playing in a room with several squishy toys

Child					Notes
Age	6	yr			
BW	20	kg			Average of the body weight indicated by ECHA and RIVM for 3-6-year-olds and for 6-11-year-olds
Inhalation volume	12.5	m^3/dy			Average of values indicated by RIVM for two age groups: 3- and 6-11 years old
Inhalation rate	0.52	m^3/hr			Derived from the daily inhalation volume per day (above)
Exposure (play) time	15	hr/dy			Assuming the child plays all day in his/her room
Intermittent Exposure Factor (IEF)	1.60				When systemic effects arise from inhalation this factor corrects for exposure duration, because DNEL values correspond to continuous (24hr) exposure
Room					
Volume	17.4	m^3			Corresponds to a floor area of 7 m^2
Air change rate (R)	0.35	hr^{-1}	0,51	hr^{-1}	Bornehag et al, Indoor Air 2005; 15: 275–280 (doi:10.1111/j.1600-0668.2005.00372.x). The smallest number is the mean value in a child's bedroom in a single-family house and the largest number is the mean value in a multi-family house.
Number of toys (units) (N_u)	40				This value is not unrealistic, considering the fact that the toys are sold at electronic shops in packages of 10-40 items.

Maximum allowed emission per toy unit (E_u)	
$$E_u\left[\frac{\mathrm{mg}}{\mathrm{hr}}\right] \leq \frac{IEF \times DNEL\ \left[\frac{\mathrm{mg}}{\mathrm{m}^3}\right] \times R\left[\frac{1}{\mathrm{hr}}\right] \times V_{\mathrm{room}}[\mathrm{m}^3]}{N_u}$$	

Inhalation Scenario 3
A 3-year-old child sleeping in a room with several squishy toys around and holding a squishy toy

Child					Notes
Age	3	yr			
BW	14	kg			Average of the body weight indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg)
Inhalation rate	0.18	m^3/hr			Average of the values indicated by ECHA and RIVM
Exposure (sleep) time	10	hr/dy			
Intermittent Exposure Factor (IEF)	2.40				When systemic effects arise from inhalation this factor corrects for exposure duration, because DNEL values correspond to continuous (24hr) exposure
Room					
Volume	17.4	m^3			Corresponds to a floor area of 7 m^2
Air change rate (R)	0.35	hr^{-1}	0,51	hr^{-1}	Bornehag et al, Indoor Air 2005; 15: 275–280 (doi:10.1111/j.1600-0668.2005.00372.x). The smallest number is the mean value in a child's bedroom in a single-family house and the largest number is the mean value in a multi-family house.
Number of toys (units) (N_u)	40				This value is not unrealistic, considering the fact that the toys are sold at electronic shops in packages of 10-40 items.

Maximum allowed emission per toy unit (E_u)	
$$E_u\left[\frac{\text{mg}}{\text{hr}}\right] \leq \frac{IEF \times DNEL\left[\frac{\text{mg}}{\text{m}^3}\right]}{\frac{N_u}{R\left[\frac{1}{\text{hr}}\right] \times V_{\text{room}}[\text{m}^3]} + \frac{1}{\dot{V}_{\text{sleep}}\left[\frac{\text{m}^3}{\text{hr}}\right]}}$$	

Table A1: Summary of the emission limits calculated for all inhalation scenarios. Bold values indicate the lowest limit value for all scenarios and each sustance.

Substance			**Toxicological reference and emission limit values**							
Name	**Abbre-viation**	**CAS No**	**DN[M]EL inhalation**	**Allocation factor**	**Is the effect systemic?**	**Inhalation Scenario 1**	**Inhalation Scenario 2 - Low air change rate**	**Inhalation Scenario 2 - High air change rate**	**Inhalation Scenario 3 - Low air change rate**	**Inhalation Scenario 3 - High air change rate**
			(µg/m^3)	%	(Yes/No)	(mg/hr)	(mg/hr)	(mg/hr)	(mg/hr)	(mg/hr)
N,N-dimethylamino-ethanol	DMAE	108-01-0	1160	100	No	0.209	0.177	0.257	0.096	0.115
N,N-dimethylformamide	DMF	68-12-2	170	10	Yes	0.007	0.004	0.006	0.003	0.004
Triethylendiamine	TEDA	280-57-9	800	100	No	0.144	0.122	0.177	0.066	0.079
Bis(2-(dimethylamino)ethyl) ether	DMAEE	3033-62-3	20	100	No	0.004	0.003	0.004	0.002	0.002
1,1,4,7,7-pentamethyl-diethylentriamin	PDT	3030-47-5	283	100	No	0.051	0.043	0.063	0.023	0.028
Cyclohexanone	CH	108-94-1	716	10	Yes	0.031	0.017	0.025	0.014	0.017
Xylenes	X	1330-20-7	130	10	Yes	0.006	0.003	0.005	0.003	0.003

Oral Scenario 1 - Mouthing A 3-year-old child putting a toy in his/her mouth			
Child			Notes
Age	3	yr	
BW	14	kg	Average of the body weight indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg)
Surface of mouth	10	cm^2	RIVM
Mouthing time	3	hr/dy	RIVM (assuming cumulative mouthing time)

Maximum allowed migration rate per toy unit (M_u)	
$M_u \ \frac{\mu g}{cm^2\ hr} \leq \frac{DNEL \ \left[\frac{mg}{kg_{BW}\ dy}\right] \times BW\ [kg]}{S_{mouth}\ [cm^2] \times t_{mouthing}\ \left[\frac{hr}{dy}\right]} \times 10^3$	A 100% bioavailability is assumed. The frequency for mouthing is assumed 1 (once per day). The concentration of the substance in the toy is assumed constant and uniform.

1

2

Oral Scenario 2 - Ingestion A 3-year-old child swallowing a piece of the squishy toy			
Child			Notes
Age	3	yr	
BW	14	kg	Average of the body weight indicated by ECHA and RIVM for 2-3-year-old children (12.4 kg) and for 3-6-year-olds (15.7 kg)
Material ingested (M_{ing})	100	mg/dy	RIVM

Maximum allowed content per toy unit ($C_{w/w,u}$)	
$C_{w/w,u} \ \frac{mg}{g} \leq \frac{DNEL \ \left[\frac{mg}{kg_{BW}\ dy}\right] \times BW\ [kg}{M_{ing} \ \frac{mg}{dy} \times 10^{-3}}$	A 100% bioavailability is assumed. It is assumed that the concentration of the substance in the toy is uniform.

3