MCL Report for: ammonium difluoro-[1,1,2,2-tetrafluoro-2-(pentafluoroethoxy)-ethoxy]-acetate (EEA-NH4)

Proposal for mandatory classification and labelling (MCL) based on Annex VI, Part 2 of the retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

CAS Number: 908020-52-0

EC Number: N/A

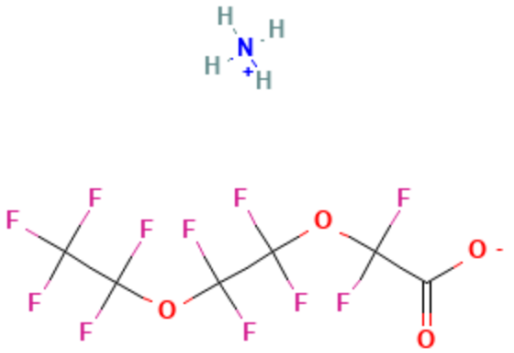
Date: February 2026

Contents

1. Identity of the substance

1.1 Name and other identifiers of the substance

Table 1. Substance identity and information related to molecular and structural formula of the substance (ECHA dissemination platform, ECHA 2024[1])

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,2-difluoro-2-(1,1,2,2-tetrafluoro-2-(1,1,2,2,2-pentafluoroethoxy)ethoxy)acetic acid amine
Other names (usual name, trade name, abbreviation)	ammonium difluoro[1,1,2,2-tetrafluoro-2-(pentafluoroalkoxy]acetate EEA-NH4
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	Not applicable
EC name (if available and appropriate)	Not applicable
CAS number (if available)	908020-52-0
Other identity code (if available)	Not applicable
Molecular formula	$C_6F_{11}O_4H_4N$
Structural formula (Taken from PubChem: https://pubchem.ncbi.nlm.nih.gov/compound/46221768)	
SMILES notation (if available)	C(=O)(C(OC(C(OC(C(F)(F)F)(F)F)(F)F)(F)F)(F)F)[O-].[NH4+]
Molecular weight or molecular weight range	363.08 g/mol

[1] Available at https://echa.europa.eu/home

Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 90% ≤ 100% w/w

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current MCL on GB MCL list (if applicable)
2,2-difluoro-2-[1,1,2,2-tetrafluoro-2-(1,1,2,2,2-pentafluoroethoxy)ethoxy]acetic acid amine; EC – N/A; CAS - 908020-52-0		None

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	The impurity contributes to the classification and labelling?
There are no impurities relevant for this classification		Not on MCL list	No

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	The additive contributes to the classification and labelling?
N/A	N/A	N/A	N/A	N/A

2 Proposed mandatory classification and labelling

Table 5: Proposed mandatory classification and labelling according to the GB CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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)		
Current GB MCL list entry	No current GB MCL list entry										
Proposed entry on the MCL list	TBD	2,2-difluoro-2-[1,1,2,2-tetrafluoro-2-(1,1,2,2,2-pentafluoroethoxy)ethoxy]acetic acid amine	-	908020-52-0	Acute Tox. 4 Eye Dam. 1 STOT RE 2 Carc. 2 Repr. 1B	H302 H318 H373 (liver, kidney) H351 H360D	GHS07 GHS05 GHS08 Dgr	H302 H318 H373 (liver, kidney) H351 H360D		ATE oral = 500 mg/kg bw	

TBD: to be determined

Table 6: Reason for not proposing mandatory classification and status under public consultation

Hazard class	Classification / reason for no classification	Within the scope of public consultation
Explosives	Not assessed	No
Flammable gases (including chemically unstable gases)	Not assessed	No
Oxidising gases	Not assessed	No
Gases under pressure	Not assessed	No
Flammable liquids	Not assessed	No
Flammable solids	Not assessed	No
Self-reactive substances	Not assessed	No
Pyrophoric liquids	Not assessed	No
Pyrophoric solids	Not assessed	No
Self-heating substances	Not assessed	No
Substances which in contact with water emit flammable gases	Not assessed	No
Oxidising liquids	Not assessed	No
Oxidising solids	Not assessed	No
Organic peroxides	Not assessed	No
Corrosive to metals	Not assessed	No
Acute toxicity via oral route	***Acute Tox. 4, H312 (ATE = 500 mg/kg bw/d)***	**Yes**
Acute toxicity via dermal route	*Data conclusive but not sufficient for classification*	**Yes**
Acute toxicity via inhalation route	Not assessed	No
Skin corrosion/irritation	*Data conclusive but not sufficient for classification*	**Yes**
Serious eye damage/eye irritation	***Eye Dam. 1, H318***	**Yes**
Respiratory sensitisation	Not assessed	No
Skin sensitisation	*Data conclusive but not sufficient for classification*	**Yes**
Germ cell mutagenicity	*Data conclusive but not sufficient for classification*	**Yes**
Carcinogenicity	***Carc. 2, H351***	**Yes**
Reproductive toxicity	***Repr. 1B, H360D***	**Yes**
Specific target organ toxicity-single exposure	Not assessed	No
Specific target organ toxicity-repeated exposure	***STOT RE 2; H373 (liver, kidney)***	**Yes**
Aspiration hazard	Not assessed	No
Hazardous to the aquatic environment	Not assessed	No
Hazardous to the ozone layer	Not assessed	No

3 History of the classification and labelling

EEA-NH4 has not previously been considered for mandatory classification and labelling (MCL) under GB CLP and does not have an existing entry on the GB MCL list. In the EU, one notifier has notified the following self-classification for this substance to ECHA's classification and labelling inventory[2]: Acute Tox. 4; H302, Eye Dam. 1; H318 and Repr. 2; H361.

No notifications have been submitted to the Agency for this substance under Article 40 of GB CLP.

[2] Inventory checked January 2026. Available at https://echa.europa.eu/home

4 Justification that action is needed

In April 2023, HSE, acting as the Agency for UK REACH and supported by the Environment Agency, published a Regulatory Management Options Analysis (RMOA) of per- and polyfluoroalkyl substances (PFAS)[3]. This concluded that PFAS have several properties that, together, pose a concern to the environment and human health.

A potential concern for developmental toxicity was identified for EEA-NH4, owing to its self-classification for reproductive toxicity. The RMOA recommended that consideration be given to the mandatory classification and labelling of EEA-NH4 to address any uncertainties and support appropriate risk management of the substance. Therefore, the Agency has prepared this targeted report to propose the mandatory classification and labelling of EEA-NH4.

[3] UK REACH Regulatory Management Options Analysis (RMOA) of per- and polyfluoroalkyl substances (PFAS); available at: https://www.hse.gov.uk/reach/assets/docs/pfas-rmoa.pdf

5 Identified uses

The substance is used at industrial sites in the manufacture of plastic products. The Chemical Safety Report (CSR) in the REACH registration dossier identifies the uses of EEA-NH4 in chemical production and refinery, transfer of substance or mixture (charging/discharging) at non-dedicated facilities, transfer of substance or mixture (charging/discharging) from/to vessels/large containers at dedicated facilities and use as a laboratory agent (ECHA 2024).

6 Data sources

This report was compiled from data in UK REACH registration dossiers, ECHA's dissemination platform (ECHA, 2024) and full study reports submitted to the Agency by AGC Chemicals Europe, Ltd.

7 Physicochemical properties

Table 7: Summary of physicochemical properties (Source: ECHA 2024)

Property	Value	Reference	Comment (e.g., measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	Anonymous, 2008	
Melting/freezing point	95.8 °C	Chilworth Anonymous, 2008	Data from a GLP-compliant guideline, fully adequate for assessment.
Boiling point	181.0 °C	Anonymous, 2008	Data from a GLP-compliant guideline, fully adequate for assessment.
Relative density	1355 kg/m^3 @ 20 °C	Anonymous, 2008	Data from a GLP-compliant guideline, fully adequate for assessment.
Vapour pressure	0.002 Pa @ 25 °C	Anonymous, 2008	Data from a GLP-compliant guideline, fully adequate for assessment.
Surface tension	59.1 mN m^{-1}	Anonymous, 2008	Data from a GLP-compliant guideline, fully adequate for assessment.
Water solubility	516 mg/L @ 25 °C	Anonymous, 2008	Data from a GLP-compliant guideline, fully adequate for assessment.
Partition coefficient n-octanol/water	1.18	Anonymous, 2008	Data from a GLP-compliant guideline, fully adequate for assessment.
Flash point	Study not technically feasible		Not applicable based on physical state (solid)
Flammability	Not flammable	Anonymous, 2008	Data from a GLP-compliant guideline study, fully adequate for assessment.
Explosive properties	Not explosive	Anonymous, 2008	Data from a GLP-compliant guideline study, fully adequate for assessment.
Self-ignition temperature	No exothermic activity observed up to 400 °C	Anonymous, 2008	Data from a GLP-compliant guideline study, fully adequate for assessment.

Property	Value	Reference	Comment (e.g., measured or estimated)
Oxidising properties	Non-oxidising	Anonymous, 2008	Data from a GLP-compliant guideline study, fully adequate for assessment.
Granulometry	10% of the material < 236.831 µm 50% of the material < 716.488 µm 90% of the material < 1416.196 µm 0.47% by volume of the sample was seen to be < 10.00 µm	Anonymous, 2008	Data from a GLP-compliant guideline study, fully adequate for assessment.
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	No data		
Viscosity	No data		

8 Evaluation of physical hazards

Not assessed.

9 Toxicokinetics (absorption, metabolism, distribution and elimination)

Table 8: Summary of toxicokinetic studies with EEA

Method	Results	Remarks	Reference
Basic toxicokinetics *in vivo* No guideline, read-across GLP compliant EEA (difluoro-(1,1,2,2-tetrafluoro-2-(pentafluoro-ethoxy)) ethoxy acetic acid; a structural analogue of EEA-NH4 Sprague-Dawley rats Distribution group: 9 animals/sex Excretion group: 3 animals/sex 10 mg/kg bw Intravenous injection Vehicle: water	Distribution in tissues: following a single injection, systemic exposure (AUC0-∞) in male rats was ~7-fold higher than in female rats. EEA remained mostly in circulation in male rats, apparent volume of distribution was around 0.2 L/kg. Extensive tissue distribution in female rats, apparent volume of distribution >2.5 L/kg Details on excretion: terminal elimination phase for EEA in serum had a half-life of 9.4 hr and 5.4 hr for female and male rats respectively. The half-life for EEA in urine was 1.8 hr and 3.2 hr for female and male rats respectively. Overall the % dose eliminated in the urine was similar in both sexes after 24 hours owing to disparity in amount of EEA available for urinary clearance in circulation.		Anonymous, 2007a
Toxicokinetics No guideline, read-across EEA (difluoro-(1,1,2,2-tetrafluoro-2-(pentafluoro-ethoxy)) ethoxy acetic acid; a structural analogue of EEA-NH4 Cynomolgus monkeys (Macaque)	Pharmacokinetic parameters for EEA in serum were similar between genders. Males appeared to have higher exposure and longer half-lives than females. On average, around 60-65% of administered EEA was recovered in the urine during the 7-days post dosing in males and females.	Mean values were skewed in this study owing to 1 male that had an AUC-∞ approximately 7-fold higher than the other males and a half-life in serum of 56 hours. Assessed by a veterinarian on study days 2 & 3 the animal presented with tremors	Anonymous, 2007b

Method	Results	Remarks	Reference
3 animals/sex 10 mg/kg bw Intravenous injection Vehicle: water		(intermittent & continuous), intermittent shivering, depressed attitude, slightly distended abdomen and a weak appearance	

9.1 Short summary and overall relevance of the provided toxicokinetic information

The toxicokinetic profile of EEA-NH4 itself has not been investigated in humans or animals. In the REACH registration dossier, the registrants have used data read-across from a structural analogue EEA to describe the expected toxicokinetics of EEA-NH4. The two available *in vivo* studies for EEA (one in rats, one in monkeys) are summarised in Table 8. As an ammonium salt, EEA-NH4 is expected to disassociate readily in aqueous, physiological conditions to the ions EEA- and NH4+, therefore the Agency agrees that the read-across of toxicokinetic data from EEA is acceptable.

The two available EEA studies followed the same dosing and sampling protocol with all animals observed twice daily for mortality and moribundity. Detailed physical examinations were performed throughout. Food consumption was recorded during the pretest period only. For pharmacokinetics assessment, blood samples were collected on wet ice prior to dosing and at ~2, 10, 20 and 30 minutes and 1, 3, 5, 7, 24 and 48 hours after dose administration. For an excretion profile, urine samples were collected over 0-6, 6-12 and 12-24 hours after dose administration. Serum and urine concentrations were measured by LCMS/MS.

Absorption

N/A

Distribution

In the rat study, the volume of distribution (Vd) of EEA was higher in females (2.5 L/kg) than males (0.2 L/kg), indicating that EEA is distributed into tissues to a far greater extent in females (AGC, 2007). No tissue samples were collected in this study, so no specifics on this could be ascertained. EEA appears to remain in circulation in the blood plasma in males.

In the monkey study, the toxicokinetic parameters for EEA in serum were generally similar between the sexes; male monkeys appeared to have higher exposure and longer half-lives than female monkeys. Additionally, apparent volumes of distribution were similar among the individual animals.

Metabolism

N/A

Elimination

In rats, the terminal elimination phase for EEA in serum had a half-life of 9.4 and 5.4 hours for females and males, respectively. The half-life for EEA in urine was 1.8 and 3.2 hours, for female and male rats, respectively. However, the percentage of EEA dose eliminated over 24 hours post-dosing was similar in both sexes (~65%). This is correlated with the amount of EEA available for urinary clearance in the circulation of female rats compared to male rats as suggested by the differences in apparent volume of distribution and overall absorption to tissues.

In monkeys, the average amount of EEA recovered in the urine was 60-65% during the 7-days post-dosing with no toxicokinetic differences based on gender. Again, the mean values were greatly influenced by the same male with high exposure and a long half-life for serum, with this animal having around 50% of the dose eliminated after 29 hours.

10 Evaluation of health hazards

10.1 Acute toxicity – oral route

Table 9: Summary of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity study Oral (gavage) OECD TG 423 GLP	Rat (Sprague-Dawley CD) 3 females/ dose Single exposure	EEA-NH4	300 & 2000 mg/kg bw	> 300 - ≤ 2000 mg/kg bw	SafePharm Laboratories, 2005

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an OECD TG 423, GLP-compliant acute oral toxicity study (SafePharm Laboratories, 2005), groups of 3 female Sprague-Dawley rats were exposed via oral gavage to EEA-NH4 at doses of 300 & 2000 mg/kg bw. At the top dose, all animals were found dead or killed in extremis. Clinical signs were reported for two of the treated animals including hunched posture, ataxia, lethargy, decreased respiratory rate, noisy respiration, dehydration and diuresis. Abnormalities noted at necropsy of the animals found dead included abnormally red lungs, dark liver, dark kidneys and a clear liquid present in the stomach. These were not seen in the animal killed at the end of the study in extremis. At 300 mg/kg bw, all animals survived and showed no clinical signs. Their body weight gains were within expected levels over the study period. No abnormalities were noted at necropsy.

Based on these results the LD_{50} was reported to be $300 < LD_{50} \leq 2000$ mg/kg bw.

10.1.2 Comparison with the GB CLP criteria

According to a guideline acute oral toxicity study in rats, the LD_{50} was between 300 and 2000 mg/kg bw. This supports classification in Category 4 according to the GB CLP criteria. For Category 4, Table 3.1.2 of Annex I to GB CLP gives a converted acute toxicity point estimate of 500 mg/kg bw.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available results, **Acute Tox. Cat. 4, H302 (Harmful if swallowed)** with an **$ATE_{(oral)}$ of 500 mg/kg bw** is proposed.

10.2 Acute toxicity – dermal route

Table 10: Summary of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity study Semi-occlusive OECD TG 402 GLP	Rats (Sprague-Dawley) 5/sex/dose	EEA-NH4 Vehicle: Water	0, 500, 1000 & 2000 mg/kg bw 24 hr	>2000 mg/kg bw	Gotemba Laboratory, 2008
Acute dermal toxicity study Semi-occlusive OECD TG 402 GLP	Rats (Wistar) 5/sex/dose	EEA-NH4	400 mg/kg bw 24 hr	>400 mg/kg bw	TNO Quality of Life, 2008a

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In an OECD TG 402, GLP-compliant acute dermal toxicity study (Gotemba Laboratory, 2008), Sprague-Dawley rats (5/sex/dose) were exposed to the test substance at doses of 500, 1000 & 2000 mg/kg bw or a vehicle control. No deaths occurred throughout the study and upon examination no animals showed clinical signs or abnormalities at any dose level. No signs of corrosivity were observed in the study. The LD_{50} was reported to be >2000 mg/kg bw.

In a second OECD TG 402, GLP-compliant acute dermal toxicity study (TNO Quality of Life, 2008), Wistar rats (5/sex/dose) were exposed to the test substance at a dose of 400 mg/kg bw for 24 hours. A dose level of 2000 mg/kg bw was not used as a prior *in vitro* study had suggested the test material would be corrosive at this dose. There were no deaths, no animals showed clinical signs and macroscopic examination upon termination of the study did not reveal any treatment-related gross changes.

Based on the available results the LD_{50} for dermal toxicity is >2000 mg/kg bw.

10.2.2 Comparison with the GB CLP criteria

Classification for acute dermal toxicity is warranted when the LD_{50} is ≤ 2000 mg/kg bw. The available data for EEA-NH4 indicate that the LD_{50} is > 2000 mg/kg bw, therefore the classification criteria are not met.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification – data conclusive but not sufficient for classification.

10.3 Acute toxicity – inhalation route

Not assessed.

10.4 Specific target organ toxicity – single exposure (STOT SE)

Not assessed.

10.5 Skin corrosion/irritation

Table 11: Summary of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
Acute dermal irritation / corrosion Semi-occlusive OECD TG 404 GLP	Japanese White rabbits 3 females	EEA-NH4 (purity unknown) Vehicle: water	0.5g of the test substance was applied to a lint sheet and clipped to the rabbits for 3 minutes, 1 or 4 hr exposure. Animals observed according to Draize method (1, 24, 48 & 72 hrs)	Primary dermal irritation index: mean score of 0.2 and a max score of 1 Erythema score: mean score of 0.4 and a max score of 4 No oedema observed. Slight erythema was seen after 4 hr, all effects were fully reversible within 48 hours.	Kannami Laboratory, 2008a

Table 12: Summary of other studies relevant for skin corrosion/irritation

<table>
<tr><th>Type of study/ data</th><th>Test substance</th><th>Observations</th><th>Reference</th></tr>
<tr><td>In vitro Skin Corrosion using EpiDerm™

OECD TG 431

Non-GLP

Human keratinocytes

3 & 60 minute exposure</td><td>EEA-NH4 (purity unknown)</td><td>
<table>
<tr><th>3-minute exposure</th><th></th><th></th></tr>
<tr><th>Test Group</th><th>Substance</th><th>A540(% of control)</th></tr>
<tr><td>Negative Control</td><td>Demineralised water</td><td>100.0</td></tr>
<tr><td>Test Substance</td><td>EEA-NH4</td><td>113.4</td></tr>
<tr><td>Positive Control</td><td>8M KOH</td><td>13.8</td></tr>
</table>
<table>
<tr><th>60-minute exposure</th><th></th><th></th></tr>
<tr><th>Test Group</th><th>Substance</th><th>A540(% of control)</th></tr>
<tr><td>Negative Control</td><td>Demineralised water</td><td>100.0</td></tr>
<tr><td>Test Substance</td><td>EEA-NH4</td><td>9.4</td></tr>
<tr><td>Positive Control</td><td>8M KOH</td><td>9.3</td></tr>
</table>
</td><td>TNO Quality of Life, 2008b</td></tr>
</table>

10.5.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In an *in vivo* OECD TG 404, GLP-compliant acute dermal irritation study (Kannami Laboratories, 2008), three female Japanese White rabbits were exposed to 0.5g of EEA-NH4 via a semi-occlusive dressing for 3 minutes, 1 hour or 4 hours. Animals were observed at 1, 24, 48 or 72 hours and scored according to the Draize method. After 4 hours of exposure, two animals exhibited erythema (score of 1) at the 24-hour observation point, but this was no longer observed after 48 hours. No oedema was observed at any time point.

An *in vitro* OECD TG 431, non-GLP skin corrosion assay with the reconstructed 3D human epidermis (RHE) model (EpiDerm™) is also available. In this study, RHE samples were exposed for 3 and 60 minutes to the test substance and the optical density was measured and compared with positive and negative controls. A positive result was observed after 60

minutes, with viability of the test sample being less than 15% (9.4%) when compared with the negative control.

10.5.2 Comparison with the GB CLP criteria

The *in vitro* skin corrosion test with reconstructed human epidermis (RHE) was conducted according to OECD TG 431. In accordance with the CLP criteria section 3.2.2.1.2.4., 'positive *in vitro* tests that give a positive result for corrosivity can be used for classification and do not generally require further investigation'. In the available study the viability of the exposed RHE after 3 minutes was greater than 50%, although after 60 minutes the viability was less than 15% (9.4%). According to the test guideline this is a clear positive result for corrosivity.

In an *in vivo* study that followed OECD TG 404, the exposed rabbits showed no erythema and only slight oedema that was fully reversible within 48 hours. This is a negative result as per the OECD test guideline.

The previously discussed dermal studies, detailed in Table 10 in the acute dermal toxicity section, support the findings of the OECD TG 404 *in vivo* study as the animals showed no clinical signs after 24 hours of dermal exposure up to 2000 mg/kg bw of the test substance. EEA-NH4 was applied neat in the OECD TG 404 study, with only slight erythema and no oedema observed during the 72-hour observation period. These corroborative findings build a weight of evidence in support of no classification; despite the positive result in the single *in vitro* study, there is clear evidence that this result does not translate into the animal model.

10.5.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the results of the available *in vivo* dermal corrosion/irritation study and acute dermal toxicity studies, no classification for skin corrosion/irritation is proposed for EEA-NH4.

No classification – data conclusive but not sufficient for classification.

10.6 Serious eye damage/eye irritation

Table 13: Summary of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores** **- Reversibility**	**Reference**
Acute eye irritation/corrosion study OECD TG 405	Rabbit (Japanese White) 3 females	EEA-NH4	0.1g 24 hr exposure	Individual mean scores are not available. Mean scores (across all rabbits/time points): - Conjunctivae: 2	Kannami Laboratory, 2008b

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset - Mean scores - Reversibility	Reference
GLP				- Iris: 1 - Corneal opacity: 1 - Chemosis: 1.3 The irritation seen in both the cornea and conjunctiva was not reversible within the 21-day observation period. Eyelid closure was observed in all animals immediately after application and in 1/3 animals from 1 hour to 4 days.	

Table 14: Summary of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Observations	Reference
In vitro eye irritation study No guideline, similar to OECD TG 438 Chicken Enucleated Eye Test (CEET)	EEA-NH4	Mean irritation scores: - Corneal swelling: 43.0 (Irritation cat IV) - Opacity: 3.0 (Irritation cat IV) - Fluorescein retention: 3.0 (Irritation cat IV) Irritation index = 163/200. Severely irritating or Category 1 “risk of serious eye damage.	TNO Quality of Life (2008c)

10.6.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

In an *in vivo* OECD TG 405 and GLP-compliant eye irritation study (Kannami Laboratories, 2008b), three female Japanese White rabbits were exposed to 0.1g of the test substance, applied to the left eye for 24 hours, followed by a 21-day observation period. Corneal vascularisation was observed in 1/3 animals on day 6 and in all animals from day 7 to 21 after application. There were no treatment-related clinical signs. The effects on the cornea (overall mean score 1; max. score 4) and conjunctiva (overall mean cornea score 2; max. score 3) were not fully reversed within 21 days. Chemosis (overall mean score 1.3) and iritis (overall mean score 1) had both fully reversed within 16 days.

A supporting *in vitro* chicken enucleated eye test (CEET) (Unnamed, 2008) is also available. This study followed a protocol similar to OECD TG 438 (Isolated Chicken Eye Test) and was non-GLP compliant. An overall irritation index was calculated of 163 out of 200, taking into account the maximum mean scores for corneal swelling, opacity and fluorescein retention. Within the parameters of this test this result was considered severely irritating.

10.6.2 Comparison with the GB CLP criteria

In an OECD TG 405 and GLP-compliant study the test substance caused severe and irreversible damage to the cornea and conjunctiva. This supports classification in category 1 for serious eye damage as per the CLP criteria (Table 3.3.1) which state '(Category 1) *A substance that produces: (a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days*.' This is further supported by a non-GLP compliant study similar to OECD TG 438, in which degradation of the cornea and severe impact on fluorescein retention were recorded. Based on the available studies the test substance meets the criteria for Eye damage, Cat. 1 – H318 (Causes serious eye damage).

10.6.3 Conclusion on classification and labelling for serious eye damage/eye irritation

The Agency concludes that, based on the available data, classification of EEA-NH4 as **Eye Dam. 1, H318 (Causes serious eye damage)** is warranted.

10.7 Respiratory sensitisation

Not assessed.

10.8 Skin sensitisation

Table 15: Summary of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Local lymph node assay (LLNA) OECD TG 429 GLP	5 female CBA mice	EEA-NH4	10%, 25% & 50% (w/v) 25µl administered daily for 3 days to the dorsum of	Stimulation index values of 1.1, 2.2 & 1.9 were calculated for 10%, 25% & 50% respectively No signs of irritation observed at any concentration	TNO Quality of Life (2008d)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
compliant			both ears 3 days after last exposure all animals received intravenous injection of ^{3}H-thymidine and lymph nodes were drained after 5 hours		

10.8.1 Short summary and overall relevance of the provided information on skin sensitisation

In the only available local lymph node assay (LLNA), completed according to OECD TG 429 and GLP-compliant, groups of 5 female CBA mice were exposed to 10, 25 & 50% (w/v) of the test substance via 25µl applications to the dorsum of the ears on 3 consecutive days. Subsequent intravenous injections of ^{3}H-thymidine were administered 3 days after the final exposure and lymph nodes were then drained after 5 hours. No signs of irritation were observed at any concentration and stimulation indices (SI) of 1.1, 2.2 and 1.9 were recorded at 10%, 25% and 50%, respectively. Concurrent negative and positive controls were conducted and performed as expected.

10.8.2 Comparison with the GB CLP criteria

In a LLNA, a SI ≥3 is considered a positive result. In the available LLNA for EEA-NH4, the SI values were less than 3 at all concentrations tested. Therefore EEA-NH4 did not demonstrate a skin sensitising potential under the conditions of the study.

10.8.3 Conclusion on classification and labelling for skin sensitisation

In a well-conducted LLNA, EEA-NH4 did not exhibit any skin sensitising potential and therefore classification is not warranted.

No classification – data conclusive but not sufficient for classification.

10.9 Specific target organ toxicity – repeated exposure (STOT RE)

The following studies in rats are available for the assessment of STOT RE: a 28-day repeated dose toxicity study; a 90-day repeated dose toxicity study; a combined chronic toxicity and carcinogenicity study. Additional information is available from two reproductive toxicity screening studies, also in rats.

Table 16: Summary of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group **Reference**	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** *** Significantly different from vehicle control at P<0.05** **** Significantly different from vehicle control at P<0.01**
28-day repeated dose toxicity study OECD TG 407 GLP-compliant Crl:CD (SD) rats 5/sex/dose Hita Laboratory, 2006	EEA-NH4 99.5% wt% Oral gavage 0, 5, 25 & 100 mg/kg bw/d (test groups) 0 and 100 mg/kg bw/d (recovery groups) 28 days exposure for all animals, followed by a 14-day recovery period for those in the recovery group Vehicle: distilled water	**Mortality and clinical signs:** no deaths or treatment-related clinical signs reported **Food consumption:** no treatment-related effects reported during the dosing period. During the recovery period, food consumption increased on Day 4 in females only. **Body weights:** no treatment-related effects **Haematology:** effects seen at 100 mg/kg bw/d. *At termination of dosing* ↓ RBC (-7.5% in males -7.2% in females), ↓Hb (-5.8% in males and -3.9% in females), ↓ Ht (-5.2% in males and -4.5% in females), ↑ reticulocyte count (+61.5%*, females only) and ↑ prolonged prothrombin time (+40.8%*, males only). *At termination of recovery period*: ↓ Hb (-3.8%*) and ↓ Ht (-4.1%*) in males. **Clinical chemistry:** effects seen at 100 mg/kg bw/d. *At termination of dosing* Males: ↑ ALT (+47.8%**) & A/G ratio (+14.7%*) and ↓ total cholesterol (-24.4%*). Females: ↓ total bilirubin (-28.6%**). *At termination of recovery* Males*:* ↑ ALT (+33.3%*) **Urinalysis & Neurobehavior:** No treatment-related findings. **Organ weights:** *Kidney* Increased kidney weights in males from 25 mg/kg bw/d. Weights at termination of dosing:- **25 mg/kg bw/d:** absolute +16.3%*, relative +18.7%* **100 mg/kg bw/d:** absolute: +19.3%**, relative: +21.3%** No differences in kidney weight compared to controls reported at the end of the recovery period.

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Liver Increased liver weights in males at 100 mg/kg bw/d. Weights at the end of dosing:- absolute: +42%** and relative: +43.3%**. No differences in liver weight compared to controls at the end of the recovery period. *Adrenal* No changes in adrenal weight at the end of the dosing period. However, at termination of recovery (100 mg/kg bw/d), relative adrenal weights were +18.1%* in males. *Heart* No changes in heart weight at termination of dosing period. However, at termination of recovery (100 mg/kg bw/d), relative heart weight ↓ in females (-10.3%**). **Necropsy:** *Termination of dosing* **100 mg/kg bw/d:** elevation of limiting ridge in the forestomach (4/5 males; 1/5 females); enlargement of the liver (2/5 males). *Termination of recovery (100 mg/kg bw/d)* No findings reported. **Histopathology: non-neoplastic findings** *Termination of dosing* **0 mg/kg bw/d:** mineralisation in corticomedullary junction of the kidney (slight, 1/5 females; moderate, 1/5 females), solitary cyst in the medulla of the kidney (slight, 1/5 females). Round cell infiltration of the prostate (slight, 1/5 males) and capsulitis (slight, 1/5 males). **5 mg/kg bw/d:** basophilic tubules (slight, 1/5 females) and mineralisation in corticomedullary junction of the kidney (slight, 2/5 females; moderate 1/5 females). **25 mg/kg bw/d:** basophilic tubules (slight, 1/5 females) and mineralisation in corticomedullary junction of the kidney (slight, 1/5 females; moderate, 2/5 females). **100 mg/kg bw/d:** hyperplasia of squamous epithelium in limiting ridge of the forestomach (slight, 4/5 males, 2/5 females); diffuse hypertrophy of hepatocytes with granular degeneration in the liver (slight, 5/5 males); focal necrosis of hepatocytes (slight, 1/5 females); cyst in medulla of the kidney (slight, 1/5 males); basophilic tubules (slight, 2/5 females) and

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		mineralisation in corticomedullary junction (slight, 1/5 females) of the kidney; hyperplasia of collecting tubular epithelium and transitional epithelium with interstitial cell infiltration in the unilateral kidney (slight, 1/5 females); round cell infiltration of the prostate (slight, 1/5 males) and capsulitis (slight, 1/5 males). *Termination of recovery (100 mg/kg bw/d)* Mineralisation in corticomedullary junction (slight, 1/5 females). No liver or forestomach findings reported in males or females.
90-day repeated dose toxicity study OECD TG 408 GLP-compliant Crl:CD (SD) rats 10/sex/dose (test group), 5/sex/dose (recovery group) Safety Research Institute for Chemical Compounds Co., Ltd., 2019	EEA-NH4 30 wt% (70% water) Oral gavage 0, 10, 50 & 250mg/kg bw/d (test groups) 0 and 250 mg/kg bw/d (recovery groups) 90-days exposure and 14-day recovery observation period	**Mortality and clinical signs:** **10 mg/kg bw/d**: 1 male found dead **50 mg/kg bw/d:** no deaths or clinical signs **250 mg/kg bw/d:** 3 males showed pale skin of the auricle and extremities (between administration days 88 and 91). Three females found dead between day 42 and day 80. **Body weight and food consumption:** *Dosing period* **250 mg/kg bw/d:** food consumption similar to controls ↓ body weight gain (males: - 15%**; females: - 17%*) ↓ body weight (males: -11%**; females: - 9%) *Recovery period (250 mg/kg bw/d)* food consumption similar to controls ↑ body weight gain (males: +92%; females: +20%) ↓ body weight (males: -11%; females: - 16%) **Ophthalmology:** No treatment-related findings **Urinalysis:** *Treatment period* **250 mg/kg bw/d** Males and females: ↓ pH, ↑ urine volume, ↓ specific gravity (all statistically significant) **Haematology:** *Treatment period* **50 mg/kg bw/d** Males: ↓RBC (-6.08%*) ↓MCHC (-2.36%**) **250 mg/kg bw/d**

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Males: ↓RBC (-37.59%**), ↓MCHC (-12.71%**), ↓ Hb (-38.22%**), ↓Ht (-30.25%**), ↑reticulocyte (+454.64%**), ↑MCV (+13.52%**), ↑WBC (+38.18%*) and ↑neutrophil (+112.16%**) Females: ↓RBC (-10.86%**), ↓Hb (-18.89%**), ↓Ht (-9.07%**), and ↓MCHC (-3.65%**), ↑reticulocyte (+61.50%**). *Recovery period* **250 mg/kg bw/d** Males: ↓RBC (-9.45%**), ↓Hb (-9.23%**), ↓MCHC (-3.89%**), ↑reticulocyte (+26.37%*), ↑WBC (+40.0%*) and ↑ lymphocytes (+46.0%*). **Clinical chemistry:** *Treatment period* **10 mg/kg bw/d** Males: ↑AST (+52.7%**), ↑ ALT (+47.0%**) and ↓ α2-G fraction (-11.5%*) **50 mg/kg bw/d** Males: ↑ AST (+62%*), ↑ALT (+77.9%**), ↑ALP (+54.82%**), ↑albumin (+12.71%**), ↓ β-G fraction (-14.38%**), ↓Crea (-14.0%*) Females: ↑TSH (+32.16%*), ↓T-Bil (-34.18%**) **250 mg/kg bw/d** Males: ↑AST (+157.0%**), ↑ ALT (+326%**), ↑ALP (+183.06%**), ↑albumin (+26.85%**), ↓ β-G fraction (-20.89%**), ↓crea (-12.63%*), ↑LDL-C (+80.95%**), ↑UN (+39.66%**), ↑K (+31.13%**), ↑IP (+9.93%**). ↓T-Bil (-31.48%**), ↓Na (-0.83%*), ↓α1-G fraction (-40.54%**), ↓T3 (-19.96%*) and ↓T4 (-50.0%**) Females: ↓AST (-39.73%*), ↓ γ-GTP (-35%*), ↓T-Bil (-61.54%**), ↑TG (+64.24%*), ↑UN (+51.78%**), ↑K (+12.31%**), ↑IP (+16.45*), ↓T3 (-36.58%), ↓T4 (-10.01%). ***Termination of recovery (250 mg/kg bw/d):*** **250 mg/kg bw/d** Males: ↑ALP (+78.79%**), ↓T-Bil (-48.39%**), ↓glucose (-17%), ↑ T-Cho (+37.9%), ↑LDL-C (+84.09%**), ↑ HDL-C (+38.3%*), ↑UN (+12.59%*), ↑ Ca (+6.6%), ↑IP (+9.62%*), ↑albumin (+22.13%**), ↑albumin fraction (+16.23%**), and ↑A/G ratio (+35.36**) and ↓α1-G fraction (-26.86%*). Females: ↑T-Cho (+33.33%*) and ↑LDL-C (+63.46%**).

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Organ weights: *Treatment period* **10 mg/kg bw/d** Males: ↑absolute kidney weight (+15.27%*), ↑absolute prostate + seminal vesicles weight (+14.88%*) **50 mg/kg bw/d** Males: ↑absolute (+21.07%**) and relative (+14.89%**) kidney weight; ↑absolute (+38.06%**) and relative (+30.43%**) liver weight. Females: ↑absolute (+15.53%*) and relative (+15.37%*) kidney weight. **250 mg/kg bw/d** Males: ↑absolute (+15.77%**) and relative (+30.28%**) kidney weight; ↑absolute (+94.15%**) and relative (+115.48%**) liver weight; ↑ relative weights of the spleen (+23.67%*), ↑heart (+21.95%**), ↑testis (+16.53%*) and ↑brain (+12.11%*). Females: ↑absolute (+23.5%**) and ↑relative (+33.58%**) kidney weight; ↑ absolute (+50.16%**) and relative (+61.27%**) liver weight; ↑relative thyroid weight (+30.05%*) ***Termination of recovery (250 mg/kg bw/d):*** Males: ↑absolute (+42.2%*) and relative (+61.03%**) liver weight; ↑ relative kidney weight (+19.39%**); ↑relative heart weight (+15.56%**); ↑ relative weight of pituitary gland (+28.22%**) and ↑ relative testis weight (+21.79%*), Females: ↑ relative liver weight (+17.5%**); ↑ relative kidney weight (+24.2%*); ↑ relative ovary weight (29.1%**) **Necropsy:** *Treatment period* **50 mg/kg bw/d** 1 female: multifocal white focus in the kidney (unilateral) **250 mg/kg bw/d** Large liver size (7 males) In females that died at this dose: 1 female red coloured area in the posterior lobe of the right lung, pale discoloration of the spleen, dilatation of the renal pelvis (unilateral), and calculus in the ureter (unilateral), whereas 2 females had yellowish white discoloration of the papilla of the kidney (bilateral) and one also had a small thymus. *Recovery period (250 mg/kg bw/d)* Males: large liver (2 animals)

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Females: yellowish brown discoloration (unilateral) and rough surface (unilateral) of the kidney (1 animal) **Histopathology:** *End of treatment period* **50 mg/kg bw/d** *Liver* Centrilobular hypertrophy of hepatocytes (males: slight in 5, mild in 1, moderate in 2) associated with granular degeneration. Centrilobular hypertrophy of hepatocytes (1 female, slight). *Kidney* Necrosis of papilla (1 female, slight). Nephroblastoma (1 female) **250 mg/kg bw/d:** *Liver - males* Centrilobular hypertrophy of hepatocytes (males: moderate in 1, severe in 9) associated with granular degeneration Centrilobular necrosis of hepatocytes (1 male, slight) Focal necrosis (males: slight in 3, mild in 1) *Liver – females* Centrilobular hypertrophy of hepatocytes (females: slight in 3, mild in 3, moderate in 2) *Kidney - females* Necrosis of papilla in the kidney (1 surviving female, mild; 2 deceased females, moderate) Dilatation of the renal tubule (2 surviving females: slight in 1, mild in 1; 3 deceased females: slight in 1, mild in 1, moderate in 1) Focal basophilic change of the renal tubule (1 surviving female, slight; 3 deceased females: mild in 2, moderate in 1). Dilatation of the renal pelvis (1 surviving female, mild) associated with calculus in the ureter (slight). Hyaline cast (1 female, slight). *Stomach* Hyperkeratosis at the limiting ridge of the stomach (1 male, slight; 1 female, slight) *Spleen* Extramedullary haematopoiesis of erythroblastic cells (1 male, slight; 1 male, mild) Atrophy of white pulp (females: mild in 2, moderate in 1)

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Bone marrow Increase in erythropoiesis in the femoral bone marrow (slight, 7 males and 2 females) *Thymus* Atrophy of the cortex (females: mild in 2, moderate in 1) *Lymph nodes* trophy of follicles in the submandibular lymph node (females: slight in 1, mild in 1) Atrophy of follicles in the mesenteric lymph node (2 females, slight) ***Termination of recovery period (250 mg/kg bw/d):*** *Liver* Centrilobular hypertrophy of hepatocytes in the liver (4 males, mild; 1 female, slight) *Kidney* One female had necrosis of papilla (mild), dilatation of the renal tubule (moderate), and focal basophilic change of the renal tubule (mild), which were similar to those found at the end of administration. In addition, this animal also showed focal fibrosis (mild) in the kidney
Combined chronic toxicity and carcinogenicity study OECD TG 453 GLP-compliant Crl:CD (SD) rats Chronic toxicity phase: 12/sex/dose Carcinogenicity phase: 66/sex/dose Labcorp (2025)	EEA-NH4 Oral gavage 0, 4, 20 and 100 mg/kg bw/d Chronic toxicity phase: 52 weeks Carcinogenicity phase: 104 weeks	**Non neoplastic findings (*for neoplastic findings, see Section 10.1 Carcinogenicity)*** **CHRONIC TOXICITY PHASE** **<u>Treatment-related mortality</u>** **100 mg/kg bw/d** 3 males, 1 female **<u>Food consumption & body weight/body weight gain</u>** No effects on food consumption. **100 mg/kg bw/d**: reduced body weight gain (↓11.6%) and body weight (↓10.1%) in males **<u>Haematological parameters</u>** **Week 13 (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↓Hb: -5.1%*, - 6.3%*, -10.8%* in males - 1.9%, -3.8%, -4.5% in females ↓RBC: -8.8%*, -9.7%*, -13.3%* in males

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		-1.8%, -5.2%, -5.6%* in females ↓Ht: -6.2%*, -7.1%*, -10.9%* in males -0.6%, -2.6%, -4.1% in females ↑ reticulocyte count: +7.7%, +11.5%, +23.1% in males **Week 26 (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↓Hb: - 5.6%*, - 5.0%*, -9.4%* in males -1.4%, -2.7%, 4.7% in females ↓RBC: -7.1%*, -8.4%*, -10.9%* in males -2.6%, -5.9%, -5.4% in females ↓Ht: -5.2%*, -4.8%, -6.8%* in males -1.9%, -3.5%, -4.5% in females ↑MCV: +2.3%, +4.2%*, +4.8% in males ↑MCH: +1.8%, +3.6%*, +1.8% in males ↓MCHC: -0.6%, -0.3%, -2.2%* in males ↑reticulocyte count: +21.7%, +26.1%, +47.8% in males : +0%, +10%, +20%* in females **Week 52 (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↓Hb: -4.9%*, -6.2%*, -17.6%* in males -2.8%, +1.0%, -5.4% in females ↓RBC: -6.2%*, -8.9%*, -13.5%* in males -1.4%, -1.2%, -4.4% in females ↓Ht: -5.8%*, -7.2%*, -12.5%* in males -0.9%, +1.6%, -4.3% in females ↑reticulocyte count: +4.3%, +8.7%, +34.8%* in males **<u>Clinical chemistry</u>** **Week 13 (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↑ ALP: +11.0%, +34%*, +116%* in males ↑ALT: +13.9%*, +22.2%*, +36.1% in males

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		↓cholesterol: -20.0%*, -30.3%*, -46.7%* in males In females ↑albumin:globulin ratio: +17%*, +34.8%*, +65.2%* in males ↓calcium: -2.7%*, -3.8%*, -3.8%* in males ↑ urea: +0%, 11.5%*, +25%* in males **Week 26 (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↑ ALP: +68.4%, +83.5%, +267%* in males ↑AST: +101%, +66.5%, +86.0% in males ↑ALT: +317%*, +161%*, +249%* in males ↑albumin:globulin ratio: +9.5%*, +28.6%*, +81.0%* in males ↑urea: +3.9%*, +19.6%*, +29.4%* in males **Week 52 (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↑ ALP: +29.1%, 161%*, +288%* in males ↑AST: +69%, +84.6%, +12.9% in males ↑ALT: +55%, >+176%, +52.5% in males ↑albumin:globulin ratio: +10.5%, +31.6%*, +94.7%* in males ↑urea: +4.3%, +4.3%, +19.6%* **<u>Organ weight changes</u>** **Week 52 (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↑ kidney weight: +14%*, +17%*, +21%* in males +20%*, +13%*, +45%* in females ↑ kidney/bw ratio: +19%*, +28%*, +37%* in males +1%, -2%, +36%* in females ↑ liver weight: -6%, +4%, +49%* in males +22%*, +12%, +46%* in females ↑ liver/bw ratio: -2%, +13%*, +66%* in males +1%, -3%, +35%* in females

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		thyroid/parathyroid: +4%, -6%, -19% in males +36%*, +50%*, +56%* in females thyroid/parathyroid/bw ratio: +9%, +4%, -8% in males +13%, +30%*, +43%* in females **<u>Macroscopic observations</u>** **Findings at 0, 4, 20 and 100 mg/kg bw/d:** **Kidney** *large*: 1/12, 1/11, 2/11 and 3/9 males 0/12, 0/10, 0/11 and 2/10 females *cyst*: 0/12, 0/11, 1/11 and 1/9 in males 0/12, 0/10, 0/11 and 0/10 females *dark*: 0/12, 0/11, 1/11 and 1/9 in males 1/12, 1/10, 2/11 and 0/10 females *irregular surface*: 0/12, 0/11, 0/11 and 1/9 males 0/12, 0/10, 0/11 and 2/10 females **Liver** *large*: 0/12, 0/11, 2/11, 8/9 males 3/12, 4/10, 4/11, 1/10 females *dark*: 0/12, 0/11, 0/11, 3/9 males 0/12, 3/10, 2/11, 5/10 females **Thyroid** *dark*: 0/12, 2/11, 3/11 and 4/9 males 1/12, 0/10, 0/11, 0/10 females **<u>Histopathology</u>** **Findings at 0, 4, 20 and 100 mg/kg bw/d** **Kidney** *Necrosis, papilla:* 0/12, 0/11, 1/10 (minimal), 4/9 (minimal →marked) in males 0/12, 0/10, 1/10 (minimal), 8/10 (minimal → severe) in females *Inflammation, papilla:* 0/12, 0/11, 0/10, 2/9 (minimal) in males 1/11 (minimal), 0/10, 0/11, 3/10 (minimal → slight) in females *Hyperplasia, urothelium:*

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		0/12, 0/11, 0/11, 1/9 (minimal) in males 4/12 (minimal), 4/10 (minimal), 3/11 (minimal), 7/10 (minimal → marked) in females *Mineralisation, cortico-medullary:* 0/12, 0/11, 1/11 (minimal), 0/9 in males 1/12 (minimal), 0/10, 1/11 (minimal), 6/10 (minimal → slight) in females *Dilatation, tubule:* 0/12, 0/11, 2/11 (minimal), 1/9 (marked) in males 0/12, 0/10, 0/11, 9/10 (minimal → moderate) in females *Pigment, tubule:* 1/12 (minimal), 4/11 (minimal → slight), 4/11 (minimal → slight), 6/9 (minimal → slight) in males 1/12 (minimal), 0/10, 0/11, 0/10 in females *Nephropathy, chronic progressive* 10/12 (minimal → slight), 6/11 (minimal → slight), 9/11 (minimal → slight), 7/9 (minimal → slight) in males 2/12 (minimal), 3/10 (minimal), 3/11 (minimal), 8/10 (minimal → slight) in females *Nephropathy, retrograde:* 0/12, 0/11, 0/11, 1/9 (marked) in males 0/12, 0/10, 0/11, 3/10 (moderated → marked) in females *Pyelonephritis:* 0/12, 0/11, 0/11, 1/9 (moderate) in males 1/12, 0/10, 0/11, 2/10 (slight → moderate in females) **Liver** *Hypertrophy, hepatocyte, centrilobular* 08/12, 4/11 (minimal), 9/11 (minimal → slight), 9/9 (minimal → moderate) in males 0/12, 0/10, 3/11 (minimal), 10/10 (slight → moderate) in females *Necrosis, focal* 1/12 (minimal), 4/11 (minimal → slight), 7/11 (minimal → moderate, 6/9 (minimal → moderate) in males 0/12, 1/10 (minimal), 0/11, 0/10 in females *Necrosis, single cell* 0/12, 0/11, 5/11 (minimal), 4/9 (minimal → slight) in males 0/12, 0/10, 0/11, 3/10 (minimal) in females

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Pigment, Kupffer cell 0/12, 2/11 (minimal), 8/11 (minimal), 7/9 (minimal → slight) in males 0/12, 0/10, 0/11, 3/10 (minimal → slight) in females *Pigment, hepatocyte, centrilobular* 0/12, 0/11, 0/11, 3/9 (minimal → slight) in males 0/12, 0/10, 0/11, 4/10 (minimal) in females *Mitosis, hepatocyte, increased* 0/12, 0/11, 0/11, 2/9 (minimal) in males 0/12, 0/10, 0/11, 2/10 (minimal → slight) in females *Hyperplasia, bile duct* 1/12 (minimal), 2/11 (minimal → slight), 2/11 (minimal → slight), 1/9 (slight) in males 0/12, 0/10, 2/11 (minimal), 0/10 in females **Thyroid** *Hypertrophy, follicular cell* 0/12, 0/11, 7/11 (minimal → slight), 8/9 (minimal → moderate) in males 0/12, 0/10, 0/11, 7/10 (minimal → moderate) in females **Lung** *Alveolar macrophages, increased* 3/12 (minimal), 3/11 (minimal → slight), 3/11 (minimal), 7/9 (minimal → slight) in males 3/12 (minimal → slight), 4/10 (minimal → slight), 3/11 (minimal), 4/10 (minimal → slight) in females **CARCINOGENICITY PHASE** **<u>Treatment-related mortality</u>** **100 mg/kg bw/d** 29 male, 16 females **<u>Food consumption & body weight/body weight gain</u>** No effects on food consumption. **100 mg/kg bw/d**: reduced body weight gain (↓12% in males; ↓21% in females) and body weight (↓12%* in males; ↓29%* in females) **<u>Haematological parameters</u>**

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Week 53 (difference to controls at 4, 20 and 100 mg/kg bw/d) ↓Hb: - 3.8%, -6.8%, -16%* in males -3.9%, -2.6%, -11.7%* in females ↓RBC: -2.2%, -4.9%*, -13.7%* in males - 6.3%, -7.0%, -12.9%* in females ↓Ht: - 3.4%, -7.3%*, -13.8%* in males -4.1%,-3.6%, -12.4%* in females **Week 77 (♂) or 78 (♀) (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↓Hb: -3.7%, -10.0%*, - 13.7%* in males -+2.7%, +2.0%, -13.5%* in females ↓RBC: -4.8%, -9.8%*, -13.0%* in males - +2.0%, -1.3%, -10.7% in females ↓Ht: -3.3%, -10.0%*, -13.8%* in males +1.0%, +1.0%, 13.6%* in females **Week 95 (♀) or 102 (♂) (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↓Hb: +0.9%, -8.6%*, 8.6%* in males ↓RBC: - +1.3, -7.9%, -4.6% in males ↓Ht: -+1.0%, -6.7%, -6.2% in males **<u>Clinical chemistry</u>** **Week 53 (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↑ ALP: +40.7%*, +95.0%*, +233.3%* in males -7.7%, +53.8%*, +46.1%* in females ↑AST: +35%, +108.8%, +72.3% in males +3.9%, +9.9%, +20.8% in females ↑ALT: +42%, +134.8%, +98.6% in males +7.0%, +11.6%, +23.2% in females ↓protein globulin: -4%, -24%*, -44%* in males

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Week 77 (♂) or 78 (♀) (difference to controls at 4, 20 and 100 mg/kg bw/d) ↑ ALP: +30.9%*, +80.2%*, +260.5%* in males -11.1%, -11.1%, +42.4%* in females ↑AST: +52.7%, +193.0%*, +116.3%* in males +33.0%, +12.8%, +19.1% in females ↑ALT: +77.2%, +224.6%*, +226.3%* in males +14.6%, +9.8%, 14.6% in females ↓protein globulin: -10.7%*, -17.9%*, -35.7%* in males **Week 95 (♀) or 102 (♂) (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↑ ALP: +2.9%, +55.1%, +275%* in males -13.4%, +17.4%, +113%* in females ↑AST: -0.8%, +90.4%, +37.5% in males ↑ALT: +2.0%, +110%*, +110% in males ↓protein globulin: -8%, -20%*, - 36%* in males **<u>Organ weight changes</u>** **Week 95 (♀) or 102 (♂) (difference to controls at 4, 20 and 100 mg/kg bw/d)** ↑ kidney weight: +11%*, +15%*, +14%* in males +11%, +12%*, +30%* in females ↑ kidney/bw ratio: +8%, +9%, +31%* in males +8%, +13%*, +54%* in females ↑ liver weight: +1%, +13%, +57%* in males +0%, +3%, +29%* in females ↑ liver/bw ratio: -1%, +6%, +80%* in males -1%, +4%, +53%* in females **<u>Macroscopic observations</u>** **Findings at 0, 4, 20 and 100 mg/kg bw/d:** **Kidney** large: 9/48, 14/42, 20/53, 24/50 males

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		0/44, 2/55, 1/40, 16/45 females cyst: 2/48, 2/42, 1/53, 0/50 males 0/44, 0/55, 0/40, 2/45 females dark: 2/48, 6/42, 6/53, 13/50 males 5/44, 2/55, 3/40, 11/45 females irregular surface: 1/48, 0/42, 0/53, 11/50 males 0/44, 1/55, 0/40, 10/45 females firm: 0/48, 0/42, 0/53, 1/50 males 0/44, 0/55, 0/40, 3/45 females **Liver** large: 14/48, 19/42, 20/53, 35/50 males 6/44, 8/55, 4/40, 13/45 females dark: 2/48, 4/42, 2/53, 20/50 males 0/44, 0/55, 0/40, 0/45 females mottled: 19/48, 16/42, 20/53, 34/50 males 25/44, 36/55, 20/40, 20/45 females **Thyroid** dark: 5/48, 10/42, 13/53, 17/50 males 2/44, 4/55, 4/40, 9/45 females **Urinary bladder:** Abnormal contents: 0/48, 0/42, 0/53, 2/50 males 0/44, 0/55, 0/40, 0/45 females Thickening: 0/48, 0/42, 0/53, 2/50 males 0/44, 0/55, 0/40, 0/45 females **<u>Histopathology</u>** **Incidence (and severity) at 0, 4, 20 and 100 mg/kg bw/d (66 animals/sex/dose examined)** **Kidney** *Necrosis, papilla* 0, 0, 0, 37 (slight → severe) in males 0, 0, 3, 52 (slight → severe) in females *Inflammation, papilla* 0, 0, 0, 24 (slight → moderate) in males 0, 0, 1 (slight), 14 (minimal → slight) in females

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		Hyperplasia, urothelial 12 (minimal → slight), 11 (minimal → slight), 15 (minimal → slight), 31 (minimal → marked) in males 41 (minimal → slight), 41 (minimal → moderate), 44 (minimal → moderate), 60 (minimal → marked) in females *Mineralisation, cortico-medullary* 0, 2 (minimal), 1 (minimal), 0 in males 32 (minimal → slight), 25 (minimal → slight), 28 (minimal → slight), 45 (minimal → moderate) *Pigment, tubule* 2 (minimal), 2 (minimal → slight), 8 (minimal → slight) in males 2 (minimal → slight), 2(minimal → slight), 0, 2 (minimal) in females *Nephropathy, retrograde* 0, 0, 0, 37 (slight → marked) in males 0, 0, 1 (minimal), 56 (minimal → severe) in females *Pyelonephritis* 0, 1 (slight), 1 (slight), 13 (minimal → marked) in males 1 (slight), 0, 1 (moderate), 32 (minimal → severe) in females **Liver** *Hypertrophy, hepatocyte, centrilobular* 0, 5 (minimal), 26 (minimal → slight), 44 (minimal → moderate) in males 0, 0, 3 (minimal), 47 (minimal → slight) in females *Degeneration, hepatocyte, centrilobular* 0, 0, 2 (minimal), 31 (minimal → slight) in males 0, 0, 0, 26 (minimal) in females *Necrosis, focal* 3 (minimal → moderate), 8 (minimal → slight), 22 (minimal → slight), 42 (minimal → marked) in males 2 (minimal), 4 (minimal → slight), 1 (minimal), 5 (minimal → slight in females) *Necrosis, single cell* 0, 0, 2 (minimal), 1 (minimal) in males 1 (minimal), 2 (minimal → moderate), 2 (minimal → slight), 8 (minimal → slight) in females *Pigment, Kupffer cell* 3 (minimal), 6 (minimal), 11 (minimal), 46 (minimal → slight) in males

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
		1 (slight), 2 (minimal), 5 (minimal), 19 (minimal → slight) in females *Pigment, hepatocyte, centrilobular* 0, 0, 0, 29 (minimal → slight) in males 0, 2 (minimal), 2 (minimal), 28 (minimal → slight) in females *Mitosis, hepatocyte, increased* 1 (minimal), 0, 0, 0 in males 5 (minimal), 5 (minimal), 3 (minimal), 7 (minimal → moderate) in females *Degeneration, cystic* 9 (minimal), 16 (minimal → slight), 26 (minimal → slight), 28 (minimal → moderate) in males 0, 1 (minimal), 0, 1 (minimal) in females **Thyroid** *Hypertrophy, follicular cell* 3 (minimal → moderate), 3 (minimal), 13 (minimal → moderate), 13 (minimal → moderate) in males 0, 0, 5 (minimal → slight), 14 (minimal → slight) in females **Testis** *Hyperplasia, Leydig cell, focal* 7 (minimal → marked), 3 (minimal → marked), 5 (minimal → marked), 16 (minimal → marked) in males **Adrenal** *Hypertrophy/hyperplasia, zona glomerulosa, diffuse* 0, 0, 0, 27 (minimal → moderate) in males 0, 0, 0, 7 (slight) in females **Spleen** *Extramedullary haematopoiesis* 56 (minimal → marked), 58 (minimal → moderate), 58 (minimal → marked), 46 (minimal → slight) in males 60 (minimal → marked), 48 (minimal → marked), 47 (minimal → marked), 41 (minimal → marked) in females

Key

Aspartate aminotransferase (AST)
Alanine aminotransferase (ALT)
Alkaline phosphatase (ALP)
γ-glutamyl transpeptidase (γ-GTP)
Total cholesterol (T-Cho)
Triglyceride (TG)
HDL-cholesterol (HDL-C)
Urea nitrogen (UN)
Creatinine (Crea)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Calcium (Ca)
Inorganic phosphorus (IP)
Total protein (TP)
Albumin/globulin ratio (A/G ratio)
Protein fraction:
1) Albumin (%)
2) α1-Globulin (α1-G, %)
3) α2-Globulin (α2-G, %)
4) β-Globulin (β-G, %)

LDL-cholesterol (LDL-C)
Total bilirubin (T-Bil)
Total bile acid (TBA)

Red blood cell count (RBC)
Hematocrit (Ht)
Hemoglobin concentration (Hb)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)

Triiodothyronine (T3)
Thyroxine (T4)
Thyroid stimulating hormone (TSH)

Mean corpuscular hemoglobin concentration (MCHC)
White blood cell count (WBC)
Prothrombin time (PT)
Activated partial thromboplastic time (APTT)

5) γ-Globulin (γ-G, %)

10.9.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In a 28-day repeated dose toxicity study (Hita Laboratory, 2006) conducted according to OECD TG 407 and GLP, EEA-NH4 was administered to Crl:CD (SD) rats (5/sex/dose) at doses of 0, 5, 25 and 100 mg/kg bw/d via oral gavage for 28 days. An additional control group and additional high dose group were included to assess recovery of the animals following cessation of dosing. The recovery period was 14 days.

The animals were observed throughout the test period for mortality and clinical signs. Food consumption and body weights were monitored throughout the dosing and recovery periods. Haematological, clinical chemistry and urinalysis measurements were taken at the end of the dosing and recovery periods. Necropsies were performed on day 28 on the animals in the test groups and on day 42 on the animals in the recovery groups, with organ weights and histopathological findings recorded for all animals.

No deaths or treatment-related clinical signs were reported during the study. The target organs were the liver, kidneys and forestomach.

Liver

In males, there was a statistically significant increase in mean absolute (↑42%) and relative (↑43.3%) liver weights at the top dose, and enlargement of the liver was recorded in 2/5 animals. This was accompanied by diffuse hypertrophy of the hepatocytes with granular degeneration (slight, 5/5 males). Slight but statistically significant changes in clinical chemistry and haematological parameters, consistent with changes in liver function, were also reported in males (↑ALT, ↑ A/G ratio, ↓total cholesterol, ↑prothrombin time). By the end of the recovery period, ALT levels remained slightly elevated (↑33.3%, statistically significant), but all of the other findings had reversed and measurements in treated animals were similar to controls.

In females, mean liver weight was only slightly increased at the top dose compared to controls (absolute: ↑13.2%; relative: ↑6%) by the end of the dosing period, and the change was not statistically significant. The only histopathology finding was focal necrosis of hepatocytes in one animal, graded as ‘slight’. By the end of the recovery period, liver findings had reversed and measurements in treated animals were similar to controls.

Although the liver findings were reversible, the Agency considers that the findings at the top dose cannot be dismissed as being an adaptive response. The considerable increase in liver weight seen in males, together with the granular degeneration (males), the necrosis (females) and the clinical chemistry/haematological findings indicative of liver damage (males), indicates liver toxicity at this dose.

Kidney

In males, there was a dose-related increase in kidney weight (mean relative weight: ↑5.3%, ↑18.7%* and ↑21.3%** at 5, 25 and 100 mg/kg bw/d). By the end of the recovery period, kidney weights had returned to normal (mean kidney weights were ↑ 5.6% in the treated animals compared to the controls, not statistically significant). One top dose male had a solitary cyst in the medulla, although the same finding was reported in a control female animal (see below) so it isn't clear whether this finding was treatment-related.

In females, there were no treatment-related effects on kidney weight. Basophilic tubules were reported in treated animals at the end of dosing (slight, 0/5, 1/5, 1/5, 2/5 at 0, 5, 25 and 100 mg/kg bw/d respectively) but were not reported in treated or control animals at the end of the recovery period (0/5 in both groups). Slight to moderate mineralisation in the corticomedullary junction of the kidney was reported in all groups at the end of the treatment period (2/5, 3/5, 3/5, 1/5 at 0, 5, 25 and 100 mg/kg bw/d respectively), and at the end of the recovery period (slight, 1/5 animals in the treated group and 1/5 animals in the control group). Given the lack of dose response in the incidence or severity of the finding, and the presence of the finding in controls, the Agency does not consider this finding to be treatment-related. A solitary cyst was reported in the kidney of one control female.

No findings were reported in the urinalysis measurements, in either sex.

Forestomach

In males, elevation of the limiting ridge of the forestomach was reported at the top dose (4/5 animals) at the end of the treatment period. This was accompanied by hyperplasia of the squamous epithelium in the limiting ridge (4/5 animals). Neither of these findings were present at the end of the recovery period.

In females, the same findings were reported at the top dose but fewer animals were affected: elevation of the limiting ridge was reported in 1/5 animals, and hyperplasia of the squamous epithelium was reported in 2/5 animals at the end of the treatment period. Neither finding was present at the end of the recovery period.

Other findings

Haematological investigations revealed slight, non-statistically significant decreases in red blood cell count, haemoglobin and haematocrit in both sexes at the top dose, and a statistically significant increase in reticulocyte count in top dose females. Taken together, the Agency considers these findings indicative of mild anaemia.

In summary, when EEA-NH4 was administered to rats by oral gavage for 28 days at doses up to 100 mg/kg bw/d, the target organs were the liver, kidneys and forestomach. The findings were generally reversible and had mostly returned to normal by the end of a 14-day recovery period. The toxicological significance and severity of the findings in the kidneys, forestomach and on the haematological parameters are not enough to support classification for STOT RE, however the Agency considers the liver findings to be of concern.

In a 90-day repeated dose toxicity study (Safety Research Institute for Chemical Compounds Co., Ltd., 2019), EEA-NH4 was administered to Crl:CD(SD) rats at dose levels of 0, 10, 50 and 250 mg/kg bw/d via oral gavage. Additional groups of control and top dose animals were established to assess the reversibility of effects during a 14-day recovery period. The toxicity study groups contained 10 males/dose, and 9 or 10 females/dose; recovery groups contained 5 males and 5 or 6 females.

At 10 mg/kg bw/d group, one male was found dead prior to dosing on administration day 65. Necropsy and histopathological examination of this animal revealed no abnormalities. Whilst the cause of death was unclear, the study authors did not consider the death to be treatment-related as no deaths were reported in males at higher doses. At 50 mg/kg bw/d, no deaths or clinical signs were reported in either males or females. At 250 mg/kg bw/d, pale ears and extremities were reported in three males between days 88 and 91. Three females died – one after dosing on day 42 (no abnormalities reported in the clinical signs prior to death), one after dosing on day 78 (after showing a decrease in motor activity, bradypnea, hypothermia and soiled perigenital fur) and one before dosing on day 80 (soiled periocular and perinasal fur was observed prior to death). These deaths were treatment-related (see discussion on kidney effects, below).

Food consumption was similar in control and all treated animals during both the treatment and recovery periods. However, at the top dose, body weight gain was statistically significantly reduced in both sexes (-15%** in males; - 17%* in females), suggesting reduced food efficiency and leading to reduced body weights in these animals by the end of the treatment period (-11%** in males and -9% in females). During the recovery period, food consumption remained similar in the control and treated animals, however body weight gain was 1.9 times higher in treated males and 1.2 higher in treated females compared to the control animals. This led to body weights in the treated animals starting to recover, although they were still lower than controls at the end of the recovery period (-11% in males and -16% in females).

The target organs were the liver, kidney, stomach and blood/haematopoietic system.

Liver

In males, statistically significant increases in liver weight were observed from 50 mg/kg bw/d (relative liver weights were +6.1%, +30.4%** and +94.2%** at 10, 50 and 250 mg/kg bw/d respectively, compared to controls), and ‘large liver’ was noted at the top dose. Histopathological examination revealed centrilobular hypertrophy of the hepatocytes associated with granular degeneration in the 50 and 250 mg/kg bw/d males, which the study authors considered to be indicative of increased xenobiotic metabolism. The authors also considered the high albumin reported in these dose groups to be indicative of increased synthetic function of the liver associated with increased metabolism. At 250 mg/kg bw/d, focal necrosis (1 male) and centrilobular necrosis (4 males) of hepatocytes were noted, accompanied by statistically significant increases in clinical chemistry parameters associated with liver damage (AST +157.0%**; ALT +326%**; ALP +183.06%**). AST, ALT and ALP were also statistically significantly increased at 50 mg/kg bw/d, however in each case the increase was less than 2-fold the control values. By the end of the recovery period, the findings in males were showing signs of recovery, although

albumin levels remained high. The Agency considers the liver effects at the top dose to be adverse, and indicative of liver damage. The liver effects at the mid-dose are largely adaptive, however the magnitude of the increase in liver weight is a concern.

In females, liver weight was statistically significantly increased at 250 mg/kg bw/d (relative liver weight was +61.27%** compared to controls), and centrilobular hypertrophy of hepatocytes was noted in the 50 and 250 mg/kg bw/d groups. There were no other histopathological changes in females, and no changes indicative of hepatic disorder were noted in the biochemical parameters. As such, the Agency considers the liver changes in top dose females to be an adaptive response to the increased xenobiotic metabolism, although the magnitude of the increase in liver weight is a potential concern.

Kidney

In males, mean relative kidney weight was statistically significantly increased (+30%**) at 250 mg/kg bw/d. There were no histopathological findings, however urinalysis revealed statistically significantly changes in several parameters (increased urine volume, reduced specific gravity, low urine pH, and increased urea nitrogen), indicating effects on renal function. The authors noted that the high potassium (K) and inorganic phosphorous (IP) measurements in top dose males may also have been related to changes in renal function. By the end of the recovery period, the kidney-related changes in males had either recovered or were recovering.

Females were more sensitive to kidney effects than males. At 50 mg/kg bw/d, mean relative kidney weight was statistically significantly increased (+15%*) and necrosis of renal papilla was noted in 1 female. At 250 mg/kg bw/d, mean relative kidney weight was increased by 33.6%** compared to controls, and the following findings were noted at necropsy: yellowish-white discolouration of the renal papilla and dilatation of the renal pelvis associated with calculus in the ureter. Histopathology revealed necrosis of the renal papilla, dilatation of the renal tubule, focal basophilic change of the renal tubule and dilatation of the renal pelvis associated with calculus in the ureter. All females that died at this dose had abnormal findings in the kidney, which the study authors considered to be the cause of death. Urinalysis of the top dose females revealed several findings (also seen in males, described above) which the study authors considered to be related to the kidney changes: increased urine volume, low specific gravity, low urine pH and high urea nitrogen UN (all statistically significant). The study authors also noted that the increased potassium and inorganic phosphorous recorded during the biochemistry measurements could also be related to the effects in the kidneys.

At the end of the recovery period, a tendency toward recovery was noted in the urinalysis, biochemistry and kidney weight changes seen at 250 mg/kg bw/d; however, necrosis of renal papilla, dilatation and basophilic change of the renal tubule were still present. In addition, rough surface and focal fibrosis of the kidney were noted at the end of recovery, which the study authors considered to be scarring and part of the repair process of the kidney lesions.

Stomach

At 250 mg/kg bw/d, hyperkeratosis at the limiting ridge of the stomach was noted in males and females. As squamous cell hyperplasia at the limiting ridge was noted in the repeated

dose 28-day study described above, the authors considered this finding to be due to the irritancy of the test article. At the end of the recovery period, these changes had reversed in both sexes.

Hematopoietic system

At 50 mg/kg bw/d, red blood cell count and mean corpuscular hemoglobin concentration were statistically significantly reduced in males, however the magnitude of the change was small (< 10%) and there were no changes in any other hematological parameters. At 250 mg/kg bw/d, red blood cell count, hemoglobin concentration, hematocrit and mean corpuscular hemoglobin concentration were all statistically significantly decreased in males and females (generally by >10%), and reticulocyte counts were increased (+455%** in males, +62%** in females). The study authors considered the increased reticulocytes to be a response to the reduced blood cells, and the statistically significant increase in mean corpuscular volume in males was considered to be caused by a marked increase in the ratio of reticulocytes, of which the volume is relatively high.

The statistically significant increases in potassium and inorganic phosphorus measured in both sexes at 250 mg/kg bw/d indicate hemolysis; however, neither hemosiderin deposition in the spleen or an increase in bilirubin were detected. Histopathological examination revealed no signs of bleeding and no prolongation was noted in the parameters of the blood coagulation system, and thus the mechanism for the observed anemia was unclear. For these changes, recovery or a tendency toward recovery was noted at the end of the recovery period. In the femoral bone marrow, an increase in erythropoiesis was noted in males and females at 250 mg/kg bw/d, and in the spleen, statistically significantly increased relative weight (+24%*) and an increase in extramedullary haematopoiesis of erythroblastic cells were noted in top dose males. The study authors considered these changes to be physical responses to anaemia. Reversibility of these changes was noted at the end of the recovery period.

In addition to the findings described above, males in the 250 mg/kg bw/d group had significantly high white blood cell count and neutrophil count, which the study authors noted could be related to the tissue necrosis seen in the liver in these animals. At the end of the recovery period, significantly high white blood cell count associated with significantly high lymphocyte count was noted; however, recovery from the change in neutrophil was noted. For the high lymphocyte count, histopathological examination of the lymphoid tissue at the end of the dosing period revealed no proliferative changes, and inflammatory changes or macroscopic findings of lymphocytes were not found in necropsy at the end of recovery. The study authors considered it possible that the high lymphocyte count was associated with an adaptive response of the bone marrow to a decrease in blood cells, and therefore considered the finding toxicologically insignificant.

Other findings

In the females that died in the top dose group, pale discoloration of the spleen and small size of the thymus were noted at necropsy, and histopathology revealed atrophy of white pulp in the spleen, atrophy of the cortex of the thymus, and atrophy of follicles in the submandibular/mesenteric lymph node, which the study authors attributed to aggravation in systemic conditions. At the end of the dosing period, clinical chemistry measurements revealed low β-G and α2- G fractions in males from 50 mg/kg bw/d, and α1-G fraction in

males in the 250 mg/kg group were low or tended to be low. The study authors interpreted these findings to mean a decrease in proteins composing β-G, α1-G and α2-G fractions, as there were no changes in total protein despite the high albumin measurement. The mechanism for these changes is unclear - histopathological examination of the kidneys revealed no abnormalities in the glomerulus and urinary protein wasn't increased, so the decrease in proteins was not caused by leakage. Furthermore, no changes were noted in the lymphoid tissue and there was no decrease in white blood cell counts. Males in the 10 mg/kg group showed significantly low α2-G fraction, which was considered toxicologically insignificant because no changes were noted in the other protein fractions, A/G ratio, albumin or TP.

T3 and T4 were reduced compared to controls in males and females at the top dose; T4 was also reduced in males in the mid-dose group. However, no changes were noted in TSH. As no changes were noted in total cholesterol, and no hypertrophy or hyperplasia of follicular cells were noted in the thyroid, the study authors considered that the amount of thyroid hormone available for the tissue was sufficient, and the low T3 and T4 were toxicologically insignificant. The study authors noted that low thyroid hormone without an associated increase in TSH or histopathological changes in the thyroid has been reported with per- and polyfluoroalkyl substances (PFAS) including perfluorooctanesulfonate (PFOS). This has been attributed to increases in tissue uptake, conjugation and excretion of thyroid hormone, which result from an increase in free thyroid hormone caused by competition of these substances at the binding site of thyroid hormone and transport protein. Since EEA-NH4 is a PFAS, the study authors considered that a similar mechanism may be in action. At the end of recovery period, recovery or a tendency toward recovery was noted in these biochemical changes.

In summary, when EEA-NH4 was administered to rats for 90 days at doses up to 250 mg/kg bw/d, the target organs were the liver, kidney, stomach and blood/haematopoietic system. The Agency agrees with the study authors that the effects in the stomach are likely due to the irritant properties of the test substance and are of limited relevance for STOT RE. There were marked sex differences in the toxicity profile, with males being more sensitive to liver effects and females being more sensitive to effects in the kidney. This profile is consistent with that seen with other PFAS substances.

In a combined chronic toxicity and carcinogenicity study (OECD TG 453, GLP), Sprague Dawley rats were administered doses of 0, 4, 20 or 100 mg/kg bw/d EEA-NH4 via oral gavage for either 52 weeks (chronic toxicity phase - 12/sex/dose) or 104 weeks (carcinogenicity phase - 66/sex/dose). Non-neoplastic findings are summarised in Table 16 and are discussed further below. Neoplastic findings are discussed in the carcinogenicity section.

There were no toxicologically significant clinical observations reported during the study. Food consumption was unaffected by treatment, however males at the top dose had reduced body weight gain (↓11.6%) compared to controls during the chronic toxicity phase. During the carcinogenicity phase, decreased body weight gains (↓12% in males; ↓21% in females) and body weights (↓12%* in males; ↓29%* in females) were noted in surviving animals of both sexes at the top dose.

Treatment-related deaths occurred at the top dose in both phases of the study. Four animals (three males and one female) in the chronic toxicity phase and 45 animals (29 males and 16 females) in the carcinogenicity phase at 100 mg/kg bw/d either died or were removed from the study due to adverse clinical observations. The cause of death/decline for all these animals was considered to be renal papillary necrosis with tubular dilatation, pyelonephritis and/or retrograde nephropathy within the kidney resulting from the administration of EEA-NH4.

The target organs were the liver, kidney, thyroid and blood/hematopoietic system.

Liver

At the end of the toxicity phase, relative liver weights were statistically significantly increased at the top dose (+66%* in males; +35%* in females). At macroscopic examination, large liver was reported in males from 20 mg/kg bw/d (incidence was 0, 0, 2 and 8 at 0, 4, 20 and 100 mg/kg bw/d) and dark liver was noted in 3 males at the top dose. Histopathology revealed centrilobular hepatocyte hypertrophy in some males administered 4 mg/kg/day and both males and females administered ≥20 mg/kg/day. The Agency considers this to be an adaptive change in response to the administration of the test substance. However, histopathology also revealed focal necrosis, single cell necrosis, Kupffer cell pigment, centrilobular hepatocyte pigment, and/or increased hepatocyte mitosis in males that were administered ≥4 mg/kg/day and occasional females that were administered 100 mg/kg/day. Compared to controls, an increased severity of bile duct hyperplasia was also noted for one male from each EEA-NH4-treated group. Clinical chemistry findings consistent with liver damage / altered liver function were also reported: increased ALP, ALT and AST levels were reported in treated males; low cholesterol and variable albumin and globulin concentrations were reported in males and females at the top dose.

At the end of the carcinogenicity phase, liver weights were statistically significantly increased at the top dose (relative liver weights were +80%* in males and +53%* in females compared to controls). Macroscopic examination revealed increased incidences of large liver (14, 19, 20, 35 in males; 6, 8, 4, 13 in females) and dark liver (2, 4, 2, 20 in males) at the top dose.

Upon macroscopic examination, test article-related findings were noted for the kidney, liver, thyroid, and urinary bladder of animals from the carcinogenicity phase. Centrilobular hepatocyte hypertrophy was recorded for the liver of males that was administered 4 mg/kg/day and for both sexes that were administered ≥20 mg/kg/day. In addition, centrilobular hepatocyte degeneration, focal necrosis, cystic degeneration, centrilobular hepatocyte pigment, Kupffer cell pigment, and/or increased hepatocyte mitosis were also variably noted for males that were administered ≥4 mg/kg/day and for females that were administered 100 mg/kg/day.

Clinical chemistry examinations of animals from the carcinogenicity phase during Weeks 53, 77, and 102 revealed high alkaline phosphatase (ALP) activity and slightly high aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in males that were administered either 20 or 100 mg/kg/day. Protein globulin concentrations were low for all groups of EEA-NH4-treated animals

Kidney

At the end of the toxicity phase, a dose-related increase in relative kidney weights was recorded in males (+19%*, +28%*, +37%* at 4, 20 and 100 mg/kg bw/d). In females, relative kidney weights were increased at the top dose only (+36%*). Macroscopic examination revealed an increased incidence of large kidney and irregular surface in both sexes at the top dose. Microscopic examination revealed papillary necrosis, papillary inflammation, urothelial hyperplasia, cortico-medullary mineralization, tubular dilatation, tubular pigment and an increased incidence and/or severity of chronic progressive nephropathy, retrograde nephropathy, and/or pyelonephritis in animals administered 100 mg/kg bw/d. In addition, papillary necrosis was also noted for a few animals that were administered 20 mg/kg bw/d, and an increased incidence and severity of tubular pigment was also noted for males that were administered ≥4 mg/kg bw/d. Clinical chemistry findings consistent with changes in kidney function were also noted (low blood calcium concentrations, increased urea and decreased protein) in males at doses ≥ 20 mg/kg bw/d. No changes to urinalysis parameters were apparent during the chronic toxicity phase of the study.

At the end of the carcinogenicity phase, relative kidney weights were statistically significantly increased in both sexes at the top dose (+31%* in males and +54%* in females). Macroscopic examination revealed an increased incidence of large kidney (in males from 4 mg/kg bw/d; in females at the top dose), cysts (2 females at the top dose), dark kidney, firm kidney and irregular surface (both sexes at the top dose). Papillary necrosis, papillary inflammation, urothelial hyperplasia, cortico-medullary mineralization, tubular dilation, retrograde nephropathy, and/or pyelonephritis were noted in the kidney of some females administered 20 mg/kg bw/d and most animals at 100 mg/kg bw/d. An increased incidence of tubular pigment was also noted in males administered ≥20 mg/kg bw/d. In occasional animals, the severity of findings evident in the kidney resulted in more widespread inflammation of the urogenital tract (urinary bladder and prostate).

Thyroid
Follicular cell hypertrophy was recorded in males from 20 mg/kg bw/d and in females at the top dose, at the end of both phases of the study. The study authors considered the hypertrophy to be a secondary/adaptive response, and in the absence of any additional evidence of tissue injury or inflammation, the finding was considered to be non-adverse within the context of the study.

Blood / haematopoietic system
Haematological examination of the animals in the toxicity phase in weeks 13 and 26 revealed slightly low haemoglobin concentrations, red cell counts, and packed cell volume in treated animals from 4 mg/kg bw/d. The finding was more pronounced in males than in females. Slightly high reticulocyte counts were observed during week 13 in males at the top dose, and in both sexes at the top dose by week 26. In week 26 only, slightly low fibrinogen concentrations were noted in treat males at all dose levels. During Week 52, low haemoglobin concentrations, red cell counts, and packed cell volume persisted in males from 4 mg/kg bw/d but was only apparent in females at the top dose. Reticulocyte counts also exhibited slightly higher levels, compared with controls, for males that were

administered 100 mg/kg/day. Slightly low fibrinogen concentrations were also apparent in all EEA-NH4 treated males.

Haematological analysis for animals in the carcinogenicity phase also revealed low haemoglobin concentrations, red cell counts, and packed cell volume for males and females at 100 mg/kg bw/d for males administered 20 mg/kg bw/d.

Other findings
At the end of the chronic toxicity phase, an increased incidence of increased alveolar macrophages was noted in the lungs of males at 100 mg/kg bw/d. At the end of the carcinogenicity period, diffuse hypertrophy/hyperplasia of the zona glomerulosa was recorded for both males and females at 100 mg/kg bw/d. A decreased incidence and/or severity of extramedullary hematopoiesis was noted in the spleen in both sexes at 100 mg/kg bw/d.

Additional information from reproductive toxicity studies:

Further findings relevant to STOT RE were noted in the two available reproductive toxicity screening studies. Full study summaries are available in Table 22 in section 10.12 below, however findings relevant to STOT RE are summarised here.

In a reproductive/developmental 28-day toxicity screening study in rats (WIL Research Laboratories, 2011), animals were dosed with 0, 5, 25 or 100 mg/kg bw/d. Males were dosed for ~28 days and females were dosed between 39 and 52 days. No mortality or clinical signs were reported in the parental generation, and there were no effects on food consumption, mean body weight or body weight gain. Higher liver weights in 25 (+24%**) and 100 mg/kg bw/d (+47%**) group males were considered related to test substance administration. Exacerbation of vacuolation/hypertrophy of basophilic cells in the anterior pituitary gland in all groups of treated males was also considered related to test substance administration.

In a reproductive/developmental 90-day toxicity screening study (Safety Research Institute for Chemical Compounds, 2014), female rats showing evidence of copulation were administered doses of 0, 10, 30, 90 or 270 mg/kg bw/d EEA-NH4 (via oral gavage) throughout gestation. One dam at 90 mg/kg bw/d showed decreased locomotor activity and hypothermia on GD 5; this dam later died on GD 6. Two dams in the 270 mg/kg group showed the same clinical signs with the addition of bradypnea on GD 1 or 2; both dams died on GD 2. A third animal showed these clinical signs on GD 21 and was euthanised because of marked exhaustion. Food consumption decreased in the 90 mg/kg group and above on GD 3 and 30 mg/kg and above on GD 6, with significant decrease in the 90 mg/kg (-9.3%**) and 270 mg/kg (-11.1%**) group. Mean body weight was not impacted, but 2 individual animals in the 90 mg/kg group had low body weight on GD 3. Animals that died or were euthanised exhibited thickened limiting ridge of the stomach, small spleen and thymus.

10.9.2 Comparison with the GB CLP criteria

When considering animal data, STOT RE is assigned on the basis of evidence that a substance causes significant or severe toxicity, either reversible and irreversible, generally

at or below the oral guidance value of 100 mg/kg bw/d (for a classification in category 2) in a 90-day rat study.

'Significant' toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

For EEA-NH4, a number of studies in rats examining different dose levels and study durations are available. Together, the studies indicate that the main targets of toxicity are the liver, kidney and haematopoietic system. Table 17 summarises some of the main findings at doses relevant for classification.

Table 17: Summary of key findings at doses relevant for classification

Study/adjusted guidance values	Effects at doses below guidance cut-off values	Agency comments
28-day oral study in rats Adjusted guidance values: Category 1: ≤ 30 mg/kg bw/d Category 2: ≤ 300 mg/kg bw/d	**100mg/kg bw/day (top dose in study)** *Liver:* Males: increased weight (>40%), 'large liver', hepatocellular hypertrophy, granular degeneration, increased ALT levels Females: focal necrosis of hepatocytes (slight, 1 female) *Kidney* Males: increased weight (~20%), cyst in medulla (1 animal). Females: basophilic tubular epithelium and transitional epithelium with interstitial cell infiltration *Haematological parameters* Reduced RBC, Hb & Ht in both sexes (all changes < 10%)	The effects in the liver are a concern – the substantial increase in liver weight in males and the necrosis reported in females are considered adverse findings. Although the necrosis was slight and only seen in 1 female, it occurred well below the guidance value for classification. Overall, liver findings support classification in **Category 2.** The findings in the kidney and on haematological parameters in this study are not considered to be significant or severe enough to support classification.

Study/adjusted guidance values	Effects at doses below guidance cut-off values	Agency comments
90-day oral study in rats Guidance values: Category 1: ≤ 10 mg/kg bw/d Category 2: ≤ 100 mg/kg bw/d	**10 mg/kg:** *Clinical chemistry parameters* Males: increased ↑AST (+157.97%) and ALT (+325.97%) and ↓ α2-G fraction (-10.54%) **50 mg/kg:** *Liver* Males: increased liver weight (+30.4%), certrilobular hypertrophy associated with granular degeneration, small (<2 fold) increases in AST, ALT and ALP *Kidney* Females: increased weight (+15%), necrosis of the renal papilla in 1 female (slight). *Haematological parameters* Small changes (<10%) in some parameters in males	At doses relevant for classification, the findings in the liver were not particularly severe in this study, although the magnitude of the increase in weight at 50 mg/kg bw/d could be considered adverse, and the small increases in AST, ALT and ALP may be an early indication of liver toxicity. Necrosis was seen at the top dose in this study (250 mg/kg bw/d), however this is considerably above the guidance value for classification. It is difficult to extrapolate the liver findings between the mid and top dose, but it seems likely that some liver toxicity would have been seen at doses around the guidance value (i.e., 100 mg/kg bw/d). The magnitude of the increase in liver weight at 50 mg kg bw/d is considered adverse and provides some limited support for classification in **Category 2.** The increased kidney weight in females associated with slight necrosis in the renal papilla of 1 female at 50 mg/kg bw/d indicates kidney toxicity at a dose well below the guidance value for classification. **Supports classification in Category 2.**

Study/adjusted guidance values	Effects at doses below guidance cut-off values	Agency comments
Combined chronic toxicity and carcinogenicity study, oral route, in rats Adjusted guidance values for chronic toxicity phase of the study: Category 1: ≤ 2.5 mg/kg bw/d Category 2: ≤ 25 mg/kg bw/d Adjusted guidance values for chronic toxicity phase of the study: Category 1: ≤ 1.25 mg/kg bw/d Category 2: ≤ 12.5 mg/kg bw/d	**CHRONIC TOXICITY PHASE** **4 mg/kg bw/d** *Liver* Males: centrilobular hepatocyte hypertrophy, focal necrosis, single cell necrosis, Kupffer cell pigment, centrilobular hepatocyte pigment, and/or increased hepatocyte mitosis. Increased ALP, AST and ALT levels. *Kidney* Males: increased weight (+19%), pigment in tubule *Haematological parameters* Decreased Hb, RBC and Ht in both sexes (changes < 10%) **20 mg/kg bw/d** *Liver* Males: increased weight (+13%), large liver, centrilobular hepatocyte hypertrophy, focal necrosis, single cell necrosis, Kupffer cell pigment, centrilobular hepatocyte pigment, and/or increased hepatocyte mitosis Females: centrilobular hepatocyte hypertrophy, dark liver *Kidney* Males: increased weight (+28%), large kidney (2 animals), papillary necrosis (1 animal, minimal), pigment in tubule, Females: papillary necrosis (1 animal, minimal) *Haematological parameters* Decreased Hb, RBC and Ht in both sexes (changes < 10%) **CARCINOGENICITY PHASE** **4 mg/kg bw/d** *Liver* Males: increased incidence of large liver, dark liver, centrilobular hepatocyte hypertrophy, focal necrosis, pigment in Kupffer cells, cystic degeneration, accompanied by slight increases in ALP, AST and ALT	**CHRONIC TOXICITY PHASE** Liver effects in males from 4 mg/kg bw/d are considered adverse are support classification in **Category 2.** Kidney effects in both sexes at 20 mg/kg bw/d are considered adverse and support classification in **Category 2.** At doses relevant for classification, the changes in haematological parameters were not significant or severe enough to support classification. **CARCINOGENICITY PHASE** Liver effects in males from 4 mg/kg bw/d are considered adverse are support classification in **Category 2.**

10.9.3 Conclusion on classification and labelling for STOT RE

Overall, the Agency proposes that that there is sufficient evidence in the relevant repeated-dose studies to support classification as **STOT RE 2, H373 (May cause damage to the liver and kidney through prolonged or repeated exposure)**.

10.10 Germ cell mutagenicity

Table 18: Summary of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Bacterial gene mutation Ames test OECD TG 471 GLP compliant	EEA-NH4 Positive control: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, sodium azide, 9-aminoacridine, benzoapyrene & 2-aminotransferase Negative control: Distilled water	S. *typhimurium strains* TA 1535, 1537, 98 & 100 E. *coli* WP2 uvr A In the presence and absence of metabolic activation from 156, 313, 625, 1250, 2500 & 5000 µg/plate	**Negative** Negative for all strains regardless of the presence or absence of metabolic activation	Genetic Laboratory (2005)
Chromosome aberration study in hamster lung fibroblasts (V79) OECD TG 473 GLP compliant	EEA-NH4	Chinese hamster lung fibroblasts (V79) Without S9 mix: 723, 868, 1040, 1250, 1500 & 1800 µg/ml With S9 mix: 603, 723, 868, 1040, 1250, 1500 & 1800 µg/ml Positive control: Mytomycin C Negative control: Distilled water	**Positive** -S9 mix/+S9 mix: The frequency of structural aberrations increased in a dose- dependent manner. The frequency of cells with numerical aberrations was not statistically significantly different to the negative controls.	Hita Laboratory (2006)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Mammalian cell gene mutation assay in mouse lymphoma (L5178Y) cells OECD TG 476 GLP compliant	EEA-NH4	Mouse lymphoma (L5178Y) cells 1st assay: Without S9-mix: 0, 0.10, 0.19, 0.39, 0.55, 0.79, 1.1, 1.3, 1.6 & 1.8 mmol/L 24hr exposure With S9-mix: 0, 0.05, 0.10, 0.20, 0.40, 0.58, 0.82, 1.2, 1.7, 2.4 mmol/L Cytotoxicity: highest dose usable for evaluating mutagenicity was 1.7 mmol/L 4hr exposure 2nd assay: Without S9-mix: 0, 0.39, 0.56, 0.79, 1.1, 1.6, 1.9, 2.2, 2.6, 3.1 mmol/L 4hr exposure With S9-mix: 0, 0.59, 0.84, 1.2, 1.7, 1.9, 2.1, 2.4, 2.6, 2.9 mmol/L 4hr exposure Metabolic activation system: Aroclor 1254-induced rat liver homogenate Vehicle: RPMI 1640 medium Positive Control: 3-methylcholanthrene	**Negative** 1st study: In both the presence and absence of metabolic activation there was no equivocal or positive result compared to the negative control. 2nd study: In both the presence and absence of metabolic activation there was no equivocal or positive result compared to the negative control.	TNO Quality of Life (2010)

Table 19: Summary of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus test in erythrocytes in rats OECD TG 474 GLP compliant	EEA-NH4	Wistar rats Oral gavage 2 daily administrations over consecutive days 0, 125, 250 & 500 mg/kg bw/d 5 rats/sex/dose Negative control: sterile water Positive control: Mitomycin C	Toxicity: 1 male was euthanised at 1000 mg/kg bw/d after the first administration and the remaining male and 2 females were found dead after the second administration. Clinical signs were observed at 500 mg/kg bw/d but were not considered severe, however 2 females were euthanised at this dose and 1 was replaced by a reserve animal. This resulted in 3 females available at the top dose. Genotoxicity: Negative, no statistically significant increase in the number of PE or MPE compared to controls.	TNO Quality of Life (2010)

10.10.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro

An Ames test (Genetic Laboratory, 2005) conducted according to OECD TG 471 using bacterial strains of S. *typhimurium* TA 1535, 1537, 98 & 100 and E. *coli* WP2 uvr A is available. Strains were tested at EEA-NH4 doses of 156, 313, 625, 1250, 2500 & 5000 µg/plate, both in the presence and absence of metabolic activation. EEA-NH4 did not induce a statistically significant increase in the number of revertant colonies compared with the negative controls in any strain, either in the presence or absence of metabolic activation. Positive and negative controls behaved as expected. This negative result shows that EEA-NH4 does not cause mutations in various bacterial strains under the conditions of this test.

A chromosome aberration study in Chinese hamster lung fibroblasts (V79) (Hita Laboratory, 2006) conducted according to OECD TG 473 is available. A preliminary cell growth inhibition test was used to determine the test concentrations of EEA-NH4. In the presence of metabolic activation, the test concentrations were 603, 723, 868, 1040, 1250, 1500, 1800 µg/mL and in the absence of metabolic activation the test concentrations were 723, 868, 1040, 1250, 1500, 1800 µg/mL. Positive and negative controls behaved as expected. There was a dose-related increase in the number of structural aberrations seen

in the short-term tests, both with and without metabolic activation. Limited details are available, but according to the REACH registration dossier the maximum frequency of cells with structural aberrations was >10%. The frequencies of numerical aberrations were <5% at all concentrations with and without metabolic activation and were not statistically significantly different to controls. Overall, the test was positive and demonstrates that EEA-NH4 is able to induce structural chromosome aberrations under these test conditions.

A gene mutation study conducted according to OECD TG 476 using L5178Y (mouse lymphoma cells) is available. Two experiments administered varying concentrations of EEA-NH4, between 0.05 and 3.1 mmol/L, to single cultures for either 24 hours in the absence or 4 hours in the presence of S9-mix (experiment 1) or 4 hours in both the absence and presence of S9-mix (experiment 2). In both experiments there was no increase in the mutant frequency (MF) by more than 88 colonies per 1,000,000, therefore the test result was considered to be negative. Positive and negative controls behaved as expected

In vivo

One *in vivo* study is available, a micronucleus test in erythrocytes (TNO QoL, 2010), conducted according to OECD TG 474. Five Wistar rats/sex were administered 0, 125, 250 & 500 mg/kg bw/d EEA-NH4 via oral gavage once per day over two consecutive days. The animals were then killed and bone marrow cells were collected from the femurs. A preliminary test was conducted also using 1000 and 750 mg/kg bw/d however due to high levels of toxicity seen at these doses they were not included in the main study. In addition, 2 female rats at 500 mg/kg bw/d were sacrificed for ethical reasons and another was replaced by a reserve animal, so only 3 female rats were studied at the top dose level. There was no statistically significant difference in the mean number of Micronucleated Polychromatic Erythrocytes (MPE) per 2000 Polychromatic Erythrocytes (PE) in the test groups when compared to the negative controls, indicating that EEA-NH4 did not cause any damage to the chromosomes and/or to the spindle apparatus of bone marrow cells in the rats under the conditions of this test. No cytotoxicity was seen in the bone marrow of male and female rats at any dose. The general toxicity reported in the study provides reassurance that the test substance reached the bone marrow. Overall, this study was negative and does not indicate a mutagenic potential for EEA-NH4.

10.10.2 Comparison with the GB CLP criteria

To be classified as a germ cell mutagen in category 1 a substance must be known to induce heritable mutations or to be regarded as if it induces heritable mutations in the germ cells of humans. There are no human epidemiological studies available, and the relevant *in vivo* study highlighted no mutagenic potential of EEA-NH4. Therefore, a classification in category 1 is not warranted.

Classification in category 2 is appropriate for substances which cause concern for humans owing to the possibility they may induce mutations in the germ cells of humans. When tested *in vitro,* EEA-NH4 did not induce mutations in bacterial or mammalian cells but did induce chromosome aberrations. However, a well conducted *in vivo* micronucleus test was

negative, therefore there is no evidence that EEA-NH4 can cause chromosome aberrations in a whole organism. As such, classification in category 2 is not warranted.

10.10.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the CLP classification criteria, the available data do not indicate a mutagenic potential of EEA-NH4. Consequently, classification for germ cell mutagenicity is not warranted.

Data conclusive but not sufficient for classification.

10.11 Carcinogenicity

Table 20: Summary of animal studies relevant for carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group **Reference**	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**
Combined chronic toxicity and carcinogenicity study OECD TG 453 GLP-compliant Crl:CD (SD) rats Carcinogenicity phase: 66/sex/dose Labcorp (2025)	EEA-NH4 Oral gavage 0, 4, 20 and 100 mg/kg bw/d Carcinogenicity phase: 104 weeks	**Neoplastic findings (*for non-neoplastic findings, see STOT RE section)*** **Incidences at 0, 4, 20 and 100 mg/kg bw/d, respectively** **Testes** *B-Adenoma, Leydig cell* 0, 1, 0, 7 in males Incidence at 100 mg/kg bw/d statistically significantly different to controls (Peto test, P-value 0.0038). Trend test was also statistically significant (Peto test, P-value 0.0001) **Mammary gland** *B-adenoma* 0, 0, 0, 0 in males 17, 21, 16, 19 in females *B-fibroadenoma* 0, 1, 2, 1 in males 28, 32, 18, 15 in females *M-adenocarcinoma* 0, 0, 0, 0 in males 12, 18, 14, 9 in females *Combined adenoma/fibroadenoma/adenocarcinoma* 0, 1, 2, 1 in males 57, 71, 48, 43 in females

Method, guideline, deviations if any, species, strain, sex, no/group Reference	Test substance, route of exposure, dose levels, duration of exposure	Results
		Pituitary *B-adenoma, pars distalis* 15, 10, 13, 8 in males 30, 40, 34, 26 in females *M-carcinoma, pars distalis* 0, 0, 0, 0 in males 7, 3, 2, 3 in females *Combined adenoma/carcinoma* 15, 10, 13, 8 in males 37, 43, 36, 29 in females **Liver** *B-adenoma, hepatocellular* 1, 1, 0, 3 in males (P value (trend test) 0.0256*) 2, 0, 1, 3 in females

10.11.1 Short summary and overall relevance of the available information on carcinogenicity.

A combined chronic toxicity and carcinogenicity study (OECD TG 453, GLP) is available. Non-neoplastic findings are discussed in the STOT RE section of this report. Neoplastic findings are discussed below. In the carcinogenicity phase of the study, Sprague Dawley rats (66/sex/dose) were administered doses of 0, 4, 20 or 100 mg/kg bw/d EEA-NH4 via oral gavage for 104 weeks.

There were no toxicologically significant clinical observations reported during the study. Food consumption was unaffected by treatment, however males at the top dose had reduced body weight gain (↓11.6%) compared to controls during the chronic toxicity phase. During the carcinogenicity phase, decreased body weight gains (↓12% in males; ↓21% in females) and body weights (↓12%* in males; ↓29%* in females) were noted in surviving animals of both sexes at the top dose.

Treatment-related deaths occurred at the top dose in both phases of the study. Four animals (three males and one female) in the chronic toxicity phase and 45 animals (29 males and 16 females) in the carcinogenicity phase at 100 mg/kg bw/d either died or were removed from the study due to adverse clinical observations. The cause of death/decline for all these animals was considered to be renal papillary necrosis with tubular dilatation, pyelonephritis and/or retrograde nephropathy within the kidney resulting from the administration of EEA-NH4. Based on these levels of toxicity, the Agency considers that the maximum tolerated dose (MTD) was exceeded at this level.

Testes

An increased incidence of Leydig cell adenoma was observed in males administered 100 mg/kg bw/day (incidence: 0, 1, 0, 7 at 0, 4, 20 and 100 mg/kg bw/d); this finding was associated with an increase in the incidence and severity of focal Leydig cell hyperplasia (total incidence: 7, 3, 5, 16 at 0, 4, 20 and 100 mg/kg bw/d). Leydig cell adenoma was characterised by nodules of interstitial cells (Leydig cells) that extended between tubules, causing compression of adjacent tubules. The increase in Leydig cell adenoma at the top dose was statistically significant compared to the control group (Peto test, P-value 0.0038). In addition, the incidences of Leydig cell adenoma were statistically significant in a trend test (Peto test, P-value 0.0001). No historical control data (HCD) are available, and there is no information available on the mode of action (MoA). The Agency therefore considers the Leydig cell tumours to be treatment-related and relevant to humans.

Liver

Benign hepatocellular adenoma occurred in three males at the top dose compared with a single incidence in controls. This result was statistically significant in a trend test (Peto test, P-value 0.0256). Similarly, in females, three hepatocellular adenomas were reported at the top dose, although the incidence in female controls was higher (two) than in male controls. The trend test in females did not report a statistically significant result. It is biologically plausible that an increase in benign hepatocellular tumours might occur as part of a continuum of effects from hepatocyte hypertrophy and/or hepatocellular degenerative changes with associated increased hepatocyte turnover. Indeed, dose-related increases in the incidence and severity of non-neoplastic findings were reported in the livers of both sexes. No HCD are available to indicate the normal background levels of this tumour in males and females at the testing laboratory. Given the biological plausibility and statistical significance, the Agency considers that these tumours cannot be dismissed as a chance finding.

Mammary gland and pituitary

A non-statistically significant decrease in the incidence of combined mammary gland tumours was noted in females administered doses ≥20 mg/kg bw/d, and a decrease in the incidence of pituitary tumours was noted in females at the top dose. A slight reduction in pituitary tumours was also noted in males at the top dose. The study authors noted that an association between body weight and/or dietary restriction and tumour incidence has long been established and a strong positive correlation between body weight and site-specific tumours such as pituitary and mammary gland tumours has previously been reported in the scientific literature. The study authors therefore considered that the findings were most likely secondary related to the chronic body weight differences noted in both sexes at 100 mg/kg bw/day. However, given the minor reduction in mammary gland tumours also noted at 20 mg/kg bw/day, in the absence of any effect on body weight, a direct effect of EEA-NH4 cannot be excluded.

Conversely, a statistically significant increase in combined mammary adenoma, fibroadenoma, and adenocarcinoma was noted in females at 4 mg/kg bw/d, compared to the vehicle control group (Log Rank test, P-value 0.0464); however, no statistically significant increase was noted for any individual tumour type. An increase in the incidence of similar tumours was not noted in males, and no increase in the incidence or severity of

preneoplastic lesions was noted in either sex. The study authors noted that mammary tumours are the second most common spontaneous tumour type observed in control female Crl:CD(SD)-rats. Therefore, this increased incidence of combined mammary tumour types was considered a chance event and not treatment-related.

Overall, the Agency considers that the Leydig cell adenomas in male rats and hepatocellular adenomas in males and females, all seen at the top dose, are relevant for classification.

10.11.2 Comparison with the GB CLP criteria

Classification in Category 1A is largely based on human data. As no human data are available for EEA-NH4, classification in Category 1A is not warranted.

Animal data can support classification in either Category 1B (presumed human carcinogen) or Category 2 (suspected human carcinogen). The classification is assigned based on the strength of the evidence plus additional considerations (i.e., weight of evidence).

Category 1B requires “sufficient evidence of carcinogenicity”, which is defined in the legal text as *“a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.”*

Considering the available data for EEA-NH4 (i.e., single study, single species, treatment-related increases in benign tumours only), the requirements for Category 1B are not met.

Category 2 is appropriate where there is “limited evidence of carcinogenicity”, which is defined in the legal text as “*the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.”*

The results of the combined chronic toxicity and carcinogenicity study in Sprague Dawley rats could potentially support classification in Category 2. Additional considerations, as part of a weight of evidence approach, are detailed in Table 21.

Table 21. Further considerations for the assessment of carcinogenicity

Consideration	Agency comments
Tumour type and background incidence	Tumour types observed are relevant to humans – increases concern. Only benign tumours observed – reduces concern. No HCD available for the testing laboratory. HCD for both tumour types would assist the assessment. In the case of the liver tumours, which occurred at low incidence at the top dose, HCD would be particularly helpful in assessing whether the tumours are treatment-related or a spontaneous finding.
Multi-site responses	Yes – increases concern.
Progression of lesions to malignancy	No – only benign tumours reported. Reduces concern.
Reduced tumour latency	No effect on tumour latency reported. Reduces concern.
Whether responses are in a single sex or both sexes	Both sexes – increases concern.
Whether responses are in a single species or multiple species	Single species (although only one species was tested). Neither increases nor decreases concern.
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	The structurally similar substance EEA is not classified as a carcinogen according to ECHA's C&L inventory, although it isn't clear whether this is based on reliable test data or an absence of information. Neither increases nor decreases concern.
Routes of exposure	Available study was via oral gavage, which is relevant for humans. Increases concern.
Comparison of absorption, distribution, metabolism and excretion between test animals and humans	No human data available. Neither increases nor decreases concern.

Consideration	Agency comments
Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity	Available data suggest that EEA-NH4 is not genotoxic. Leydig cell tumours: no information on MoA available. Considered relevant for humans. Increases concern. Liver tumours: biologically plausible that the tumours are part of the continuum of effects of toxicity to the liver. Therefore, the liver tumours are considered to be relevant for humans. However, the tumours could be the result of excessive toxicity in the liver, which would lessen the relevance for classification.
The possibility of a confounding effect of excessive toxicity at test doses	The MTD is exceeded at the top dose in this study, based on the high levels of kidney toxicity and associated deaths. The Agency is also proposing STOT RE 2 for effects on the liver. The increased incidences of tumours were seen at the top dose only. Tumours seen at dose levels causing excessive toxicity are of questionable relevance for classification. Reduces concern.

Overall, the Agency considers this to be a borderline case between Category 2 and no classification. Treatment-related benign tumours (Leydig cell adenomas in males and hepatocellular adenomas in both sexes) were seen in a single study, at a dose level which caused excessive toxicity. At this stage, the Agency proposes classification in Category 2 but will consider the arguments further in the technical report, in light of any new information submitted during the public consultation.

10.11.3 Conclusion on classification and labelling for germ cell mutagenicity

The Agency proposes that EEA-NH4 should be classified as **Carc. 2; H352 (Suspected of causing cancer.**

10.12 Reproductive toxicity

The potential reproductive toxicity of EEA-NH4 has been investigated in two reproduction/ developmental toxicity screening studies in rats based on OECD TG 421 and according to GLP.

Table 22: Summary of animal studies for reproductive toxicity

Method, guideline, deviations if any, species, strain, sex, no/group **Test substance, dose levels, duration of exposure** **Reference**	**Results** *** Significantly different from vehicle control at P<0.05** **** Significantly different from vehicle control at P<0.01**
Reproductive/ developmental toxicity screening Study OECD TG 421 GLP compliant 12/sex/dose Crl:CD (SD) rats EEA-NH4 0, 5, 25 & 100 mg/kg bw/d Vehicle: sterile water Oral gavage Males received daily doses at least 14 days prior to mating, throughout the mating period and 1 day prior to sacrifice Females received daily doses at least 14 days prior to pairing, through lactation day 3 or 1 day prior to euthanasia if they had not mated or failed to deliver WIL Research Laboratories, 2011	**F0 parental** Mortality and clinical signs 100mg/kg bw/d: 2 females with total litter loss were euthanised on lactation day 1. No test substance-related clinical findings noted at any dosage level. Body Weights No changes to mean body weight or body weight gain and food consumption unaffected during premating and gestation at any dose level. During lactation, lower mean BW and BWG corresponded with reduced mean food consumption in 100mg/kg bw/d females. Reproductive Performance No statistically significant differences on fertility indices, male copulation index, female conception index, mean days between coitus and mating or gestation lengths compared to controls Gross Observations No test substance-related gross observations No change in the mean number of corpora lutea, although an increase in the mean number of unaccounted-for sites (1.9 sites) was noted in the 100 mg/kg bw/d group compared to controls (0.4 sites). (Maximum mean value is 1.4 sites in the WIL historical control data). Organ Weights Increased liver weights in males (absolute: ↑ 24%**; 47%**, relative: ↑ 24%**; 47%**) at 25 and 100 mg/kg/day, respectively Histopathology Exacerbation of vacuolation/hypertrophy of basophilic cells in the anterior pituitary gland in 1/12 (minimal), 5/12 (minimal), 5/12 (4 minimal, 1 mild), and 5/12 (3 minimal, 2 mild) males in the 0, 5, 25 & 100 mg/kg bw/d groups respectively. **F1 pups**

Method, guideline, deviations if any, species, strain, sex, no/group Test substance, dose levels, duration of exposure Reference	**Results** *** Significantly different from vehicle control at P<0.05** **** Significantly different from vehicle control at P<0.01**

Litter size/survival

Lower mean live litter size at 100 mg/kg bw/d (mean 10.3 pups born vs 14.4 in the control, not statistically significant)

Reduced postnatal survival (including total litter loss) from birth to PND 4 at 25 and 100 mg/kg bw/d were noted:

Parameter	**Dose Level (mg/kg bw/d)**			
	0	5	25	100
PND 0 (relative to number born)	99.3	98.1	97.6	76.4*
PND 0-1	100.0	96.8*	92.8*	58.0++*
PND 1-4	99.4	100.0	97.0	90.1*
Birth to PND 4	98.7	94.9	88.1*	38.7++*

PND = Postnatal day

++ = Significantly different from the concurrent control group at 0.01 using Dunn's test.

* = Outside the historical control data range.

Parameter	**WIL Historical Control Data** **Mean ± SD**
PND 0 (relative to number born)	98.3 ± 1.28
PND 0-1	98.9 ± 1.03
PND 1-4	98.5 ± 1.70
Birth to PND 4	95.8 ± 2.42

Method, guideline, deviations if any, species, strain, sex, no/group **Test substance, dose levels, duration of exposure** **Reference**	**Results** *** Significantly different from vehicle control at P<0.05** **** Significantly different from vehicle control at P<0.01**
	<u>Body Weights</u> Lower mean body weights (up to 10.8% and 27.7%) were noted in the 25 and 100 mg/kg bw/d pups, respectively during PND 1 and 4. <u>Gross Observations</u> Clinical findings in the pups included a body that was cool to the touch and small stature at 100 mg/kg bw/d <u>Histopathology</u> No test substance related findings.
Reproductive/developmental 90-day toxicity screening study Based on OECD TG 421 GLP compliant 10 females/dose Crl:CD (SD) rats EEA-NH4 0, 10, 30, 90 & 270 mg/kg bw/d Vehicle: Purified water Oral gavage Pregnant females received daily doses from gestation day (GD) 0 to lactation day 0 and for females that did not deliver, up to GD 25	**<u>F0 Parental</u>** <u>Mortality and clinical signs</u> 90 mg/kg group: decreased locomotor activity and hypothermia in 1 dam on GD 5 which later died on GD 6. 270 mg/kg group: decreased locomotor activity, hypothermia and bradypnea in 2 dams on GD 1 or 2 which both died on GD 2. A third animal showed these clinical signs on GD 21 and was euthanised because of marked exhaustion <u>Body weight and food consumption</u> Food consumption decreased in the 90 mg/kg group and above on GD 3 and 30 mg/kg and above on GD 6, with significant decrease in the 90 mg/kg (-9.3%**) and 270 mg/kg (-11.1%**) group Mean body weight was not impacted, but 2 individual animals (including the animal that died on GD 6) in the 90 mg/kg group had low body weight on GD 3 <u>Necropsy</u> Animals that died or were euthanised exhibited thickened limiting ridge of the stomach, small spleen and thymus <u>Reproductive effects</u> No changes in number of corpora lutea or implantations, implantation index or gestation period Gestation index was significantly reduced in the 90 mg/kg group (-32%**) and the 270 mg/kg group (-100%**)

Method, guideline, deviations if any, species, strain, sex, no/group Test substance, dose levels, duration of exposure Reference	Results * Significantly different from vehicle control at P<0.05 ** Significantly different from vehicle control at P<0.01
	Delivery was not observed in one litter however only implantation sites were observed in this dam. **F1 pups** Developmental effects **30 mg/kg bw/d**: The viability indexes of offspring were low on PND 1 (89.06%), 4 (89.06%) and 6 (89.06%). **90 mg/kg bw/d**: On PND 0 all pups were dead upon delivery in 1 litter. Gestation index was low (77.8%) Birth index was statistically significantly low (44.66%**) Viability index was statistically significantly low on PNDs 0 (57.76%**), 1 (81.04%*), 4 (81.04%*) and 6 (79.86%*). **270 mg/kg bw/d:** On PND 0 all pups were dead upon delivery in all of the litters (7) A large proportion of pup deaths occurred on PND 0 or 1 and only 1 pup in the 90 mg/kg group died on PND 2 Clinical observations On PND 0-2 dark purple discolouration was noted in the lumber, hindlimbs, or tail of 1 and 3 pups in the 30 and 90 mg/kg groups respectively, reducing to 1 and 2 pups after PND 2 Pup body weight Body weight was statistically significantly reduced in pups on PND 0 and 4 in the 30 (M –12%** & -14%* F –11%** & -14%*) and 90 mg/kg (M –20%** & -20%** F –20%** & -20%**) groups. Body weight in male and female offspring in the 90 mg/kg group tended to be low on PND 6. Necropsy Necropsy of dead pups on PNDs 0-6 revealed black discolouration of the digit of the hindlimb or the tail in 1 and 2 pups in the 30 and 90 mg/kg groups, respectively. Cleft palate was also reported in 1 pup in the 270 mg/kg group, but this was considered a spontaneous finding by the study authors.

10.12.1 Short summary and overall relevance of the available information for the assessment of adverse effects on sexual function and fertility and adverse effects on development

The available studies used to assess adverse effects on sexual function and fertility are summarised in Table 21.

Reproductive/developmental toxicity screening study (WIL Research Laboratories, 2011)

In this GLP-compliant study conducted according to OECD TG 421, 12 Crl:CD (Sprague-Dawley) rats/sex/group were administered EEA-NH4 via oral gavage. The dose levels of 0, 5, 25 & 100 mg/kg bw/d were selected based on the results of a previous 28-day RDT (Shiraishi, 2006). Males were dosed at least 14 days prior to mating and through to 1 day before sacrifice, whereas females were dosed 14 days prior to pairing and through to lactation day 3 or 1 day before sacrifice, if there was no evidence of mating or a failure to deliver. All animal observations were conducted according to the test guideline. Only selected tissues were examined microscopically from all F0 animals in the control and high dose group.

Parental Toxicity

There were no test substance-related clinical findings noted at any dosage level. Clinical observations noted in the treated groups, including red material around the nose and/or mouth and hair loss on various body surfaces, occurred infrequently, at a similar frequency in the control group, and/or in a manner that was not dose-related. Mean body weights, body weight gains, and food consumption were unaffected by test substance administration during the entire treatment period for the 5, 25, and 100 mg/kg bw/d group males and during the pre-mating and gestation periods for the 5, 25, and 100 mg/kg bw/d group females. During lactation, lower mean body weights and body weight gains with corresponding reduced mean food consumption were noted in the 100 mg/kg bw/d group females. Mean lactation body weights, body weight gains, and food consumption in the 5 and 25 mg/kg bw/d group females were similar to that of the control group.

There were no test substance-related gross observations. However, treatment-related increases in liver weight were reported in males at 25 and 100 mg/kg bw/day. Exacerbation of vacuolation/hypertrophy of basophilic cells in the anterior pituitary gland in males (all doses) was also reported which varied between mild to moderate severity.

Effects on sexual function and fertility

There were no test substance-related effects on male and female mating and fertility indices, male copulation index, or female conception index. The mean number of days between pairing and coitus and mean gestation durations in the 5, 25, and 100 mg/kg bw/d groups were similar to those in the control group. The mean numbers of corpora lutea and implantation sites were unaffected by test substance administration. However, a higher mean number of unaccounted-for sites was noted in the 100 mg/kg bw/d group compared with the control group (1.9 unaccounted for sites were noted in the 100 mg/kg/day group

compared with the control group (0.4 sites) and the maximum mean value (1.4 sites) in the WIL historical control data).

Developmental effects/offspring toxicity

Mean live litter size in the 100 mg/kg bw/day group (10.3 pups born) was lower than the control group (14.4 pups born). The decrease compared to controls was not statistically significant, however it fell below the minimum mean value in the WIL historical control data (11.6 pups born), and this finding was considered to be test substance-related. Mean live litter size in the 5 and 25 mg/kg bw/day groups was similar to the control group values and the mean numbers of pups born and percentage of males at birth at all dosage levels were unaffected by F0 maternal test substance administration.

Table 23: Summary of mean live litter size

Dosage Level (mg/kg/day)	**0**	**5**	**25**	**100**
Mean Live Litter Size (PND 0)	14.4	13	13.5	10.3

Reduced postnatal survival was noted in the 100 mg/kg bw/d group on PND 0 (relative to the number born), 0-1, 1-4, and from birth to PND 4; the differences were statistically significant ($p < 0.01$) during PND 0-1 and from birth to PND 4. This reduction was considered to be related to administration of the test substance by the study authors. Two females in the 100 mg/kg bw/d group had total litter loss on lactation day 1. In the 25 mg/kg bw/d group, there was an overall reduction in survival from birth to PND 4, primarily resulting from a decrease in survival from PND 0-1, compared to the concurrent control group and the mean values in the WIL historical control data; these reductions were considered to be test substance-related.

Table 24: Postnatal Survival (% per litter). Taken from the study report.

	Dosage Level (mg/kg/day)				WIL HC[a]
Parameter	0	5	25	100	Mean ± SD
PND 0 (relative to number born)	99.3	98.1	97.6	76.4	98.3 ± 1.28
PND 0-1	100.0	96.8	92.8	58.0++	98.9 ± 1.03
PND 1-4	99.4	100.0	97.0	90.1	98.5 ± 1.70
Birth to PND 4	98.7	94.9	88.1	38.7++	95.8 ± 2.42

[a] = WIL historical control data
SD = Standard deviation
PND = Postnatal day
++ = Significantly different from the concurrent control group at 0.01 using Dunn's test.

An increased number of pups were found dead or missing in the 25 and 100 mg/kg/day groups compared to the control group, which correlated to the reduced postnatal survival. The numbers of pups (litters) found dead in the control, 5, 25, and 100 mg/kg bw/day groups were 2(2), 7(6), 8(6), and 74(11), respectively. In addition, 0(0), 2(2), 11(5), and 21(8) pups (litters) in the control, 5, 25, and 100 mg/kg bw/day group were missing and presumed to have been cannibalized. Test substance-related clinical findings for the pups

included a body that was cool to the touch and small stature in the 100 mg/kg bw/day group. There were no other clinical findings attributed to F0 maternal test substance administration by the study authors.

Treatment-related lower mean pup body weights (both sexes) were noted at 25 and 100 mg/kg bw/day on PND 1 compared to the concurrent control group. However, mean body weight gains in these groups were similar to the control group during PND 1-4. As a result, mean pup body weights in the 25 and 100 mg/kg bw/day groups were also lower than the concurrent control group on PND 4.

Table 25: Pup body weights and body weight changes. Taken from the study report.

	Dosage Level (mg/kg/day)				WIL HC[a]
Parameter	0	5	25	100	Mean (Range)
Body Weights [g]					
PND 1 (males)	6.9	7.0 (1.4%)	6.2 (-10.1%)	5.0 (-27.5%)**	7.1 (6.6-7.6)
PND 1 (females)	6.5	6.7 (3.1%)	5.8 (-10.8%)	4.7 (-27.7%)**	6.7(6.1-7.1)
PND 4 (males)	9.2	9.8 (6.5%)	8.5 (-7.6%)	7.5 (-18.5%)*	9.9 (8.7-11.0)
PND 4 (females)	8.7	9.5 (9.2%)	7.9 (-9.2%)	7.1 (-18.4%)*	9.4 (8.1-10.4)
Body Weight Changes [g]					
PND 1-4 (males)	2.3	2.9	2.3	2.5	-
PND 1-4 (females)	2.2	2.8	2.1	2.3	-

[a] = WIL historical control data
PND = Postnatal day
* = Significantly different from the concurrent control group at 0.05 using Dunnett's test.
** = Significantly different from the concurrent control group at 0.01 using Dunnett's test.

Under the conditions of this screening study, no adverse effects on reproduction were observed at doses < 25 mg/kg bw/d. Neonatal toxicity was observed in the 25 and 100 mg/kg bw/d groups. A higher mean number of unaccounted-for sites was noted in the 100 mg/kg bw/d group compared to the control group as well as reduced live litter size in the 100 mg/kg bw/d group and lower postnatal survival (including total litter loss) during birth to PND 4 in the 25 and 100 mg/kg bw/d groups were noted; the difference in postnatal survival was statistically significant in the 100 mg/kg bw/d group. Test substance-related clinical findings noted for the 100 mg/kg bw/d group pups included a body that was cool to the touch and small stature. In addition, reduced mean pup body weights (up to 10.8% and 27.7%) were noted in the 25 and 100 mg/kg bw/d groups, respectively, during PND 1 and 4; the differences were statistically significant for the 100 mg/kg bw/d male and female pups. These findings were reported in the absence of severe maternal toxicity at all dose levels.

Reproductive/ developmental toxicity screening study (Safety Research Institute for Chemical Compounds Co., Ltd, 2014)

The Agency notes that the study report indicates that this GLP study was performed based on OECD TG 421, however EEA-NH4 was orally administered via gavage at 0, 10, 30, 90 and 270 mg/kg bw/d to female Crl:CD(SD) rats showing evidence of copulation (10 females/group) during gestation period to lactation day 0 and for females that did not deliver, up to GD 25. OECD TG 421 states that dosing of both sexes should begin at least 2 weeks prior to mating, dosing should then continue in both sexes during the mating period and males should be further dosed after the mating period at least until the

minimum total dosing period of 28 days. Dosing of parental females should continue throughout pregnancy and at least up to, and including, day 13 post-partum or the day before sacrifice. The Agency considers that this study still provides information on the potential effects of EEA-NH4 on maternal rats and on development of embryos and foetuses as well as growth of the offspring.

Parental Toxicity

Mortality and Clinical Signs

One dam in the 90mg/kg bw/d group showed a decrease in locomotor toxicity and hypothermia on GD 5 and later died on GD 6. Two dams in the 270 mg/kg bw/d group also showed a decrease in locomotor toxicity, hypothermia and bradypnea on GD 1 or 2; both died on GD 2. One further dam at this dose showed a decrease in locomotor toxicity, hypothermia and bradypnea on GD 21 and was euphonised due to exhaustion.

Body Weight and Food Consumption

No statistically significant differences in mean body weight gain or body weight were reported during the gestation period between any dose group and the control group. However, 2 individual dams in the 90 mg/kg bw/d group showed body weight decreases of -38g and -17g at GD 3 respectively, the Agency notes that the dam with the 38g decrease was the dam at this dose that died on GD6.

Food consumption was statistically significantly low on GD 9 in the 10 mg/kg bw/d group and on GD 6-15 in the 30 mg/kg bw/d group. Food consumption was also statistically significantly low in the 90 and 270 mg/kg bw/d groups on GD6. In the two dams with decreased body weight in the 90 mg/kg bw/d group food consumption was markedly decreased on GD 3. During lactation the period food consumption was statistically significantly low on PND 4-6 in the 90 mg/kg bw/d group. The Agency notes that food consumption in nursing rats increases depending on the litter size, and therefore this low food consumption may be related to the low number of offspring in this group.

Necropsy

Necropsy reported findings of thickening of the limiting ridge of the stomach in all dams at 270 mg/kg bw/d except the two that died on GD 2. Small size spleen or small size thymus were reported in the dams that died or were euthanised due to exhaustion in the 90 and 270 mg/kg bw/d groups, however no organic changes that the study authors considered to be likely the cause of death were reported.

Effects on sexual function and fertility

There were no changes noted in the number of corpora lutea, implantations or implantation index and no effects were observed on preimplantation loss of parental females. The length of gestation period was unaffected, and no abnormalities such as prolonged parturition or dystocia were noted.

Developmental effects/offspring toxicity

The gestation index was statistically significantly reduced in the 90 and 270 mg/kg bw/d groups. At the top dose, none of the 7 pregnant dams delivered live pups. On an individual animal level, there were only implantation sites in the uterus of one animal where delivery was not observed in the 90 mg/kg bw/d group.

The birth index, number of offspring, number of live offspring and viability index were also reduced compared with the controls at 90 mg/kg bw/d. On PND 1, 4 and 6 the viability indexes tended to be low in the 30 and 90 mg/kg groups.

Table 26: Summary of the delivery data for the F0 generation. Taken from the study report.

Delivery data
Generation : F0
Species : Rat

Test article Dose		Gestation period (day)	Number of implantation	Birth index (%)	Number of offspring	Number of live newborns M	Number of live newborns F	Number of live newborns Total	Sex rate (offspring) (%)	Number of dead newborns Dead	Number of dead newborns Cannibalism	Number of dead newborns Total	Gestation index (%)
EEA-NH4	n	10	10	10	10	10	10	10	82/140	10	10	10	10/10
0 mg/kg	Mean	22.00	15.2	92.42	14.1	8.2	5.8	14.0	58.6	0.1	0.0	0.1	100.0
	S.D.	0.00	3.3	5.55	3.2	3.0	2.4	3.0		0.3	0.0	0.3	0.00
EEA-NH4	n	10	10	10	10	10	10	10	70/141	10	10	10	10/10
10 mg/kg	Mean	22.00	14.6	96.67	14.1	7.0	7.1	14.1	49.6	0.0	0.0	0.0	100.0
	S.D.	0.00	1.4	3.52	1.4	2.0	2.6	1.4		0.0	0.0	0.0	0.00
		EF											
EEA-NH4	n	10	10	10	10	10	10	10	64/138	10	10	10	10/10
30 mg/kg	Mean	22.00	15.0	92.96	14.4	6.4	7.4	13.8	46.4	0.6	0.0	0.6	100.0
	S.D.	0.00	2.6	9.16	2.3	2.5	2.0	1.9		1.6	0.0	1.6	0.00
		EF											
EEA-NH4	n	8	9	9	9	8	8	8	33/59	8	8	8	7/9
90 mg/kg	Mean	22.00	13.8	44.66	11.1	4.1	3.3	7.4	55.9	5.0	0.1	5.1	77.8
	S.D.	0.00	4.7	38.47	4.8	3.1	2.6	5.2		5.3	0.4	5.2	0.00
		EF		ST **				ST *		ST *		ST **	
EEA-NH4	n	7	7	7	7	7	7	7	0	7	7	7	0/8
270 mg/kg	Mean	22.00	14.9	0.00	6.7	0.0	0.0	0.0		5.4	1.3	6.7	0.0
	S.D.	0.00	1.7	0.00	4.3	0.0	0.0	0.0		4.6	1.8	4.3	
		EF		ST **	ST *	ST **	ST **	ST **	ED	ST **	ST *	ST **	FT $$

M : Male, F : Female
Significantly different from EEA-NH4 0 mg/kg: $$ P<0.01
Significantly different from EEA-NH4 0 mg/kg: * P<0.05, ** P<0.01
FT : Fisher's exact test (two-side), ST : Steel test (two-side)
ED : The number of samples of a treated group is less than 2.
EF : The averages of all groups are same and all data is 0 in frequency. (all data of all groups is identical.)

The low viability index of the offspring was also detected in the older reproduction/ developmental toxicity screening study at 25 and 100 mg/kg bw/d where EEA-NH4 was administered from pre-mating throughout gestation and lactation. This study highlighted that administration during only gestation induced similar effects.

No changes were noted in the body weight of parental animals on LD 0, no abnormalities were noted in nursing behaviour and body weight of the offspring was statistically significantly low on PND0 in the 30 and 90 mg/kg bw/d groups.

Clinical observation/necropsy of the offspring revealed dark discolouration of the hindlimb or the tail on and after PND 2 or 3 in the 30 and 90 mg/kg bw/d groups. The study authors considered that this suggests a possibility of necrosis of the terminal tissue attributable to hematogenous disorder possibly contributing to the deaths of the offspring.

10.12.2 Comparison with the GB CLP criteria

EEA-NH4 has been tested two reproductive/developmental toxicity screening studies. The Agency notes that the dosing schedule in the second of these studies performed by Safety Research Institute for Chemical Compounds Co., Ltd (2014) (during gestation period to lactation day 0 and for females that did not deliver, up to GD 25) provides less information compared to the dosing schedule in the study performed by WIL Research

Laboratories (2011), (animals dosed at least 14 days prior to mating) on the possible effects of EEA-NH4 on sexual function and fertility. However, the 2014 study does still provide information on the effects of EEA-NH4 on maternal rats and on development of embryos and foetuses as well as growth of the offspring.

Sexual Function and Fertility

In the WIL Research Laboratories (2011) study no effects on sexual function and fertility were reported at any tested dose, there were no test substance-related effects on F0 male or female reproductive performance, or on mean gestation length, parturition, implantation sites, and corpora lutea. The Agency notes the lack of any significant maternal toxicity reported in this study up to the highest tested dose of 100 mg/kg bw/day.

In the Safety Research Institute for Chemical Compounds Co. Ltd. (2014) study, no adverse effects on sexual function and fertility were reported, no changes were noted in the number of corpora lutea, implantations, implantation index and no effects were observed in the preimplantation loss of parental females. Further, no changes were observed in the length of the gestation period with no abnormalities such as prolonged parturition or dystocia noted up to the highest tested dose of 270 mg/kg bw/d.

On the basis that there is no evidence in the available studies that EEA-NH4 causes any adverse effects to sexual function or fertility, classification for this end point is not warranted.

Developmental toxicity

In the WIL Research Laboratories (2011) study, developmental toxicity was reported in the 25 and 100 mg/kg bw/d groups. A higher mean number of unaccounted-for sites was noted in the 100 mg/kg bw/d group compared to the control group. Reduced live litter size in the 100 mg/kg bw/day group and lower postnatal survival (including total litter loss) from birth to PND 4 in the 25 and 100 mg/kg bw/d groups were noted; the difference in postnatal survival was significant in the 100 mg/kg bw/d group. Test substance-related clinical findings noted for the 100 mg/kg bw/d group pups included a body that was cool to the touch and small stature. In addition, reduced mean pup body weights (up to 10.8% and 27.7%) were noted in the 25 and 100 mg/kg bw/day groups, respectively, during PND 1 and 4; the differences were significant for the 100 mg/kg bw/day male and female pups. The adverse effects on development reported in this study occurred in the absence of severe maternal toxicity.

In the Safety Research Institute for Chemical Compounds Co., Ltd, 2014 study, developmental toxicity was reported from 30 mg/kg bw/d up to the top dose of 270 mg/kg bw/d. In the 90 mg/kg bw/d group delivery was not observed in one litter, and only implantation sites were observed in the uterus of the relevant dam. Furthermore, in another dam in the 90 mg/kg bw/d dose group all of the offspring were delivered dead and all of the pups that were delivered in the 270 mg/kg bw/d group were also dead. The gestation index was statistically significantly low or tended to be low in the $\geq$90 mg/kg bw/d dose groups; this was in addition to birth index, number of offspring, number of live offspring and viability of offspring that were statistically significantly low or tended to be low on PND 0. The number of dead pups on PND 0 was statistically significantly

high at ≥90 mg/kg bw/d. The viability indexes of offspring in the 30 and 90 mg/kg bw/d groups were low, or tended to be low on PND 1, 4 and 6 however the study authors noted that deaths occurred in few offspring on, and after PND 2, therefore they considered that the mortality seen from implantation to PND 1 was due to an effect of EEA-NH4 administration. Maternal toxicity in this study included marked decreases in food consumption and body weight gain on GD3 in two animals in the 90 mg/kg bw/d group, with one of these animals also showing a decrease in locomotor activity and hypothermia on GD5. This animal died on GD 6. In the 270 mg/kg bw/d group similar clinical signs were noted and mortality was reported on GD 2 in 2 animals and in another animal on GD 21 which was euphonised due to exhaustion. The Agency notes that severe maternal toxicity appears to be limited to these individual animals, while no changes were noted in the group mean body weights. Necropsy of parental females revealed thickening of the limiting ridge of the stomach in the 270 mg/kg bw/d group and small sized spleen or thymus in animals that died or were euthanised due to exhaustion in the 90 and 270 mg/kg bw/d groups.

EEA-NH4 is not a known human developmental toxicant; therefore, classification in Category 1A is not necessary.

For classification in Category 1B, there must be clear evidence from animal studies of an adverse effect on development occurring in the absence of other toxic effects. The Agency notes clear evidence of an adverse effect on development reported in two separate reproductive/developmental toxicity screening studies. Effects including higher mean number of unaccounted-for sites, reduced live litter size, lower postnatal survival (including total litter loss) were reported in In the WIL Research Laboratories (2011) study in the absence of maternal toxicity at any dose level. This is supported by the findings from the Safety Research Institute for Chemical Compounds Co., Ltd, (2014) study which reported a reduction in gestation index, birth index, number of offspring, number of live offspring and viability of offspring in the ≥90 mg/kg dose groups.

The Agency does note the maternal toxicity reported in individual animals in the 90 and 270 mg/kg bw/d groups in the 2014 study but considering Annex I: 3.7.2.4.2 of the guidance on the application of the CLP criteria, Part 3 Version 5.0 which states '*Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies*.' The developmental effects reported in this study are still considered as supporting evidence relevant to the classification of EEA-NH4 for developmental toxicity.

Overall, based on the developmental effects seen in two separate screening studies, the Agency considers that EEA-NH4 warrants classification as **Repr. 1B; H360D (May cause damage to the unborn child).**

Lactation

The two reproduction/developmental toxicity screening studies used in the sexual function and fertility and development sections are the only studies available to assess any adverse effects on or via lactation, please see the previous sections and table 22 for details.

There is no convincing evidence from humans or animals to suggest that EEA-NH4 has an adverse effect on lactation or via lactation. The results from absorption, distribution, metabolism and excretion studies do not indicate a likelihood of EEA-NH4 accumulating to potentially toxic levels in breastmilk. Therefore, the Agency considers that classification for this endpoint is not warranted, based on the available data.

10.12.3 Conclusion on classification for reproductive toxicity

EEA-NH4 warrants classification as **Repr. 1B; H360D (May cause damage to the unborn child).**

10.13 Aspiration hazard

Not assessed.

11 Evaluation of environmental hazards

Not assessed.

12 Evaluation of additional hazards

Not assessed.

13 Additional labelling

Not applicable.

14 References

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15 Annexes

Not applicable.

Further information

For information about health and safety, or to report inconsistencies or inaccuracies in this guidance, visit the Agency website.

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