Agency technical report on the classification and labelling of: 2,2'-iminodiethanol; diethanolamine

EC Number: 203-868-0
CAS Number: 111-42-2

March 2026

Contents

Brief summary

The conclusion of the Agency technical report is that 2,2'-iminodiethanol; diethanolamine meets the classification criteria for:

Acute Tox. 4; H302 (Harmful if swallowed), ATE = 1100 mg/kg bw

STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure)

Carc. 2; H351 (Suspected of causing cancer)

Repr. 1B; H360FD (May damage fertility. May damage the unborn child)

Is this in agreement with the RAC opinion? **YES**

At the time of publication, this mandatory classification and labelling (MCL) has not been agreed and/or adopted in Great Britain.

This is a targeted technical report which only considers carcinogenicity, germ cell mutagenicity, reproductive toxicity, acute toxicity (oral, dermal, inhalation) and STOT RE. These were the only hazard classes considered in the EU Committee for Risk Assessment (RAC) Opinion.

This substance has an existing MCL which includes Skin Irrit. 2, H315 and Eye Dam. 1, H318. Skin irritation and eye damage are not assessed in this technical report, therefore they should be retained in the GB MCL.

Introduction

Under Article 37 of the GB CLP Regulation[1], the Agency[2] is required to produce a technical report for each substance on which the Committee for Risk Assessment (RAC) of the European Chemicals Agency produces an opinion[3].

This technical report documents an independent scientific assessment, conducted by HSE technical specialists of the classification and labelling of 2,2'-iminodiethanol; diethanolamine.

Table 1. Information considered in the scientific assessment

Document	Included in assessment
EU CLH report	Yes
Annexes to the EU CLH report	Yes
RAC opinion	Yes
Background document	Yes
Information submitted during the EU public consultation process (RCOM table, including attachments)	Yes
RAC minority opinion(s)	Not applicable
Other information:	No

This information has been evaluated against the classification and labelling criteria set out in the GB CLP Regulation.

[1]The retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

[2] HSE acting in its capacity as the GB CLP Agency

[3] Under Article 37(4) of Regulation (EU) No 1272/2008 on classification, labelling and packaging of substances and mixtures

Overview of current and proposed classification and labelling

Table 2. Current and proposed classification and labelling

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram, Signal Word Code(s)	Hazard Statement Code(s)	Suppl. Hazard Statement Code(s)		
GB MCL List entry	603-071-00-1	2,2'-iminodiethanol; diethanolamine	203-868-0	111-42-2	Acute Tox. 4 * STOT RE 2 * Skin Irrit. 2 Eye Dam. 1	H302 H373 ** H315 H318	GHS08 GHS05 GHS07 Dgr	H302 H373 ** H315 H318			
EU dossier submitter's proposal	603-071-00-1	2,2'-iminodiethanol; diethanolamine	203-868-0	111-42-2	**Add** Acute Tox. 4 Carc. 2 Repr. 1B **Modify** Acute Tox. 4 STOT RE 2	**Add** H332 H351 H360FD **Modify** H302 H373 (haematopoetic system, kidney, nervous system)	**Retain** GHS08 GHS05 GHS07 Dgr	**Add** H332 H351 H360FD **Modify** H302 H373 (haematopoetic system, kidney, nervous system)		**Add** oral: ATE = 1100 mg/kg bw inhalation: ATE = 2.8 mg/L (dusts or mists)	
EU RAC opinion	603-071-00-1	2,2'-iminodiethanol; diethanolamine	203-868-0	111-42-2	**Add** Carc. 2 Repr. 1B **Modify** Acute Tox. 4 STOT RE 2	**Add** H351 H360FD **Modify** H302 H373	**Retain** GHS08 GHS05 GHS07 Dgrr	**Add** H351 H360FD **Modify** H302 H373 (damage to organs)		**Add** oral: ATE = 1100 mg/kg bw	
Agency technical report conclusion	603-071-00-1	2,2'-iminodiethanol; diethanolamine	203-868-0	111-42-2	**Add** Carc. 2 Repr. 1B **Modify** Acute Tox. 4 STOT RE 2	**Add** H351 H360FD **Modify** H302 H373	**Retain** GHS08 GHS05 GHS07 Dgrr	**Add** H351 H360FD **Modify** H302 H373 (damage to organs)		**Add** oral: ATE = 1100 mg/kg bw	

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram, Signal Word Code(s)	Hazard Statement Code(s)	Suppl. Hazard Statement Code(s)		
Resulting MCL entry on GB MCL list	603-071-00-1	2,2'-iminodiethanol; diethanolamine	203-868-0	111-42-2	Carc. 2 Repr. 1B Acute Tox. 4 STOT RE 2 Skin Irrit. 2 Eye Dam. 1	H351 H360FD H302 H373 H315 H318	GHS08 GHS07 GHS05 Dgr	H351 H360FD H302 H373 H315 H318		oral: ATE = 1100 mg/kg bw	

Background

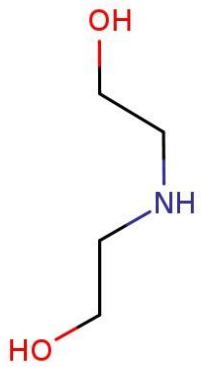
Active substance in Plant Protection Products: ☐

Active substance in Biocidal Products: ☐

Chemical registered under REACH: ☒

Diethanolamine has a range of consumer uses such as fuels, washing and cleaning products, disinfectants, cosmetics and personal care products. It is used by professionals in building and construction work and in scientific research as a laboratory chemical (ECHA, 2025).

Figure 1: the structural formula of diethanolamine, from ECHA CHEM[4]



Diethanolamine is currently listed in Annex VI of EU CLP and the GB MCL list. Its current classification was carried over into EU CLP from the previous European Directive 67/548/CEE and includes minimum classifications of Acute Tox. 4*; H332 and STOT RE 2*; H373. An EU CLH report was prepared by the dossier submitter (DS; Germany) in 2024 to assess the substance's carcinogenic, mutagenic and reproductive toxicity (CMR) properties, address the minimum classifications and assess a new Extended One Generation Reproductive Toxicity Study (EOGRTS) submitted in 2018 after diethanolamine underwent Substance Evaluation in the EU (CLH, 2024).

[4] https://chem.echa.europa.eu/100.003.517/overview?searchText=111-42-2

Scientific assessment of the physical, human health and environmental hazard classes

Physical Hazards

Not assessed in the CLH report or RAC opinion.

Health Hazards

Acute Toxicity

Classification agreed by RAC:

Acute toxicity – oral route

For acute toxicity by the oral route, 1 key study and 6 supporting studies were available (all non-guideline). The key study was similar to OECD TG 401 but preceded the introduction of the test guideline and was not GLP-compliant (BASF AG, 1966a). No information on the purity of diethanolamine used was available. Groups of 10 rats (strain not specified, 5/sex) were administered doses of 200, 800, 1000, 1250, 1600, and 3200 mg/kg bw. In the groups administered 2000 and 2500 mg/kg bw, only 5 male animals were used. Diethanolamine was administered by oral gavage and animals were observed daily for 14 days. No mortality was observed in any dose group up to 1000 mg/kg bw. Reported clinical signs included tumbling, staggering gait, twitches, convulsions, dyspnoea, abdominal lateral position and scrubby coat. Gross pathology revealed hydrothorax, local adhesions of the gut and signs of irritation on the gastro-intestinal tract. The LD_{50} was determined to be 1100 mg/kg bw/d in females, and 2500 mg/kg bw/d in males.

Of the remaining non-guideline studies, 4 were conducted in rats and of these, only Korsrud (1973) had dosing details available. In the Korsrud study, male SD rats were administered doses of 0 (9 animals), 100 (9 animals), 200 (7 animals), 400 (7 animals), 800 (8 animals), 1600 (8 animals), 3200 (8 animals) and 6400 mg/kg bw (8 animals). A major limitation of this study was that animals were only observed for 18 hours after exposure. At the top dose, 7/8 animals died; no mortality was observed in any of the other groups. A dose-dependent increase in liver and kidney weights was noted but no specific information regarding this was available.

In the other rat studies, no information regarding dose levels were available but LD_{50} values were noted as: 1820 mg/kg bw (Smyth, 1969); 710 mg/kg bw (Smyth, 1970) and 700-1700 mg/kg bw in females and 1700-2800 mg/kg bw in males (Knaak, 1997).

Two further studies were conducted with multiple species. The first was conducted in rats, mice, rabbits and guinea pigs (strains were not specified). No information regarding dosing, numbers of animals used or observations made were available but LD_{50} values were noted as: rat 3460 mg/kg bw, mouse 3300 mg/kg bw, rabbit 2200 mg/kg bw and guinea pig 2200 mg/kg bw (Izmerov *et al*., 1982).

The second multi species study was conducted in rats and mice. The strains, number of animals used and dose information was not available. LD_{50} values were noted as 2300 mg/kg bw in rats and 4570 mg/kg bw in mice (EPA-US, 1989).

In the key study, the LD_{50} in female rats (the most sensitive sex) was 1100 mg/kg bw, which falls within the range for Category 4 ($300 < ATE \leq 2000$ mg/kg bw). Therefore, RAC concluded that diethanolamine should be classified as Acute Tox. 4; H302 (Harmful if swallowed), with an ATE of 1100 mg/kg bw.

Acute toxicity – dermal route

One review was available to assess acute toxicity by dermal exposure. Knaak *et al*. (1997) reported LD_{50} values of 8100-12200 mg/kg bw for rabbits, but no further study details were available. RAC noted that these values were above the upper limit for Category 4 classification (2000 mg/kg bw), but based on a lack of study information, concluded that classification for acute dermal toxicity was not warranted owing to insufficient data.

Acute toxicity – inhalation route

For acute toxicity by the inhalation route, there were 4 studies available. The first study was an inhalation risk test (IRT), which was similar to OECD TG 403 and was not GLP compliant (BASF AG, 1956). Groups of 6 rats (strain not specified) were exposed to diethanolamine vapour. The mean concentration was reported as 0.2 mg/L of air, but there was no verification of the test atmosphere concentration. Animals were exposed for 8 hours followed by a 7-day observation period. No mortalities occurred and no other observations were reported. RAC considered this study unsuitable for classification purposes.

A second IRT was performed: groups of 12 rats (6/sex, strain not specified) were exposed to diethanolamine vapour for 8 hours followed by a 7-day observation period (BASF AG, 1956). The mean concentration was reported as 1.9 mg/m^3. No mortalities occurred, no other observations were noted. RAC considered this study unsuitable for classification purposes.

A non-guideline study was available, though information was limited: no information regarding the animals used was available (Hartung, 1970). A short-term inhalation of vapour (200 ppm) or aerosols (1400 ppm) is the only detail provided. The only reported observation is that “some deaths occurred”. RAC considered this study unsuitable for classification purposes.

A non-guideline study (Foster, 1971) was available from the REACH registration dossier. SD rats (4/sex/group) were exposed to unheated diethanolamine aerosol by whole body exposure. Groups were exposed for 4 hours to concentrations of 0.13, 0.55, 1.18 and 3.35 mg/L.

A second set of animals were exposed for 80 and 105 minutes to a concentration of 6.4 mg/L of diethanolamine vapour and mist generated by heating the compound to 110^{O}C. Concentration of diethanolamine was determined via a colourimetric method. The observation period was not specified. No mortalities occurred in any of the 4-hour exposure groups. At the 6.4 mg/L exposure a total of 5/8 rats died; 3/4 after 80 minutes exposure and 2/4 after 105 minutes.

If the dose at which 5/8 animals died is extrapolated from 105 minutes to 240 minutes (4-hour exposure), an LD_{50} of 2.8 mg/L is derived (105/140*6.4 mg/L), which is indicative of a classification for acute inhalation toxicity Category 4. However, RAC noted that the first part of the study showed no mortality at the higher concentration of 3.35 mg/L. This may be accounted for as the methods of producing the vapour/aerosol differ; in the 4-hour exposure, the diethanolamine was not heated whereas it was heated to 110^{o}C in the 105/80 minute exposure studies.

RAC considered that heating the substance to 110^{o}C in the second part of the study may have intensified the toxicity experienced by the animals in those groups. They considered the reliability of these results to be low and raised animal welfare concerns relating to the temperatures used.

RAC additionally noted that the Foster (1971) study was missing information such as whether full body or nose only exposure was used, whether the concentrations in the vapour/aerosol were verified and whether there were any clinical signs of systemic toxicity.

Based on the uncertainties in Foster (1971), RAC concluded that the study could not be used to inform the classification. They concluded that classification for acute toxicity via inhalation was not warranted for diethanolamine.

Classification proposed by the Agency:

Acute toxicity – oral route

The Agency agrees with RAC's conclusion on classification. Diethanolamine meets the criteria for classification as **Acute Tox. 4; H302 (Harmful if swallowed), with an ATE of 1100 mg/kg bw.**

Acute toxicity – dermal route

The Agency agrees with RAC's conclusion on classification. Diethanolamine does not warrant classification for acute toxicity via the dermal route.

Acute toxicity – inhalation route

The Agency agrees with RAC's conclusion on classification. Diethanolamine does not warrant classification for acute toxicity via the inhalation route.

Specific target organ toxicity – single exposure (STOT SE)

Not assessed in the CLH report or RAC opinion.

Skin corrosion/irritation

Not assessed in the CLH report or RAC opinion.

Serious eye damage/irritation

Not assessed in the CLH report or RAC opinion.

Respiratory sensitisation

Not assessed in the CLH report or RAC opinion.

Skin sensitisation

Not assessed in the CLH report or RAC opinion.

Specific target organ toxicity – repeated exposure (STOT RE)

Classification agreed by RAC:

There were 11 studies available for the assessment of STOT RE. The DS proposed classification for the haematopoietic system, nervous system and kidney.

Studies available via the oral route (findings relating to effects for STOT RE are listed here, findings relevant for other hazard classes are discussed in the corresponding sections of this document).

The first study available was an OECD TG 443 Extended One Generation Reproductive Toxicity study (EOGRTS), which was GLP-compliant with no deviations. Wistar rats (30/sex/group for the parent generation, 75/sex/group F1 generation) were administered diethanolamine daily via drinking water from 16 days pre-mating until the end of the study. Doses were calculated to be 0, 6.8, 21.5, 73.4 mg/ kg bw/d in males, and 0, 10.2, 29.4, 103.9 mg/kg bw/d in females pre-mating, 0, 11.5, 34.9, 116.8 mg/kg bw/d in females during gestation and 0, 24.0, 66.3, 173.3 mg/kg bw/d during lactation. In the F1 generation, doses were calculated to be 11, 34 and 123 mg/kg bw/d in males and 13, 39 and 137 mg/kg bw/d in females. The CLH report and RAC Opinion do not clearly indicate whether the findings below were statistically significant, and if so, the level of the statistical significance.

Signs of general toxicity in parental animals included reduced water consumption in females from the mid dose (at the top dose this was up to ↓ 18% during GD 14–18, and up to ↓ 45% during lactation). Food consumption was also reduced in females from the mid dose (at the top dose this was up to ↓ 29% during lactation). In males, food consumption was lower than in controls at the top dose during premating (↓ 9%).

Body weight gain was decreased in females from the mid-dose during GD 4-7 (up to ↓5%), and at the top dose from premating day 7, throughout gestation and lactation (up to ↓33% during gestation). In males, body weight gain was decreased at the mid dose (↓ 23%) and the top dose (↓ 25%). Therefore, lower body weights were noted at the start of mating in the top dose group (↓5.1% in males and ↓4.1% in females). At the end of the study, in the mid dose group this progressed to ↓5.3% in males and ↓2.5% in females and in the top dose group ↓10% in males and ↓8.1% in females. In females, in the top dose group, body weight was reduced by up to ↓15% during gestation.

The target organs in this study were the blood system, liver and kidneys.

Haematology showed microcytic anaemia in both sexes with several parameters affected including reduced haemoglobin (HGB) in the mid dose group (↓ 5.7% in males and ↓ 3.2% in females) and at the top dose (↓21.1% in males and ↓14.8% in females), reduced haematocrit (HCT) (↓3.9% and ↓15.1% in females at the mid and top doses, respectively,

and ↓18.6% in males at the top dose), and mean corpuscular haemoglobin content (MCH) in males at the mid dose (↓5.6%) and females at the top dose (↓4.8%). Mean corpuscular volume (MCV) in males was reduced in all dose groups (↓2.9, ↓4.4 and ↓6.4% at the low, mid and top doses, respectively). At the top dose, there was also a reduced red blood cell count (RBC) (↓12.9% males and ↓10.4% females) and a reduced Mean Corpuscular Haemoglobin Concentration (MCHC) (↓2.9%) in males only.

Examination of coagulation parameters showed an increase in platelet counts in males (↑ 14%) at the mid dose and ↑ 30% at the top dose. Prothrombin time was also decreased at the mid dose (↓ 6.1% in males and ↓ 4.7% in females), and at the top dose (↓11.1% in males and ↓7.6% in females.

Treatment-related increases in relative liver weight were reported in both sexes: relative liver weights were ↑ 4%, 7%, and 2% in males and ↑ 9%, 15% and 25% in females at the low, mid and high dose respectively. Centrilobular hypertrophy was noted at the top dose in 4/30 males and 10/30 females.

In the kidney, increases in absolute and relative weights were seen in all dose groups: relative weights increased ↑ 5%, 12% and 18% in males and ↑ 13%, 19% and 24% in females at the low, mid and high dose respectively. Tubular degeneration was noted in the mid and top dose groups with the following incidences and severities: in the mid dose, grade 1 degeneration was found in 5/21 males and 9/21 females, and grade 2 degeneration was found in 10/21 females. In the top dose, degeneration was found in all female animals only, with 6/20 at grade 1, 10/20 at grade 2 and 4/20 at grade 3. This was accompanied by multifocal mineral deposits in males at the top dose and females from the mid dose. There was also an increase in urine volume and decrease in urine specific gravity in males from the mid dose group.

Other findings included increases in absolute brain weight in female animals (↑ 2% at the low dose, ↑ 2% at the mid dose, the top dose were not studied), increases in relative brain weights were ↑ 3% in males and ↑ 5% in females at the mid dose, ↑ 11% in males and ↑ 9% in females at the top dose. There was also a decrease in absolute heart weights; ↓ 3% in males at the low dose, ↓ 6% in males at the mid dose, ↓ 10% in males at the top dose and ↓ 9% at the top dose.

The second study was a modified reproductive/developmental toxicity screening study (OECD TG 421), which was a GLP-compliant range finding study. Groups of 10 Wistar rats/sex/group were administered diethanolamine daily via drinking water for 14 days pre-mating, 6 days during the mating period and then males were dosed for a further week (for a total of 4 weeks) and females were dosed throughout gestation and lactation until PND 4 (for a total of 8 weeks). Doses were calculated to be 0, 46, 95, 137 and 144 mg/kg bw/d.

General toxicity included decreased water consumption in top dose females (↓ 36% during gestational days 10-18). Decreased food consumption was observed in males from the

low dose (reaching ↓18% at the top dose), and in females from 95 mg/kg bw/d (during premating this reached ↓46%, and during lactation fluctuated between ↓37% and ↓52%). Decreased body weight gain and decreased terminal body weights were observed in all treated groups. Terminal body weights were ↓4, 7, 11 and 12% in males and ↓4, 7, 8 and 11% in females at 0, 46, 95, 137 and 144 mg/kg bw/d respectively.

The target organs were the blood system, liver and kidneys.

Blood parameters were measured on day 28 in females and day 56 in males. These assessments revealed reduced RBC at the top two doses in males (↓ 11.4% and ↓ 17.9%, respectively). In females, RBC was reduced in all treated groups (↓ 9.6%, 9.2%, 19.3% and 24.6% at 0, 46, 95, 137 and 144 mg/kg bw/d respectively. Reduced HGB was noted in all treated males and from 137 mg/kg bw/d in females (levels were ↓5.7%, ↓11.4%, ↓18.2% and ↓22.7% in males and ↑13.0%, ↑15.2%, ↓22.8% and ↓28.3% in females at 0, 46, 95, 137 and 144 mg/kg bw/d respectively).

Reduced choline content was found in plasma in males and females in the 137 and 144 mg/kg bw/d groups. No further detail regarding the magnitude of this finding was available in the CLH report.

There were a number of effects found in the liver. Increases in absolute and relative liver weights were found in all treated groups; relative liver weights were ↑ 13%, 21%, 33% and 34% in males and ↑23%, 32%, 39% and 38% in females at 0, 46, 95, 137 and 144 mg/kg bw/d respectively. Organ weight changes were accompanied by histopathological findings. Centrilobular hypertrophy was found in females in the top two dose groups and diffuse hypertrophy was found in males in the top two dose groups. A clay discolouration of the liver was noted in males and females in the top two dose groups. Peripheral fatty changes were found in males and females in all but the low dose groups; no details regarding the magnitude of these findings were available.

In the kidney, increased absolute and relative weights were noted in all groups; relative weights were ↑ 16%, 17%, 15% and 18% in males and ↑ 23%, 22%, 33% and 35% in females at 0, 46, 95, 137 and 144 mg/kg bw/d respectively. The changes in organ weight were accompanied by histopathological findings. Tubular degeneration was noted in all treated groups; this finding was not reported in controls of either sex. Specific incidences are provided in the tables 3 and 4 below.

Table 3: kidney degeneration in males

Kidney degeneration in males	Grade 1	Grade 2	Grade 3	Grade 4
46 mg/kg bw/d	10/10	0/10	0/10	0/10
95 mg/kg bw/d	0/10	6/10	4/10	0/10
137 mg/kg bw/d	1/10	5/10	4/10	0/10
144 mg/kg bw/d	0/10	7/10	3/10	0/10

Table 4: kidney degeneration in females

Kidney degeneration in females	Grade 1	Grade 2	Grade 3	Grade 4
46 mg/kg bw/d	10/10	0/10	0/10	0/10
95 mg/kg bw/d	1/10	5/10	4/10	0/10
137 mg/kg bw/d	0/10	0/10	6/10	4/10
144 mg/kg bw/d	0/10	0/10	3/10	7/10

Tubular casts were found in all treated group of females, and in treated males from 95 mg/kg bw/d. No information regarding incidence or severity was available in the CLH report. Mineralisation was also found in both sexes from 95 mg/kg bw/d. Reduced choline content was also noted in the kidney in all treated animals. No further detail regarding the magnitude of this finding was available in the CLH report.

The third study available was a repeat-dose toxicity (RDT) study considered to be equivalent to an OECD TG 408 90-day oral toxicity study. Groups of 10 Fischer 344 rats/sex/group were administered doses of 0, 25, 48, 97, 202, 436 mg/kg bw/d (males) and 0, 14, 32, 57, 124, 242 mg/kg bw/d (females) orally via drinking water daily for 13 weeks.

At the top dose, 2/10 males died. Clinical signs of toxicity included decreased body weight gain. In males this was ≥10% in all but the lowest dose group, up to a maximum of ↓ 44% at the top dose. In females this was ≥10% in all dose groups, up to a maximum of ↓ 25% at the top dose. Water consumption was decreased in males from 48 mg/kg bw/d and in females at the top dose. Other clinical observations consisted of tremors, emaciation, abnormal posture and a rough coat, which were observed in males and females in the top 2 dose groups.

The target organs in this study were the blood system, kidney and nervous system.

Haematological effects included microcytic anaemia; decreased MCV was noted in all groups and across both sexes. In males, this was ↓1.9%, ↓ 3.7%, ↓ 7.4%, ↓ 9.3% and ↓ 9.3% at 25, 48, 97, 202 and 436 mg/kg bw/d, respectively. In females, MCV was ↓ 1.8%, ↓ 3.6%, ↓ 5.4%, ↓ 8.9% and ↓ 12.5% at 14, 32, 57, 124 and 242 mg/kg bw/d, respectively.

Reduced RBC was reported in males from 48 mg/kg bw/d and in females from 32 mg/kg bw/d. In males, RBC was ↓ 6.71%, ↓16.6%, ↓27.19% and ↓35% at 48, 97, 202 and 436 mg/kg bw/d, respectively. In females, RBC was ↓6.67%, ↓10%, ↓ 19.29% and ↓ 23.45% at 32, 57, 124 and 242 mg/kg bw/d.

Reduced HGB was also reported in all treated males and in females from 32 mg/kg bw/d. HGB was ↓3.4%, ↓ 10.1%, ↓ 14.9%, ↓ 33.8% and ↓ 39.9% in males at 25, 48, 97, 202 and 436 mg/kg bw/d, respectively. In females, HGB was ↓ 8.6%, ↓ 13.9% ↓ 25.2% and ↓ 30.5% at 32, 57, 124 and 242 mg/kg bw/d.

In the kidney, absolute weights were increased in females compared to controls, and relative weights were increased in both sexes. Relative kidney weights were ↑ 11%, ↑13%, ↑ 12% and 2% in males at 48, 97, 202 and 436 mg/kg bw/d, respectively. In females, relative kidney weights were ↑ 36%, ↑ 39%, ↑ 36%, ↑ 53% and ↑ 87% at 14, 32, 57, 124 and 242 mg/kg bw/d respectively.

The increase in kidney weights was accompanied by an increased incidence of nephropathy, found to be present in all dose groups. In males, this was 2/10 animals with a severity of 1.0 in the 25 mg/kg bw/d, 2/10 animals with a severity of 1.0 in the 48 mg/kg bw/d group, 3/10 animals with a severity of 1.0 in the 97 mg/kg bw/d group, 6/10 animals with a severity of 1.0 in the 202 mg/kg bw/d group, and 10/10 animals with a severity of 2.4 in the 436 mg/kg bw/d group. In females, this was 9/10 animals with a severity of 1.0 in the 14 mg/kg bw/d group, 10/10 animals with a severity of 1.5 in the 32 mg/kg bw/d group, 10/10 animals with a severity of 1.4 in the 57 mg/kg bw/d group, 9/10 animals with a severity of 1.0 in the 124 mg/kg bw/d group, and 2/10 animals with a severity of 1.0 in the 242 mg/kg bw/d group.

Tubular necrosis was also noted in 10/10 top dose males. In females, necrosis was noted in the top 2 dose groups; in 1/10 animals at 124 mg/kg bw/d and in 3/10 animals at 242

mg/kg bw/d. All findings were graded at a severity rating of 1.0; there were no findings of necrosis in any of the control groups.

Tubular mineralisation was noted in males in the top 3 dose groups. There was an incidence of 1/10 animals with a severity of 1.0 in the 97 mg/kg bw/d, 10/10 animals with a severity of 1.8 in the 202 mg/kg bw/d group, and 10/10 animals with a severity of 1.7 in the 436 mg/kg bw/d group. Tubular mineralisation was reported in all treated females, and there was a dose-related increase in severity, with an incidence of 10/10 animals with a severity of 2.0 in the 14 mg/kg bw/day group, 10/10 animals with a severity of 2.5 in the 32 mg/kg bw/d group, 10/10 animals with a severity of 3.0 in the 57 mg/kg bw/day group, 10/10 animals with a severity of 2.4 in the 124 mg/kg bw/d group and 10/10 animals with a severity of 1.7 in the 242 mg/kg bw/d group.

Demyelination (minimal to mild) of the brain (medulla) and spinal cord was noted in all animals in the top 2 dose groups (both sexes).

The fourth study available was another RDT study (again, considered similar to OECD TG 408); groups of 10 B6C3F1 mice/sex/group were administered diethanolamine at doses of 0, 104, 178, 442, 807, 1674 mg/kg bw/d (males) and 0, 142, 347, 884, 1154, 1128 mg/kg bw/d (females) via drinking water for 13 weeks. RAC noted that the male low dose was the only one that fell within the GVs for STOT RE classification.

All animals in the top two dose groups died before the end of the study. These groups were excluded from any further reporting.

The target organs in this study were the liver, kidney and heart.

In the liver, dose-related increases were reported in absolute and relative weights in both sexes. Relative liver weights were ↑ 18%, 29% and 56% at 104, 178 and 442 mg/kg bw/d in males and ↑ 25%, 53% and 124% at 142, 347 and 884 mg/kg bw/d in females. Changes in liver weights were also accompanied by histopathological changes in the liver, which were present from the lowest doses: hypertrophy with increased eosinophilia, disruption of hepatic cords was seen in 9/10 males with a severity of 2.0 at the low dose, all males in the mid dose with a severity of 2.8 and all males in the top dose group with a severity of 3.0; it was also noted in all female animals in all dose groups with a severity of 1.9 in the low dose group, 2.8 in the mid dose group and 3.0 in the top dose group. None of these findings were present in control groups. The CLH report also notes increased nuclear pleomorphism, multinucleated hepatocytes with increasing severity grades (from 1.9 to 3.0) and increased incidence of hepatocellular necrosis from the low dose. Increases in enzyme activities were also noted: ALT was in females at the mid dose (↑ 28%), and in both sexes at the top dose (↑ 196% in males, ↑ 128% in females). Sorbitol-DH levels were also increased in top dose males (↑ 84%).

In the kidney, there were increases in absolute weights in males; ↑ 10% at the mid dose and ↑ 14% at the top dose. There were increases in relative kidney weights: in males ↑ 20% at the mid dose and ↑ 26% at the top dose; and in females ↑ 13% at the mid dose and ↑ 31% at the top dose. Increases in kidney weights were accompanied by an increased incidence of nephropathy in males: 5/10 at the mid dose and 8/10 at the top dose. No further information regarding severity was available.

In the heart, increases in absolute weight was seen in females at the top dose. Relative heart weights were increased in males at the top dose and females in mid and top doses. This was accompanied by minimal-to-marked degeneration and necrosis of cardiac myocytes from 442 mg/kg bw/d in males and 884 mg/kg bw/d in females.

Studies available via the inhalation route (findings relating to effects for STOT RE are listed here, other findings are discussed in the relevant section of this document)

The first study was a subacute inhalation toxicity study, conducted according to OECD TG 412 and GLP. Groups of 10 Wistar rats/sex/dose were administered diethanolamine via the inhalation route in an aerosol form. Exposure was nose/head only with a MMAD of 3.7-4.8 µm at levels of 0, 110, 210 and 400 mg/m^3, 6 hours per day, 5 days per week for 2 weeks.

Males at the top dose had a slightly decreased body weight and had a body weight gain equivalent to 77% of control animals.

Males and females showed slightly reduced cholesterol values (~15–23% reduction).

In the liver, there was an increase in absolute weight of ↑ 13% in top dose females.

The second study available was a 90-day subacute inhalation toxicity study conducted according to OECD TG 413 and GLP. Groups of 13 Wistar rats/sex/dose were administered diethanolamine via the inhalation route in an aerosol form. Exposure was nose/head only with a MMAD of 0.6 – 1.9 µm at levels that were analytically determined to be 0, 15, 153 and 410 mg/m^3 for 6 hours per day, 5 days per week.

Local effects were observed from the lowest dose and consisted of grade 1/2 focal squamous metaplasia of the laryngeal epithelium in all animals. From the mid dose, there was a concentration-dependent increase in laryngeal squamous hyperplasia along with local inflammation of the larynx and trachea.

Effects on the blood system were noted at the top dose only and included a decrease in MCV (↓ 4% in males and ↓ 3% in females), a decrease in RBC (↓ 6.2% in males and ↓ 8.5% in females), and a decrease in HGB (↓ 10.2% in males and ↓ 13.9% in females).

In the liver, there were increases in relative weights: at the mid dose, this was ↑ 10% in females, and at the top dose was ↑ 9% in males and ↑ 19% in females. The weight effects

were accompanied by slightly increased ALP in males and females and decreased ALT in males from the mid dose.

In the kidney, there were increases in relative weights (↑ 10% in males and ↑ 12% in females at the mid dose, and ↑ 13% in males and ↑ 16% in females at the top dose). This was accompanied by minimal/slight tubular hyperplasia in some females and intratubular lithiasis in males.

Urinalysis showed an increase of blood in the urine of top dose animals. In males, there was an increase in excretion of renal tubular epithelium cells including casts from the mid dose. No information regarding the incidence or severity of these effects was available in the CLH report.

The third study was a 90-day subacute inhalation toxicity study conducted according to OECD TG 413 and GLP. Groups of 10 Wistar rats/sex/dose were administered diethanolamine via the inhalation route in an aerosol form. Exposure was nose/head only with a MMAD of 0.6 – 0.7 µm at levels that were analytically determined to be 0, 1.57, 3.43 and 8.18 mg/m^3 for 6 hours per day, 5 days per week. In addition, 10 females/dose group were observed for a 3-month recovery period.

All observed effects in this study were local only. At the mid dose, 3/10 male rats were observed to have focal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis, considered to be minimal/adaptive. At the top dose, 9/10 males and 9/10 females were observed to have focal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis, 3/10 males and 2/10 females were observed to have submucosal inflammation, and 2/10 males were found to have squamous metaplasia at the ventral pouch and arytenoid cartilages.

Studies available via the dermal route (findings relating to effects for STOT RE are listed here, other findings are discussed in the relevant section of this document)

The first study was a 90-day sub-chronic dermal toxicity study conducted to NTP standards, considered similar to OECD TG 411, which was GLP-compliant. Groups of 10 Fischer 344 rats/sex/dose group were administered doses of 0, 32, 63, 125, 250 and 500 mg/kg bw/d via the dermal route. Diethanolamine was applied to the shaved back of each animal from the mid back to the interscapular region. The application site was not occluded and the test substance was applied daily, 5 days per week for 13 weeks.

Local effects consisted of skin lesions (ulceration, inflammation, hyperkeratosis and acanthosis) at the application site at all dose levels. In terms of systemic toxicity, the target organs were the blood system, kidneys and nervous system.

Haematological effects consistent with microcytic anaemia were reported. There were treatment-related decreases in MCV in both sexes (↓ 1.9%, 3.8%, 8.0%, 10.2% and 12.5%

in males; ↓ 1.8%, 3.6%, 14.5%, 9.4% and 13.7% in females at 32, 63, 125, 250 and 500 mg/kg bw/d, respectively), accompanied by decreased RBC in males from 125 mg/kg bw/d (↓ 3.5%, 12.27% and 30.44%) and in females from 32 mg/kg bw/d (↓ 3.81%, 7.92%, 14.5%, 22.29% and 35.63%). HGB was also decreased in females from the low dose (↓4.7%, 9.8%, 17.4%, 29.2% and 47.6% at 32, 63, 125, 250 and 500 mg/kg bw/d) and in males from 63 mg/kg bw/d (↓ 2.6%, 8.4%, 20.2% and 40.9%).

In the kidney, increases in absolute and relative weights were observed in males and females. No information regarding the magnitude of the changes was available in the CLH report. An increase in incidence and severity of nephropathy was also reported in females; 9/10 with a severity of 1.3 in the 32 mg/kg bw/d group, all animals with a severity of 1.4 in the 63 mg/kg bw/d group, all animals with a severity of 1.7 in the 125 mg/kg bw/d group, 7/10 animals with a severity of 1.1 in the 250 mg/kg bw/d group and 4/10 animals with a severity of 1.1 in the 500 mg/kg bw/d group.

These findings were accompanied by tubular mineralisation. Findings were observed at all doses in females and in top dose males: 9/10 with a severity of 1.0 in females in the 32 mg/kg bw/d group, all females with a severity of 1.6 in the 63 mg/kg bw/d group, all females with a severity of 1.9 in the 125 mg/kg bw/d group, all females with a severity of 1.1 in the 250 mg/kg bw/d group and 4/10 females with a severity of 1.0 and 9/10 males with a severity of 1.9 in the 500 mg/kg bw/d group. Tubular necrosis was also reported in 2/10 females with a severity of 1.0 in the 250 mg/kg bw/d group and all females with a severity of 1.0 in the 500 mg/kg bw/d group.

In the brain, minimal demyelination was found in the medulla oblongata in the top 2 dose groups. At 250 mg/kg bw/d, of the finding was observed in 7/10 females; at 500 mg/kg bw/day, there was an incidence of 10/10 in males and 9/10 in females.

The second study available was considered to be equivalent or similar to an OECD TG 451, combined long term toxicology and carcinogenicity study. Groups of 50 Fischer 344 rats/sex/group were administered doses of 0, 16, 32 and 64 mg/kg bw/d for males and 0, 8, 16 and 32 mg/kg bw/d for females, via the dermal route daily, 5 days per week, for 103 weeks.

In the liver, a decrease in the incidence of basophilic foci was noted in females from the low dose and in males from 32 mg/kg bw/d.

In the kidney, nephropathy was noted in females at all doses: 47/50 females at 8 mg/kg bw/d with a severity grading of 1.5; 48/50 females at 16 mg/kg bw/d with a severity grading of 1.9, 48/50 females at 32 mg/kg bw/d with a severity grading of 2.7.

There was a lower incidence of fibroadenoma in top dose females; 5/50 females at 32 mg/kg bw/d compared to 14/50 females in the control group.

The third study was another 90-day subchronic dermal toxicity study conducted to NTP standards, considered similar to OECD TG 411, which was GLP-compliant. Groups of 10 B6C3F1 mice/sex/dose were administered doses of 0, 80, 160, 320, 630 and 1250 mg/kg bw/d via the dermal route. Diethanolamine was applied to the shaved back of each animal from the mid back to the interscapular region. The application site was not occluded and the test substance was applied daily, 5 days per week for 13 weeks.

In the liver, dose-related increases in absolute and relative weights were noted. Relative weights were ↑17%, 31%, 36% and 57% in males (at 160, 320, 630 and 1250 mg/kg bw/d) and ↑ 11%, 19%, 33%,45% and 89% in females (at 80,160, 320, 630 and 1250 mg/kg bw/d, respectively). Liver weight changes were accompanied by hepatocellular necrosis in males and increased nuclear pleomorphism in males from 80 mg/kg bw/d and females from 160 mg/kg bw/d. An increase in Sorbitol-DH activity was noted in males from 320 mg/kg bw/d. ALT activity was increased in males from 630 mg/kg bw/d, and in females at the top dose.

In the kidney, absolute weights were increased in all treated groups compared to controls. In males, there was a dose-related increased in relative kidney weights from the low dose (↑ 8%, 11%, 15%, 18% and 36% at 80, 160, 320, 630 and 1250 mg/kg bw/d), whereas in females, relative kidney weights were increased at the top two doses only (↑ 15% and 23%). Kidney weight changes were accompanied by minimal to mild renal tubular necrosis in males and females from 630 mg/kg bw/d, no information was available in the CLH report regarding incidence or severity.

In the heart, absolute weights were increased in both sexes at the top dose, although no information regarding the magnitude of the changes was available. Cardiac myocyte degeneration was noted but no further information regarding this was available in the CLH report.

The fourth study was considered to be equivalent or similar to an OECD TG 451, combined long term toxicology and carcinogenicity study. Groups of 50 B6C3F1 mice/sex/group were administered doses of 0, 40, 80, and 160 mg/kg bw/d via the dermal route daily, 5 days per week for 103 weeks.

In the liver, increases in the incidences of hepatocyte changes were noted, including cytoplasmic changes in males, with an incidence of 17/50 in the low dose group, 17/50 in the mid dose group and 14/50 in the top dose group, compared to 1/50 in controls. Syncytial alterations were noted in both sexes from the mid dose group with an incidence of 30/50 males and 17/50 females at the mid dose and 23/50 males and 18/50 females in the top dose group, compared with no findings in the control group.

In the kidney, there was an increased incidence of renal tubule hyperplasia in males; 10/50 in the top dose group compared with 1/50 controls.

There was also an increased incidence of follicular cell hyperplasia in the thyroid gland; 22/50 males and 28/50 females in the low dose group, 30/50 males and 32/50 females in the mid dose group and 42/50 males and 39/50 females in the top dose group compared with 18/50 in control groups.

Discussion

Blood system

Microcytic anaemia was reported in several studies. The EOGRTS study, reproductive/developmental toxicity screening test and repeated dose 90-day oral toxicology study reported reduced levels of several parameters indicative of microcytic anaemia (especially HGB and RBC, but also HCT, MCV and MCH) at doses below or slightly above the GV classification in STOT RE 2. Blood parameters, such as HGB, were reduced by ≥ 20 %. RAC noted that according to the CLP guidance (ECHA, 2024b), a ≥ 20 % reduction in HGB is considered to be an adverse effect on haematology. Dermal administration of diethanolamine also resulted in dose-dependent reductions of HGB levels, which exceeded 20 % in females exposed to doses of 250 mg/kg bw/d (i.e., just above the upper GV for STOT RE 2 of 200 mg/kg bw/d). A 90-day sub-chronic inhalation toxicity study also reported decreased HGB, RBC and MCV-levels, but these decreases were < 20 % in magnitude and occurred at doses above the GV for STOT RE classification.

Overall, several of the studies reported microcytic anaemia after exposure to diethanolamine and four of the studies reported reduction of blood parameters by > 20 % at doses below or slightly above the GV for STOT RE 2 classification. RAC concluded that classification for STOT RE 2 (blood system) was warranted.

Liver

Liver weight was increased in a majority of the available studies and for all exposure routes. Centrilobular hypertrophy and enzyme activity (reduced ALT, increased AST and ALP) were also present in more than one study. In the 90-day oral toxicity study in mice, an increase in hypertrophy with increased eosinophilia and disruption of hepatic cords was also reported. These effects were observed at the GV for classification in males and slightly above the GV for classification in females. In the 90-day oral toxicity study in mice, there was also an increase in enzyme activity.

In the 90-day dermal toxicity study in mice, hepatocellular necrosis and cytological changes with increased nuclear pleomorphism were reported in both sexes below the GV for classification. This was accompanied by increased enzyme activities (ALT and Sorbitol-DH).

RAC concluded that classification for STOT RE 2 (liver) was warranted.

Kidney

Kidney weight was increased in the majority of the available studies and for all exposure routes. Other adverse effects in the kidney such as degeneration/regeneration of tubular epithelium, nephropathy, tubular cast, tubular mineralisation and increased cell proliferation were all noted after oral exposure. Similar adverse effects were also noted in dermal toxicity studies. The 90-day dermal toxicity study reported nephropathy and increased severity at concentrations below GV for STOT RE 2 and tubular necrosis in females slightly above the GV for STOT RE 2 classification and the rat carcinogenicity study also reported nephropathy. One inhalation study also reported tubular hyperplasia in some females, tubular lithiasis, increased excretion of renal tubular epithelium including casts and blood in urine at doses below guidance values for classification.

RAC concluded that classification for STOT RE 2 (kidney) was warranted.

Nervous system

Nervous system effects were reported in the 90-day oral toxicity and dermal toxicity studies on F344 rats. In the 90-day oral exposure study, tremors and abnormal posture was reported at 48 mg/kg bw/d in males and 242 mg/kg bw/d in females. Demyelination in the medulla oblongata and spinal cord were reported in all females slightly above (≥ 124 mg/kg bw/d) the GV for STOT RE 2. Demyelination was reported in males at a higher concentration. In the 90-day dermal exposure study, demyelination in the medulla oblongata was reported in females above (≥ 250 mg/kg bw/d) the GV for STOT RE 2 classification (≤ 200 mg/kg bw/d). In males the effects occurred at higher doses.

RAC concluded that classification for STOT RE 2 (nervous system) was warranted.

Conclusion

Overall, RAC concluded that diethanolamine warranted classification for STOT RE 2 for effects on the blood system, kidney, nervous system and liver. They referred to Section 3.9.4.1 of the Guidance on the Application of the CLP Criteria (ECHA, 2024b), which recommends using the general term ‘damage of organs’ in the hazard statement where more than three primary target organs have been identified. Therefore, RAC concluded that diethanolamine should be classified as STOT RE 2; H373 (damage of organs).

Classification proposed by the Agency:

The Agency agrees with RACs conclusion on classification. Diethanolamine meets the criteria for classification for STOT RE 2 (H373) with regard to the blood system, liver, kidney and nervous system. Since more than three organs are included in this classification, the Agency agrees that the classification and hazard statement should be **STOT RE 2; H373 (damage to organs)**.

Germ cell mutagenicity

Classification agreed by RAC:

For germ cell mutagenicity, 6 *in vitro* studies were available. The first was a bacterial reverse mutation assay considered similar to OECD TG 471. It was not GLP-compliant and deviated from the TG by only using concentrations of up to 4000 µg/plate. The following bacterial strains were used: *S. typhimurium* TA, 1535, TA 1537, TA 1538, TA 98, TA 100, *E. coli WP2* and *WP2uvrA*. Plates were incubated at the following concentrations of diethanolamine: 0, 125, 250, 500, 1000, 2000 and 4000 µg, in the presence and absence of S9. Results were reported as 'negative' with no further elaboration.

The second study was an *in vitro* gene mutation study in bacteria, considered similar to OECD TG 471, with no information regarding GLP-compliance. The study deviated from the TG by only using 4 bacterial strains and a maximum concentration of diethanolamine of 3333 µg/plate. The bacterial strains used were: *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100. Concentrations of diethanolamine used were 0, 33, 100, 333, 1000 and 3333 µg/plate in the presence and absence of S9. Results were reported as negative, stating that cytotoxicity was noted in the 3333 µg plate. RAC considered this study to be supportive only.

A non-guideline chromosome aberration study was also available. This was not GLP compliant and deviated from current test guidelines by only evaluating 100 cells, as well as lacking individual data. The study used rat liver cell lines[5] with diethanolamine concentrations of 0, 0.125, 0.25, 0.5 of GI50 (50% growth inhibition), with and without S9 for 24 hours. Results were reported as negative, with no cytotoxicity or precipitation specified. RAC considered this study to be supportive only.

A chromosome aberration study similar to OECD TG 473 was available. No information regarding GLP-compliance was provided and the purity of diethanolamine used was not specified. In the study, CHO (Chinese Hamster Ovary) cells were incubated with diethanolamine at the following concentrations without S9: 0, 101, 505, 2010 µg/ml; and with S9 at 0, 303, 1010, 3010 µg/ml. Results were reported as negative with no further detail available in the CLH report.

An *in vitro* gene mutation in mammalian cell study, considered similar to OECD TG 476 was available, conducted according to GLP compliant. L5178Y mouse lymphoma cells were incubated with diethanolamine at concentrations of 0, 25, 50, 100, 200, 300, 400, 600 µg/ml in the presence and absence of S9. Cytotoxicity was reported at 400 µg/ml, and the results were reported as negative with no further details available in the CLH report.

[5] Cell lines are listed as RL1 and RL4 in the CLH report, but RL2 and RL4 in the RAC opinion.

The final *in vitro* study available was a DNA damage and/or repair study (*in vitro* sister chromatid exchange in mammalian cells) considered similar to an OECD TG 479 study. No details regarding GLP compliance were available and the purity of diethanolamine used was not specified. In the study, CHO cells were incubated with diethanolamine at the following concentrations: 0, 150, 500, 1500 µg/ml in the presence and absence of S9. Cytotoxicity was observed, although further details are not available, and the overall result was reported as negative. RAC considered this study to be supportive only.

One *in vivo* study regarding diethanolamine was also available for consideration, referred to as an "*In vivo* mammalian somatic cell study: cytogenicity/erythrocyte micronucleus". The study protocol was similar to the OECD TG 474 procedure, with the one deviation being that signs of toxicity were noted in the lowest dose tested. This study was not GLP-compliant and RAC considered it to be a supporting study only. During the study, 20 B6C3F1 mice (10 males and 10 females per group) were administered diethanolamine dermally at the following doses: 0, 80, 160, 320, 630, 1250 mg/kg bw 5 times a week for 13 weeks. Local and systemic signs of toxicity were observed down to the lowest concentration; no further information was available regarding this. The results from this study were negative.

In the absence of any positive *in vitro* or *in vivo* data, RAC concluded that classification for germ cell mutagenicity was not warranted.

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on classification; diethanolamine does not warrant classification for germ cell mutagenicity.

Carcinogenicity

Classification agreed by RAC:

There were 3 studies available for the assessment of carcinogenicity for diethanolamine. The first was a carcinogenicity study, which was GLP-compliant and followed a protocol similar to OECD TG 451. Fifty male F344 rats/ group were administered diethanolamine at 0, 16, 32 and 64 mg/kg bw/d and 50 females/group were administered 0, 8, 16 and 32 mg/kg bw/d, 5 days per week for 103 weeks, with a post-exposure period of 10 days. The substance was administered dermally using ethanol as a vehicle. General toxicity and non-neoplastic findings are discussed in the STOT RE section.

There were no neoplastic findings. Other reported findings included a 7% decrease in body weight in top dose males, some hyperkeratinosis at the application site and exudate. In females, nephropathy was also noted (incidences of 40, 47, 48 and 48 in each dose group, respectively).

The next study was a dermal carcinogenicity study, conducted according to GLP and following a protocol similar to OECD TG 451. Groups of 50 B6C3F1 mice/sex/group were administered diethanolamine at doses of 0, 40, 80 and 160 mg/kg bw/d, 5 days per week for 103 weeks, with a post exposure period of 10 days. Administration was via the dermal route with ethanol as the vehicle. General toxicity and non-neoplastic findings are discussed in the STOT RE section.

In female mice, there was a statistically significant decreased survival rate at all doses: 44/50 in controls, 33/50 in the low dose group, 33/50 in the mid dose group and 23/50 in the top dose group. Study authors attributed the deaths to the liver neoplasms observed. There was no effect on survival in males.

This was accompanied by a bodyweight decrease which showed a dose-response relationship (up to 24% for females in the top dose group and 11% for males in the top dose group, further details available in the STOT RE section).

Liver effects consisted of cytoplasmic alterations in males (1/50 in controls, 17/50 in the low dose group, 17/50 in the mid dose group and 12/50 in the top dose group). Syncytial alteration was noted in 0/50 (m) and 0/50 (f) in control groups, 28/50 (m) and 2/50 (f) in low dose groups, 38/50 (m) and 17/50 (f) in mid dose groups and 23/50 (m) and 18/50 (f) in the top dose groups.

Neoplastic findings were present in the liver in males and females. This included an increase in hepatocellular adenoma, carcinoma and hepatoblastoma, as follows:

Table 5: neoplastic findings in the liver in the B6C3F1 mouse study, adapted from page 29 of the RAC opinion (ECHA, 2025)

Statistically significant trends highlighted in **bold**.

Concentration mg/kg bw/d	0	40	80	160	HCD
Hepatocellular adenoma	31/50 (m) 62% 32/50 (f) 64%	**42/50 (m) 84%** **50/50 (f) 100%**	**49/50 (m) 98%** **48/50 (f) 96%**	**45/50 (m) 90%** **48/50 (f) 96%**	118/249 (47.4% ±8.9%) (m) 133/252 (52.8% ±11.4%) (f)
Hepatocellular carcinoma	12/50 (m) 24% 5/50 (f)	17/50 (m) 34% **19/50 (f)**	**33/50 (m) 66%** **38/50 (f)**	**34/50 (m) 68%** **42/50 (f)**	54/249 (21.7% ±2.5%) (m)

	10%	**98%**	**76%**	**84%**	35/252 (13.9% ±7.3%) (f)
Hepatoblastoma	0/50 (m)	2/50 (m) 4%	**8/50 (m)** **16%**	**5/50 (m)** **10%**	1/249 (0.4% ±0.9%) (m)
Combined hepatocellular adenoma, carcinoma or hepatoblastoma	39/50 (m) 78% 33/50 (f) 66%	**47/50 (m)** **94%** 50/50 (f) 100%	**50/50 (m)** **100%** 50/50 (f) 100%	**49/50 (m)** **98%** 50/50 (f) 100%	154/249 (61.8% ±9.1%) (m) 149/252 (59.1% ±6.4%) (f)

Hepatocellular carcinomas and hepatoblastomas metastasised to the lungs in all groups. In controls this had an incidence of 3 males only; in low dose groups this was 4 males and 3 females; in mid dose groups this was 9 males and 6 females; and in the top dose groups the incidence was 7 males and 1 female. The size of the liver tumours was larger in animals exposed to diethanolamine, but no further information regarding this was available.

There were neoplastic findings in the kidney for male mice, which followed a dose response trend but were not statistically significant. The study included an extended analysis of proliferative lesions in the kidneys, which lead to the identification of more adenomas. Combined, the standard and extended evaluations showed a statistically significant dose-related trend of renal tubule adenomas in the two highest dose groups. Neoplastic kidney findings in male mice are summarised in Table 6, below.

Table 6: neoplastic findings in the kidney in the B6C3F1 mouse study, adapted from page 30 of the RAC opinion (ECHA, 2025)

Statistically significant trends and incidences highlighted in **bold**.

Concentration mg/kg bw/d	**0**	**40**	**80**	**160**	**HCD**
Adenomas (standard evaluation)	1/50 2%	4/50 8%	6/50 12%	6/50 12%	2/299 (0.7 % ± 1.0 %)

Carcinomas (standard evaluation)	2/50 4%	1/50 2%	0/50	2/50 4%	2/299 (0.7 % ± 1.0 %)
Adenomas and carcinomas (standard evaluation)	3/50 6%	5/50 10%	6/50 12%	8/50 16%	4/299 (1.3 % ± 2.4 %)
Adenomas (standard and extended evaluation)	1/50 2%	6/50 12%	**8/50 16%**	**7/50 14%**	N/A
Carcinomas (standard and extended evaluation)	2/50 4%	1/50 2%	0/50	2/50 4%	N/A
Adenomas and carcinomas (standard and extended evaluation)	3/50 6%	7/50 14%	8/50 16%	9/50 18%	N/A

The final study available was a short term, non-guideline carcinogenicity study in transgenic mice. Groups of 15-20 homozygous female Tg.Ac transgenic mice/sex/group were administered 0, 5, 10 or 20 mg of diethanolamine via the dermal route. GLP compliance was unspecified. The study only investigated skin tumours (no kidney or liver investigations); there were no neoplastic findings.

Discussion of mode of action

Diethanolamine is not mutagenic (see germ cell mutagenicity section). However, there was weak evidence of genotoxicity in the liver tumours observed in the mouse study, indicated by mutations in ß-catenin *Catnb* genes, which suggested oxidative DNA damage. The DS and RAC noted that these mutations were different to the H-*ras* mutations often found in spontaneous liver tumours. The DS suggested two possible mechanisms for carcinogenesis: either altered choline homeostasis, inducing a choline deficiency, or displacement of ethanolamine by diethanolamine in phospholipids that may

result in reduced endogenous production of choline. RAC acknowledged these suggestions but also noted that the mechanisms of diethanolamine-induced carcinogenesis may be more complex and not fully understood. RAC referred to several publications from the literature, including Lehmann-McKeeman *et al.* (2002), who demonstrated hepatic choline deficiency in mice after dermal exposure to diethanolamine, and publications by Nakae *et al*. (1992) and Denda *et al*. (2002), who demonstrated a link between a choline-deficient diet and increased liver tumours in rats and spontaneous liver tumour-resistant mouse strains. They also acknowledged publications suggesting associations between choline deficiency and liver/muscle dysfunction and fatty liver in humans, highlighting that several publications in the literature demonstrate that an increased dietary intake of choline can reduce cancer risk in humans (Sun *et al.,* 2016; Zhou *et al*., 2017; Xu *et al.,* 2008). RAC considered that it was possible that choline depletion could alter the expression of genes that regulate growth and tumour development, referring to Bachman *et al*. (2006), who suggested that choline depletion reduces S-adenosyl methionine (SAM) availability, resulting in hypomethylation of DNA and therefore altered gene expression. Studies suggest that there are species differences in the oxidation of choline to betaine, which is an important pathway in rodents for maintenance of hepatic SAM; this pathway is less important in humans, therefore resulting in mice being more sensitive than humans to choline deficiency.

Species differences in diethanolamine metabolism have also been reported in several literature publication. Accumulation of the substance in the liver has been demonstrated in rats and mice, with the DS suggesting that this may be driven by saturable membrane transporters in rodents. *In vitro* studies have also shown that diethanolamine is taken up by human hepatocytes. RAC suggested that diethanolamine uptake may increase with increased choline deficiency. Species differences have also been reported for hepatic DNA synthesis and decreased GJIC in hepatocytes after diethanolamine exposure, with *in vitro* data suggesting lower sensitivity in human hepatocytes.

RAC also considered that diethanolamine may disrupt phospholipid metabolism by displacing ethanolamine, as ethanolamine and choline are both precursors for phospholipid biosynthesis. This would in turn affect cell and organelle membrane function and the fatty acid second messengers. They highlighted an NTP study that described reductions in hepatic choline, phosphocholine, phosphatidylcholine, glycerophosphocholine and SAM in mice after exposure to diethanolamine (NTP, 1999b), noting that these results were similar to those observed in studies with choline deficient mice.

With regards to the kidney tumours observed in male mice, there was no indication of diethanolamine-induced choline deficiency. RAC noted that potential pre-neoplastic lesions were observed in the form of renal hyperplasia, but concluded that the mechanism of renal tumour formation had not been investigated.

Weight of evidence assessment

RAC considered the results of the studies described above in a weight of evidence assessment. Neoplastic findings were observed in one species, in the B6C3F1 mouse study, at multiple sites. These included increased incidences (above the HCD range) of hepatocellular adenomas and carcinomas in all treatment groups compared to the control. However, RAC noted that the control incidences of these findings were also higher than the HCD range. Metastasis of liver tumours to the lungs was reported in both sexes. With regards to malignancy, both carcinomas and adenomas were reported. In the absence of interim evaluations of the animals, RAC assumed it likely that liver adenomas may progress to carcinomas. Incidences of hepatoblastoma were also statistically significantly increased in males at the mid and top dose (above the HCD range), with RAC noting that this was a rare finding (0 incidences in the concurrent control). In the combined evaluation (standard evaluation + extended evaluation), there were also statistically significant increases in renal adenomas in males at the mid and top dose, another rare finding according to the concurrent control.

No information was available on tumour latency or structural similarity of diethanolamine to other carcinogenic substances. No excessive toxicity was observed in the mouse study.

Mice were exposed to the test substance via the dermal route; the potential for oral exposure (i.e., by licking the application site) was unknown. RAC noted that *in vivo* and *in vitro* dermal exposure ADME data indicated species differences in dermal absorption of diethanolamine, with highest skin penetration in mice, followed by rabbits, rats and humans. Tissue distribution was comparable in rats and mice, with accumulation of the substance observed in the liver and kidneys. Some species differences in diethanolamine metabolism have been reported (see 'Discussion of mode of action' section above). RAC considered that possible MOAs for the liver tumours included choline deficiency and ethanolamine displacement in phospholipids, but no MOA was hypothesised for the kidney tumours. Overall, RAC concluded that human relevance could not be excluded. They concluded that the observed liver and kidney tumours were adverse and relevant to humans.

Conclusion

There were no human data available to warrant classification in Category 1A. According to Table 3.6.1 of the CLP Regulation, classification in Category 1B is '*based on strength of evidence together with additional considerations*'. Animal data should provide '*sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen)*'. Classification in Category 2 can be based on '*limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies*'.

RAC considered that data from a single study/species may be sufficient to fulfil this criterion, if malignant neoplasms are observed to an unusual degree, or if there are strong

neoplastic findings at multiple sites. RAC concluded that the B6C3F1 mouse study was well-conducted and provided sufficient evidence for carcinogenicity in one species, in the form of liver adenomas and carcinomas (both sexes, with metastasis) and hepatoblastoma and renal adenomas (males). However, they considered that the study had some uncertainties, such as high incidence of hepatocellular adenoma in the concurrent control, lack of malignancy in the kidney tumours, and that similar results were not obtained in the rat carcinogenicity study. They additionally noted that, as the study was performed using dermal exposure, the carcinogenic effects could have been underestimated as oral exposure has been shown to have a higher absorption rate in rats. Due to the uncertainties in the mouse study, along with the fact that carcinogenic findings were not obtained in any other studies, RAC concluded that classification as Category 2 was appropriate.

The DS calculated a T25 value based on the hepatocellular adenomas and carcinomas observed in the mouse study. The values were 12.34 mg/kg bw/d for males and 7.14 mg/kg bw/d for females for hepatocellular adenoma and 9.82 mg/kg bw/d for males and 7.14 mg/kg bw/d for females for hepatocellular adenoma and carcinoma. These correlate to the medium potency group in section I.2.3 of the Guidance on the Application of the CLP Criteria (ECHA, 2024b)(those with a T25 value of between 1 and 100 mg/kg bw/d) and therefore RAC concluded that the GCL should apply. Overall, RAC concluded that diethanolamine should be classified as Carc. 2; H351 (Suspected of causing cancer).

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on classification. Diethanolamine meets the criteria for classification as Carc. 2; H351 (Suspected of causing cancer).

Reproductive toxicity

Classification agreed by RAC:

Adverse effects on sexual function and fertility

There were 8 studies available for the assessment of sexual function and fertility.

EOGRTS, OECD TG 443, rats

The first study was an OECD TG 443 EOGRTS, which was GLP-compliant with no deviations. Wistar rats (30/sex/dose for the parent generation, 20/sex/dose for the F1A cohort and 25/sex/dose for the F1B cohort) were administered diethanolamine daily via drinking water from 16 days pre-mating until the end of the study. In the P generation, doses were calculated to be 0, 6.8, 21.5 and 73.4 mg/ kg bw/d in males, and 0, 10.2, 29.4, 103.9 mg/kg bw/d in females during pre-mating, 0, 11.5, 34.9, 116.8 mg/kg bw/d in

females during gestation and 0, 24.0, 66.3, 173.3 mg/kg bw/d during lactation. In the F1 generation, doses were calculated to be 0, 11, 34 and 123 mg/kg bw/d in males and 13, 39 and 137 mg/kg bw/d in females.

In the F0 generation, there were no effects on the oestrus cycle during premating or mating, and there were no effects on fertility indices in males or females. The gestation length was increased from 22.0 days in controls to 22.5 days at the top dose, which was statistically significant. There was also a decrease in the number of implantations (an average of 12.3 in the controls, to 12.2 at the low dose, 11.4 at the mid dose and 7.8 at the top dose), this was statistically significant at the top dose and consequently led to decreased litter size in treatment groups (11.9, 11.8, 11.1 and 7.3 at the control, low, mid and top doses, respectively).

In the F1A cohort, in male animals, feminisation of the mammary gland was found in 4/16 animals. The DS described this as effects on the tubule-alveolar structure, decreased cytoplasm and increased basophilia and was graded from slight to severe. Diffuse hyperplasia was also noted in 1/16 males. Six females in the same cohort showed increased secretion of the mammary gland; this incidence was statistically significant.

At the top dose, decreased size and immature testicles were observed in 3/20 males in cohort 1A and 3/25 in cohort 1B; these were graded from moderate to extreme. Tubular degeneration was also noted in 3/20 males from cohort 1A and 4/25 in cohort 1B. Macrovesicular vacuolation in the ductus deference was noted in 4/20 males at the mid dose and 12/20 males at the top dose. These were graded at 1-3. Animals in this dose group were also reported to have aspermia in the epididymis, though no specific incidences were provided.

Prolonged and irregular oestrus cycles were noted in cohorts 1A and 1B. In cohort 1A cycles were 4.1 days in controls and 5.5 days at the top dose, whilst in cohort 1B cycles were 4.2 days in the control group and 5.0 days at the top dose. Both increases were statistically significant, but the DS noted that no particular cycle phase was prolonged.

Decreased macroscopical ovary size was noted at the top dose in 4/25 females in cohort 1B. A statistically significantly decreased number of primordial and growing follicles was also noted in the ovary in cohorts 1A and 1B at the top dose (88% compared to 100% in the control). Ovarian atrophy was found at the top dose: 1/20 animals at grade 2 and 4/25 at grades 4-5; these were for females in cohorts 1A and 1B respectively.

Luteal cysts were found at the mid dose in 1/20 females in cohort 1A and 1/25 females in cohort 1B. At the top dose they were found in 6/20 females in cohort 1A and 6/25 in cohort 1B. Absence of corpora lutea was observed in 2/25 females in cohort 1B at the top dose.

Body weight changes in this study included ≤ 10% reductions in mid- and top-dose P0 males, which RAC did not consider marked enough to account for the observed effects in top-dose males. In P0 females, decreases were statistically significant from premating through gestation and lactation (↓0.1, 4.1*, 5.1*, 15.1*, 9.8*, 8.5* and 8.1* %) and in the mid-dose at study termination (↓2.5%). However, these female top dose body weight measurements were not corrected for litter size; once this had been corrected, body weight decreases did not exceed 10% in any P0 female dose group, and RAC concluded that the statistically significant uncorrected values could be explained by the reduced numbers of implantations and litter size. As with the males, RAC concluded that the female body weight changes were not sufficient to explain the observed effects on fertility.

Reproductive/developmental toxicity screening study, rats

In a modified reproductive/developmental toxicity screening (OECD TG 421), which was GLP-compliant, 10 Wistar rats/sex/dose were administered diethanolamine daily via drinking water for 14 days pre-mating, 6 days during the mating period and then males were administered for a further week (for a total of 4 weeks) and females through gestation and lactation until PND 4 (for a total of 8 weeks). Doses were calculated to be 0, 46, 95, 137 and 144 mg/kg bw/d.

In the parent generation findings at the top dose included cystic corpora lutea in 2/10 females. Only the top dose and control group were investigated for this.

There was a dose-related decrease in the number of implantation sites: 12.1, 10.7, 8.3, 5.9 and 4.0 at 0, 46, 95, 137 and 144 mg/kg bw/d respectively; the decrease was statistically significant from 95 mg/kg bw/d. A decreased number of pups were delivered, with mean litter sizes of 11.6, 9.8, 6.6, 3.9 and 4.0 in the control, 46, 95, 137 and 144 mg/kg bw/d groups, respectively; this decrease was also statistically significant from 95 mg/kg bw/d

Gestation length also increased with treatment: 22.2 days in controls, 22.4 days in the 46 mg/kg bw/d group, 22.7 days at 95 mg/kg bw/d group, 23.1 days in the 137 mg/kg bw/d group and 22 days in the 144 mg/kg bw/d group. This was statistically significant at 137 mg/kg bw/d only, although at the highest dose there was only one litter born. A statistically significant decreased gestation index was also noted at the top dose: 33% compared to 100% in controls.

Fertility index was 100%, 100%, 100%, 90% and 30% at 0, 46, 95, 137 and 144 mg/kg bw/d, respectively. The decrease at the top dose was statistically significant.

Female body weights were reduced by 10.7, 17.5 and 18.4% at the end of gestation at 95, 137 and 144 mg/kg bw/d, respectively, but RAC noted that these were not corrected for the lower numbers of implantations and smaller litter sizes. Body weight reductions were < 10% in all dose groups on lactation day (LD) 1.

90-d repeated dose study (oral, rats)

The third study was a 90-day repeated dose toxicity (RDT) study considered to be similar to OECD TG 408. Groups of 10 Fischer 344 rats/sex/group were administered diethanolamine at doses of 0, 25, 48, 97, 202, 436 mg/kg bw/d (males) and 0, 14, 32, 57, 124, 242 mg/kg bw/d (females) orally via drinking water.

RAC noted that from 97 mg/kg bw/d, males showed reductions in testis and epididymis weight, though no specific details were discussed. Testicular degeneration was found in 3/10 males in the 202 mg/kg bw/d group and in all males at the top dose of 436 mg/kg bw/d.

Other findings from 97 mg/kg bw/d included hypospermia in the cauda epididymis and atrophy of the seminal vesicles and prostate glands (no further details provided). Reduced sperm motility was also observed in the 202 mg/kg bw/d group. No further details were provided on these findings.

RAC noted that body weights were decreased in all male treated groups (reductions of 5, 11, 18, 28.7 and 44.2% compared to the control at 24, 48, 97, 202, 436 mg/kg bw/d, respectively). They considered that the effects on organ weights could be related to the reduction in body weight. However, they noted that specific reproductive effects in the form of testis degeneration, hypospermia and organ atrophy were observed at 97 mg/kg bw/d,and could not be explained by the 18% reduction in body weight at this dose level.

No effects on female reproductive organs, oestrous cycle length or stages were found.

90-d repeated dose study (oral, mice)

Another RDT study was available; groups of 10 B6C3F1 mice/sex/group were administered diethanolamine at doses of 0, 104, 178, 442, 807, 1674 mg/kg bw/d (males) and 0, 142, 347, 884, 1154, 1128 mg/kg bw/d (females) via drinking water for 13 weeks. There were no findings relevant to effects on sexual function and fertility.

90-d repeated dose study (dermal, rats and mice)

The fifth study available was a 90-day sub chronic dermal RDT study considered to be equivalent to OECD TG 411. Groups of 10 Fisher rats/sex/group or 10 B6C3F1 mice/sex/group were administered doses of 0, 32, 63, 125, 250, 500 mg/kg bw/d (rats) and 0, 80, 160, 320, 630, 1250 mg/kg bw/d (mice) dermally. The back of each animal was shaved and the test substance applied from the mid back to the interscapular region, unoccluded. Formulation was applied daily, 5 days a week for 13 weeks. There were no findings relevant to effects on sexual function and fertility.

90-d repeated dose study (inhalation, rats)

The sixth study available was a 90-day sub chronic inhalation toxicity study, performed in line with OECD TG 413 and GLP. Groups of 13 Wistar rats/dose/group were administered diethanolamine by aerosol, with nose/head only exposure at levels of 0, 15, 152 and 410 mg/m³ with a MMAD 0.6–1.9 µm. Exposure was for 90 days (6 h/day, 5 days/week, totalling 65 exposures).

Diffuse testicular atrophy and minimal to slight atrophy of the prostate were noted in single animals at the top dose.

There were no effects on organ weights. RAC noted that decreased body weight gain was reported in males, but the extent of this was not specified.

There were no findings in female animals.

Reproductive/developmental study (dermal, mice)

The next available study was a non-guideline reproductive and developmental toxicity study; diethanolamine was dissolved in ethanol and applied to a 2 cm^2 area on the back of C57BL/6 mice (15 males/group and 10 pregnant females per group) at doses of 0, 20, 80 and 320 mg/kg bw/d. Males were exposed for 28 days, then paired with control females, and females were exposed from GD 6 to PND 21, for approximately 40 days. This study was conducted by the Korean FDA and an unofficial translation was provided.

In the P0 generation, there was a reduced percentage of motile sperm in all dose groups, with a dose-dependent decrease in all sperm motility parameters. No further information was available regarding this.

In the F1 offspring of P0 exposed males, there were reduced absolute epididymis weights at post-natal day (PND) 21 at the mid and top dose, and on PND 70 at the top dose. Reduced absolute testis weight was observed at PND 70 at the top dose, and reduced absolute prostate weight at PND 70 at all doses (statistically significant at the mid dose only). There was a reduced percentage of motile sperm at PND 70, which was described as statistically significant at the top dose.

In females, there were increased absolute uterus weights at PND 21 and 70 at all dose levels. No further details regarding incidence or the magnitude of the observations were available.

Slight body weight reduction was reported in males, but RAC noted that information on this was scarce. No information was available on systemic effects

Neuro-developmental toxicity study (dermal, mice)

In a non-guideline dermal neuro-developmental toxicity study, 6 female c57BL/6 mice were exposed to diethanolamine at doses of 0, 20, 80, 160, 320 and 640 mg/kg bw/d between GD 7-17. There were statistically significant reductions in the number of viable foetuses per litter at the top three dose levels (mean numbers of 4.8, 3.3 and 2.7 compared to 8.2 in the control). One dam died at the top dose.

Mechanistic study (mice)

The final animal study available was a non-guideline mechanistic study. Groups of 10 Swiss strain male albino mice were administered 0, 110, 165, and 330 mg/kg bw/d diethanolamine only, or 330 mg/kg bw/d diethanolamine + 10, 25 and 50 mg/kg bw/d curcumin dissolved in olive oil. The test substance was administered orally for a total of 45 days; it was unclear whether this was via gavage. Curcumin is reported to dose-dependently negate the effects of diethanolamine.

Findings included statistically significant and dose-dependent decreases in serum testosterone levels and reductions in testicular cholesterol/total lipid levels in all dose groups. No further information regarding the magnitude of these effects were available. Statistically significantly decreased activity of testicular 3β- and 17β-hydroxysteroid dehydrogenase was also found at the top dose and was reported to be dose-dependent.

In vitro data

One *in vitro* study was available regarding effects on sexual function and fertility on human sperm parameters. Sperm were taken from a group of 10 healthy adult donors between the ages of 23 and 25 and suspended in a 0.9% sodium chloride solution containing 0, 100, 200, 300, 400, or 500 µg/mL of diethanolamine. Only samples with sperm counts > 50 mio/mL, > 50% viability, and normal morphology were used. Sperm parameters (motility, viability, morphology (conducted by Gimesa stain) were measured after 0, 15, 30, 45, and 60 minutes.

Statistically significant decreases in motility and viability were found in all concentrations at all time intervals when compared to controls. Both parameters showed a dose-response relationship. There was also a dose-dependent decrease in the number of sperm with normal morphological presentation.

Conclusion

RAC referred to section 3.7.2.2.1.1 of the Guidance on the Application of the CLP Criteria (ECHA, 2024b), which advises against classifying for reproductive toxicity based on effects observed in the presence of marked systemic toxicity. They noted that body weight decreases generally did not exceed 10% when they occurred alongside effects on sexual

function and fertility; the exception to this was the occurrence of effects on male reproductive organs and sperm alongside an 18% reduction in male body weight at 97 mg/kg bw/d in the 90-day oral rat study. Overall, RAC concurred with the DS that effects on sexual function and fertility did not occur in the presence of marked systemic toxicity, and that observed effects were relevant for classification.

RAC discussed disruption of choline uptake and metabolism as a potential mode of action, referring to the EFSA Scientific Opinion on Dietary Reference Values for Choline (EFSA, 2016), which describes the importance of choline in both animal and human reproduction. However, RAC also noted that other possible MOAs had not been ruled out.

RAC considered the studies described above to show clear adverse effects on sexual function and fertility. In males, these effects consisted of weight decreases and histopathological changes in male reproductive organs, along with reductions in the numbers and quality of sperm. In females, effects consisted of reduced numbers of implantations, decreased litter size, prolonged oestrus cycles and gestation lengths, reductions in the numbers of developing follicles and the presence of luteal cysts.

In the absence of human data, RAC determined that classification in Category 1A was not appropriate. They concluded that the evidence of adverse effects on sexual function and fertility were sufficiently clear and consistent for diethanolamine to be placed in Category 1B, rather than Category 2.

Overall, RAC concluded that diethanolamine should be classified as **Repr. 1B; H360F (May damage fertility).**

Effects on development

There were several studies available regarding effects on development.

EOGRTS, OECD TG 443, rats

The first study was an OECD TG 443 EOGRTS, which was GLP-compliant with no deviations (also discussed under ‘Adverse effects on sexual function and fertility’). Wistar rats (30/sex/group for the parent generation, 75/sex/group F1 generation) were administered diethanolamine daily via drinking water from 16 days pre-mating until the end of the study. Doses were calculated to be 0, 12.75, 37.68 and 128.35 mg/kg bw/d. The F1 generation was divided into the following cohorts: 1A/1B (reproductive toxicity; 20/25 animals/sex/dose, respectively), 2A/2B (developmental neurotoxicity; both with 10 animals/sex/dose), and 3 (developmental immunotoxicity (10 animals/sex/dose).

Signs of parental toxicity included reduced body weight in males at the mid and top dose levels (≤10% at all stages of the study) and in females at the top dose (≤10% at all stages after correction for litter size). Effects on organs included microcytic anaemia in top-dose

males, liver hypertrophy at the top dose, liver enzyme changes from the mid dose, and slight brain weight increases (further discussed under 'STOT RE' above).

In F1 animals, a statistically significant lower pup viability index was noted between PND 0-4 at the top dose (↓93% compared with 100% in the control), including 2 litters completely lost. As discussed under 'Adverse effects on sexual function and fertility', maternal body weights were reduced by < 10% at this dose, when corrected for reduced numbers of implantations. While there were no differences in pup birth weight, pup weights were statistically significantly lower (↓13.2% males, ↓13.4% females) by PND 21. Mortalities occurred in the F1 generation at the top dose: 1/40 in cohort 1A, 2/50 in cohort 1B and 1/20 in cohort 2A.

This occurred concurrently with some signs of systemic toxicity; this included effects on water consumption (↓13% at the mid dose and ↓16% at the top dose) and food consumption (↓17.2% at the top dose), body weight effects (↓6% males, ↓ 7% females, top dose, PND 4), piloerection in F1 animals at the top dose but not in the parent generation and eosinophilic cysts at all dose levels at the top dose, which was also not observed in parent animals. Further details of these can be found in the STOT RE section.

Increased mean time to vaginal opening was reported in the treatment groups; from 29.7 days in controls to 30.2 days in the low dose, 30.6 days in the mid dose and 32.0 days at the top dose. This was statistically significant at the mid and top doses. The study authors noted that the concurrent control had a low pubertal age compared to HCD, and therefore only the value of 32.0 days at the top dose fell outside of the HCD range. F1 animals also showed delays in preputial separation, with the mean number of days reported to be 41.1, 41.2, 41.8 and 43.3 in the control, low, mid and high dose groups, respectively. This was statistically significant at the top dose; however, all values were within the HCD, and the effect was therefore considered by RAC to be a consequence of delayed development.

Hormone changes were reported in the form of an increasing trend in T4 levels compared to the control in both sexes in F1 animals. In females, the median increases on PND 4 were ↑12% and ↑27% at the low and mid doses, respectively (no plasma available at the high dose), whilst in males on PND 4, the median increase at the mid dose was ↑11.8%. On PND 22 the median increases in females reached ↑24%%, ↑46.4% and 48.9%, and in males reached ↑16.6%, 14.8% and 22.6% at the low, mid and high doses, respectively. By PND 92, the median increases were ↑5.2%, 21.3% and 37.3% in females and ↑6.8%, ↑6.7% and ↑18.2% in males at the low, mid and high dose, respectively.

In terms of developmental neurotoxicity, a high stepping gait was noted in several top dose F1 animals: 2/20 males in cohort 1A; 2/25 males and 3/25 females in cohort 1B; 1/10 females in cohort 2A; 1/10 males and 1/10 females in cohort 3, this was not observed in parental animals. At the top dose, the hippocampus and cerebellum both measured larger

in males (↑7% and ↑12% respectively), the parietal cortex measured 20% larger in females. Only top dose and control animals in the 2A cohort were investigated for this. Degeneration of the medulla oblongata and spinal cord in males and females was observed at PND 77 in cohort 2A. This finding was not observed in PND 22 in cohort 2 or in parental animals. An auditory startle response test was performed on cohort 2A on PND 22. At the top dose, there was no habituation to the test environment. The maximum amplitude of both sexes at the top dose was reduced compared to controls.

With regards to developmental immunotoxicity, for cohort 3, in the top dose group, there was a significantly lower percentage of CD4 lymphocytes (↓10.8%) and an increase in CD8 lymphocytes (↑17.8%), compared to the control. This equated to a decreased CD4:CD8 ratio of ↓22.9% compared to the control.

Reproductive/developmental toxicity screening test, modified OECD TG 421 (rats)

In the modified reproductive/developmental toxicity screening (OECD TG 421) described under 'Adverse effects on sexual function and fertility' above, an increase in post implantation losses was noted: 4.1% in controls, 9.9% in the 46 mg/kg bw/d group, 22.4% in 95 mg/kg bw/d group, 31.1% in the 137 mg/kg bw/d group and 81% in the 144 mg/kg bw/d group. This was statistically significant at the top dose.

Pup survival was reduced at 137 mg/kg bw/d; the viability index in each group was 99% in controls, 100% in the 46 mg/kg bw/d group, 92% at 95 mg/kg bw/d group, 32% in the 137 mg/kg bw/d group and 100% in the 144 mg/kg bw/d group (only one litter was born in the top dose group). The reduction at 137 mg/kg bw/d was statistically significant.

Parental toxicity included reduced female body weight at the end of gestation (↓5.3%, ↓10.7%, ↓17.5% and 18.4% compared to the control, at each increasing dose); by LD1, female body weight reductions were < 10% in all dose groups. Anaemia was observed from 46 mg/kg bw/d, and nephrotoxicity and liver effects were observed from 137 mg/kg bw/d (discussed under 'STOT RE' above).

Reproductive/developmental study (dermal, mice)

The third study available was a non-guideline reproductive and developmental toxicity study (also described under 'Adverse effects on sexual function and fertility', above); diethanolamine was dissolved in ethanol and applied to a 2 cm^2 area on the back of C57BL/6 mice (15 males/group and 10 pregnant females per group) described further above. This study was conducted by the KFDA and an unofficial translation was provided.

In the offspring of exposed females, reduced body weight gain and body weight were noted until PND 28 in males and females in the top dose group. Further details regarding the magnitude of this effect were not available.

In the offspring of exposed males there was delayed fur appearance in the top dose group, and delayed testes descent in the mid dose group. Both of these findings were considered not statistically significant, but no further details regarding incidence or magnitude were available.

In the behavioural tests conducted as part of the study, there was an increased latency time in the hot plate test in all male and female offspring from exposed females, in female offspring from exposed males this was dose-dependent. There was increased latency in the passive avoidance test in all offspring from exposed females, male offspring showed a dose dependent relationship which was not significant.

Range-finding developmental toxicity study (inhalation, rats)

The fourth study available was a non-guideline range finding study; 10 pregnant female Wistar rats/group were administered doses of 0, 100, 200 and 400 mg/m^3 of diethanolamine via the inhalation route, head/nose only, diethanolamine was administered as an aerosol with an MMAD of 0.6-1.2 µm.

The parental animals experienced increased absolute liver weight at the top dose, and increased relative liver weight from the mid dose. Decreased serum cholesterol and triglyceride was noted from the mid dose and increased sodium and creatinine were noted from the mid dose.

Decreased mean placental weight was noted from the mid dose, although RAC noted that the study reporting of this effect was unclear (summary says one time “decreased” another time “increased”). No effects on foetal weights or external findings were noted.

PNDT, OECD TG 414 (inhalation, rats)

The range-finding study was a precursor to the fifth study available, which was an OECD TG 414 prenatal developmental toxicity study. Twenty-five pregnant Wistar rats/group were administered doses of 10, 50 and 200 mg/m^3 via the inhalation route, head/nose only, diethanolamine was administered as an aerosol with an MMAD of 0.6-1.2 µm from GD 6 to 15.

In the parental group, vaginal haemorrhages were noted in 8/21 pregnant rats in the top dose group.

In offspring animals at the top dose there was an increase in foetuses with skeletal variations (mainly cervical ribs). No further details regarding magnitude, etc. were available.

There were no adverse findings in the mid or low dose groups.

PNDT, equivalent to OECD TG 414 (dermal, rats)

The sixth study available was a study judged to be the equivalent to an OECD TG 414 prenatal developmental toxicity study. Groups of 25 pregnant SD rats were administered diethanolamine at doses of 0, 150, 500 and 1500 mg/kg bw/d via the dermal route on GD 6-15. Due to a dosing error, the mid dose group received only 380 instead of 500 mg/kg bw/d.

In parental animals, moderate to severe skin irritation was noted in the mid and top dose groups. There was reduced body weight gain and corrected body weight; in the top dose group this was up to ↓ 4.5%. There was also an increase in absolute and relative kidney weights in the mid and top dose, although no details regarding the magnitude of these effects were available. Regarding haematology, parental animals experienced ↓HCT, ↓ MCV and in male animals ↓ MCH content in all dose groups, ↓ RBC in the mid and top dose groups and ↓HGB and ↓platelet counts in the top dose group. No details regarding the magnitude of these effects were available.

Increases in skeletal variations, consisting of delays in ossification in the proximal hindlimb phalanges and forelimb metacarpals were noted in offspring at the top dose.

PNDT, OECD TG 414 (dermal, rabbits)

In another OECD TG 414 prenatal developmental toxicity study, 15 pregnant New Zealand White rabbits per group were administered diethanolamine at doses of 0, 35, 100 and 350 mg/kg bw/d dermally to clipped backs on GDs 6-18. Signs of systemic toxicity were noted in parental animals and are discussed in the STOT RE section. No reproductive or developmental toxicity effects were observed.

Developmental toxicity study (oral, rats)

The eighth study available was non-guideline and information regarding GLP was not available. Groups of 12 pregnant SD-derived rats were administered doses of 50, 125, 200, 250 and 300 mg/kg bw/d via oral gavage from GDs 6-19.

Two females were euthanised on GD 11. Remaining top dose animals were terminated on GD 12 and excluded from the study results. One female in the 200 mg/kg bw/d group was also euthanised on GD 22, attempting to deliver a litter of 15 pups, all of which were found dead in utero at necropsy.

In the 250 mg/kg bw/d group, one animal was found dead on GD 15 and one was euthanised on GD 21 with 12 dead pups *in utero*. Under the conditions of this study, the LD_{50} was calculated to be 218 mg/kg bw/d.

In parental animals, decreased water consumption was noted during GDs 9-12, this was resolved by GD 19. There was also reduced feed intake which was significant in the top

two dose groups, reduced body weight and body weight gain and increased absolute kidney weights in all groups except the low dose. Further details regarding this can be found in the STOT RE section.

Increased post implantation losses were noted in all groups: 2.5% in the control group, 5.8% at 50 mg/kg bw/d group, 3.4% at 125 mg/kg bw/d, 17.3% at 200 mg/kg bw/d and 51% in the 250 mg/kg bw/d. There was an increase in postnatal mortality on PND 0-4; 0% in the control group, 0.6% at 50 mg/kg bw/d group, 1.8% at 125 mg/kg bw/d, 2.8% at 200 mg/kg bw/d and 13.4% in the 250 mg/kg bw/d.

In the pups, birth weights were ↑3.9%, ↑3.8%, ↓7.4% and ↓13.9% compared to controls at 50, 125, 200 and 250 mg/kg bw/d, respectively. Post-natal pup body weight gain was also reduced at higher doses. On PND21, pup body weights were ↑4.3%, ↑5.2%, ↓9.6% and ↓10.4% compared to controls at 50, 125, 200 and 250 mg/kg bw/d; the differences were statistically significant in the top two dose groups.

Post-natal screening test (oral, mice)

The ninth study available was a non-guideline post-natal mouse screening test/Chernoff and Kavlock test which was GLP-compliant. Groups of 48-49 pregnant Swiss Albino mice were administered doses of 0 or 450 mg/kg bw/d of diethanolamine by oral gavage on GDs 6-15. Pups were maintained until PND 3.

No maternal toxicity was observed, but body weight was statistically significantly higher after treatment compared to the control on PND 0 (↑ 6.3%) and statistically significantly lower on PND 3 (↓6.1%).

Other effects included an increase in gestational length, from 18.2 days in controls to 18.5 days in the dosed group. Although the number of live litters born was reduced from 89% in controls to 79%, this was not statistically significant. Full litter loss occurred in 11% of pregnancies in the control group and 21% in dosed animals.

Neonatal survival was 95% in control animals compared with 77% in the dosed group.

In pups, there was decreased body weight gain in PNDs 0-3; body weight was ↓23.8% compared to control.

Neuro-developmental study (dermal, mice)

The tenth study available was a non-guideline neuro-developmental toxicity study which was not GLP-compliant. Groups of 6 pregnant female c57BL/6 mice per group were administered diethanolamine at doses of 0, 20, 80, 160, 320 and 640 mg/kg bw/d dermally on GDs 7-17.

One animal in the 640 mg/kg bw/d group died. There was reduced liver content of choline and its metabolites in the 80 mg/kg bw/d; this was the only group studied for this. No other information regarding parental toxicity was available.

There was a reduced number of viable foetuses per litter: 8.2 in the control group, 8.0 in the 20 mg/kg bw/d group, 6.3 in the 80 mg/kg bw/d group, 4.8 in the 160 mg/kg bw/d group, 3.3 in the 230 mg/kg bw/d group and 2.7 in the 640 mg/kg bw/d group. This was statistically significant from 160 mg/kg bw/d.

In the hippocampus, there was decreased mitosis (56±14% based on phospho-histone 3) and increased apoptosis (170±10% based on TUNEL and 178±7% based on activated caspase 3) in the 80 mg/kg bw/d group. No other dose groups were studied for these effects.

Mechanistic study (mice)

The eleventh study available was an *in vivo* mechanistic study; it was non-guideline and GLP compliant. Pregnant c57BL/6 mice were dermally administered 80 mg/kg bw/d of diethanolamine, and 2 pups from different dams were analysed for diethanolamine metabolites. Diethanolamine was found in 0.023 and 0.026mM in the foetal brain, whilst phospho-diethanolamine was found in concentrations of 1.6mM and 1.3mM.

Neuro-developmental study (mice)

The final study available was a non-guideline neurodevelopmental *in vivo/in vitro* mechanistic study, that was not GLP compliant. For the *in vivo* part of the study, 3 groups of 7 pregnant females were maintained as controls while 7 pregnant females per dose group were administered 5, 40, 60 and 80 mg/kg bw/d of diethanolamine by the dermal route. A group of 5 non-pregnant mice of the same strain were treated with either the vehicle or 80 mg/kg bw/d of diethanolamine for 11 days.

The following parameters were measured *in vivo:* mitosis was decreased; approximately 50% of the controls at the top dose. Apoptosis was statistically significantly increased in the hippocampus; approximately 175% of the controls. Choline and diethanolamine metabolites were measured in the liver and plasma of the non-pregnant females and increased concentrations of diethanolamine and its metabolites were found in both. Choline and choline metabolite levels were decreased in the liver. There was no further information regarding the magnitude of or significance of these effects.

For the *in vitro* part of the study, cortical neuronal precursor cells at E14 were exposed to 70 µM choline chloride and 3 mM diethanolamine, or 210 µM choline chloride + 3 mM diethanolamine.

The following parameters were measured *in vitro*:

BrdU incorporation showed decreased mitosis after treatment with diethanolamine (3 mM) after 48 h (3.8% vs 16% in controls) and 72 h (2.6% vs 15.4% in controls). Choline supplementation (3 mM DEA + 210 µM choline chloride) restored mitosis after 48 h (11.7% vs 16% in controls) and 72 h (14.0% vs 15.4% in controls). There was no difference in mitosis between control and choline supplemented group (210 µM choline chloride) after 48 h and 72 h.

Analysis via the TUNEL method showed that after 72 h, diethanolamine increased apoptosis (14.8% vs 4.8% in controls). Choline supplementation (CS-DEA: 3 mM DEA + 210 µM choline chloride) prevented this effect of diethanolamine (5.6% vs 4.8% in controls).

Choline uptake into neural precursor cells was measured and found to be decreased by diethanolamine treatment by 59% compared with controls. Choline supplementation mitigated this, restoring choline uptake to about 87% of controls.

Choline metabolites were measured. Diethanolamine decreased the concentration of choline and phosphocholine. Treatment with diethanolamine and choline combined showed prevention of the decrease of choline concentrations, but not the decrease of phosphocholine levels. Choline supplementation increased the intracellular concentrations of choline and phosphocholine.

Choline kinase levels were also measured, choline kinase has a lower affinity for diethanolamine, and diethanolamine inhibited the phosphorylation of choline. This was considered significant at levels of 40 mM.

Conclusion

From the 10 animal and 2 mechanistic studies available, RAC concluded that there was clear and consistent evidence of an adverse effect on development. Effects included post implantation losses, lower offspring survival and growth, developmental neurotoxicity and developmental immunotoxicity, and were observed across several reproductive toxicity studies, in more than one species and more than one route of exposure. RAC noted that, in some studies, maternal toxicity in the form of body weight reductions, organ effects (liver, haematopoeitic system, kidney, nervous system) and general toxicity was observed at dose levels where developmental effects were reported. However, they noted that body weight reductions were generally below 10%, and therefore not regarded as ‘marked’ toxicity. Additionally, RAC noted that some developmental effects were observed at doses below those at which general toxicity occurred. The available dataset did not demonstrate a causal relationship between maternal toxicity and developmental toxicity that would preclude classification.

RAC considered disruption of choline uptake and metabolism to be a plausible MOA for the observed developmental effects. They did not consider species differences in

tolerance for choline deficiency (discussed under ‘Carcinogenicity’ above) to be a sufficient argument to dismiss the developmental toxicity findings as relevant for humans.

Overall,RAC concluded that diethanolamine met the classification criteria for **Repr. 1B; H360D (May damage the unborn child).**

Effects on or via lactation

No data were available to indicate whether diethanolamine had an adverse effect on or via lactation. RAC noted that some of the developmental toxicity studies in rodents showed reduced offspring survival and growth early after birth; however, they also noted that ADME data indicate that the substance has a long elimination time and therefore it was plausible that these effects were developmental rather than related to lactation. However, they concluded that there were no data to confirm or dismiss this. Therefore, RAC concluded that classification for effects on or via lactation was not warranted.

Conclusion

RAC concluded that diethanolamine should be classified as **Repr. 1B; H360FD (May damage fertility. May damage the unborn child).** They concluded that classification for effects on or via lactation was not warranted.

Classification proposed by the Agency:

The Agency agrees with RAC’s conclusion on classification. Diethanolamine should be classified as **Repr. 1B; H360FD (May damage fertility. May damage the unborn child).** Classification for effects on or via lactation is not warranted.

Aspiration hazard

Not assessed

Environmental hazards

Hazardous to the aquatic environment

Not assessed

Other hazards

Hazardous to the ozone layer

Not assessed

Overall conclusion

The Agency has evaluated the RAC Opinion, its rationale and any additional scientific evidence that may have been made available to HSE against the criteria for classification and labelling in the GB CLP Regulation and technical guidance.

The Agency technical report **agrees** with the classification proposed by RAC for the following hazards:

Acute Tox. 4; H302 (Harmful if swallowed); ATE = 1100 mg/kg bw

STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure)

Carc. 2; H351 (Suspected of causing cancer)

Repr. 1B; H360FD (May damage fertility. May damage the unborn child)

Overall, the conclusion is to **agree** with the RAC opinion.

References

ECHA (2024a) Guidance on the Application of the CLP Criteria, Part 2: Physical Hazards. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 4.0, ref: ECHA-24-G-07-EN. Available at https://www.echa.europa.eu/

ECHA (2024b) Guidance on the Application of the CLP Criteria, Part 3: Health Hazards. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 5.0, ref: ECHA-24-G-06-EN. Available at https://www.echa.europa.eu/

ECHA (2024c) Guidance on the Application of the CLP Criteria, Part 4: Environmental Hazards; and Part 5: Additional Hazards. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 4.0, ref: ECHA-24-G-05-EN. Available at https://www.echa.europa.eu/

For all other references, please see the EU CLH report and the EU RAC opinion (available at: https://echa.europa.eu/registry-of-clh-intentions-until-outcome)

CLH (2024) CLH report (including Annexes): Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: 2,2'-iminodiethanol; diethanolamine; Date: 2024; Written by: Germany; Accessed date: 02/2026

ECHA (2025) Committee for Risk Assessment (RAC) Opinion (including Annexes) proposing harmonised classification and labelling at EU level of 2,2'-iminodiethanol; diethanolamine; Reference CLH-O-0000007532-76-01/F; Date: 07/03/2025, Accessed date: 02/2026

Documents published as part of the EU CLH process: Source: European Chemicals Agency, http://echa.europa.eu/

Glossary of terms used in Agency technical reports

Agency, the	HSE, acting in its capacity as the GB CLP Agency
AR	Applied radioactivity
ATE	Acute toxicity estimate
BCF	Bioconcentration factor
BOD	Biological Oxygen Demand
bw	Body weight
CAR	Competent Authority Report
CAS	Chemical Abstracts Service
CI	Confidence interval
CL	Confidence limits
CLH	Harmonised Classification and Labelling
CLP	Classification, labelling and packaging (of substances and mixtures)
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
CV	Coefficient of Variation
d	Day
DAR	Draft Assessment Report
DOC	Dissolved Organic Carbon
DS	Dossier Submitter
DT	Dissipation time OR degradation time (also DissT or DegT where apparent)
DT_{50}	Dissipation half-life OR degradation half-life (hours or days), see also above
dw	Dry weight
ECHA	European Chemicals Agency
ECx	x% effect concentration
EFSA	European Food Safety Authority
E_rCx	x% effect concentration based on growth rate
EU	European Union
HCT	Haematocrit
HGB	Haemoglobin
GLP	Good Laboratory Practice
GV	Guidance value
h	Hours
K_{OC}	Organic carbon-water partition coefficient
K_{OW}	Octanol-water partition coefficient
LC_x	x% lethal effect concentration

MCH	Mean corpuscular haemoglobin
MCL	Mandatory Classification and Labelling
MCV	Mean corpuscular volume
M-factor	Multiplying factor
MW	Molecular weight
NOEC	No-observed effect concentration
OECD	Organisation for Economic Co-operation and Development
QSAR	Quantitative structure-activity relationship
RAC	Risk Assessment Committee
RAR	Renewal Assessment Report
RCOM	Response to comments document
RBC	Red blood cell count
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals regulation
STOT-RE	Specific target organ toxicity – repeated exposure
STOT-SE	Specific target organ toxicity – single exposure
TG	Test Guideline
US EPA	United States Environmental Protection Agency
wt	Weight
wwt	Wet weight

Further information

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