Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

2-methoxyethyl acrylate

EC Number: 221-499-3

CAS Number: 3121-61-7

Index Number: -

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1 PHYSICAL HAZARDS

1.1 Explosives

Statement based on the chemical structure of the substance.

1.2 Flammable gases (including chemically unstable gases)

Not relevant.

1.3 Oxidising gases

Not relevant

1.4 Gases under pressure

Not relevant.

1.5 Flammable liquid

Study reference:

Tremain, S.P., 2012, 2-methoxyethyl acrylate: Determination of Flash Point and Auto-Ignition Temperature (Liquids and Gases), Report 41202568

Test type

EU method A.9 – Flash Point (closed-cup method)

GLP-Guideline study

Test material

- 2-MEA
- Degree of purity: 99.9%
- Batch number: C220424SP6

Study summary and results:

An aliquot (approximately 2 g for temperatures up to 100 °C and approximately 4 g for temperatures above 100 °C) of the test item was transferred at the set temperature. The test flame was introduced into the sample cup for approximately 2.5 seconds by sliding the cup shutter open. Observations were made for ignition of the vapor. If no ignition occurred, the temperature was increased and the test flame re-introduced. This was repeated until the lowest reproducible temperature at which a flash occurred, using a fresh sample, was determined.

The flash point was corrected for the atmospheric pressure using the following equation:

Corrected flash point = C + 0.23 (101.325 - p)

where:

C = observed flash point (°C)

p = ambient atmospheric pressure (kPa)

Results

59°C ± 2°C

1.6 Flammable solids

Not relevant.

1.7 Self-reactive substances

Statement based on physical state of the substance.

1.8 Pyrophoric liquids

Statement based on physical state of the substance.

1.9 Self-heating substances

Statement based on physical state of the substance.

1.10 Substances which in contact with water emit flammable gases

Statement based on experience in handling and use and on the chemical structure of the substance.

1.11 Oxidising liquids

Statement on the chemical structure of the substance.

1.12 Corrosive to metals

Study reference:

Shimbori, K., 2012, Report MUN127/12(A)

Test type

Based on test method of Part III sub-section 37.4 of the UN RTDG, Manual of Tests and Criteria

Test material

- 2-MEA

- Degree of purity: ≥99.9%

Study summary and results:

The aluminium and steel test pieces are immersed in a liquid substance at 55 °C for 7 days. Corrosion rate is calculated by loss of weight of test piece after testing. Classification of the substance is judged on whether corrosive rate exceed 6.25 mm a year or not.

Test Piece:
- steel type, S235JR+CR(1.0037 resp. St 37-2)
- aluminium, non-clad, types 7075-T6

Results

Corrosion rate:
- Aluminium Test Piece: Max 0.06 mm/year
- Steel Test Piece: Max 0.03 mm/year

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No experimental data.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

3.1.1.1 Acute oral toxicity - Study 1 (1968)

Study reference: *Union Carbide Corporation, 1968*

Test type

Equivalent to OECD guideline 401

Non GLP

Deviations: No details on analytical purity of the test substance; limited details on test animals and environmental conditions; prior to GLP

Test substance

- 2-MEA
- Degree of purity: no data
- Batch number: no data

Test animals

- Albino Wistar rats
- 5 males/ group
- Age and weight at the study initiation: 90-120g, no data on age

Administration/exposure

- Duration of test/exposure period: single dose by oral gavage
- Doses: 0.5 mL/kg bw corresponding to 505 mg/kg bw, 1.0 mL/kg bw corresponding to 1010 mg/kg bw, 2.0 mL/kg bw corresponding to 2020 mg/kg bw; based on a test substance density of 1.01 g/cm³. Dose levels were chosen based on previous data and/or a limit test, and successive dose levels were tested until levels were found in which, within 14 days, all, some, or no animals died.
- No vehicle (undiluted test material)
- 14-day observation period following administration
- Clinical observations and mortalities were recorded.

Statistics:

- The LD_{50} value was calculated by the probit method.

Results and discussion

Mortality:

505 mg/kg bw: no animals died

1010 mg/kg bw: 4 males died

2020 mg/kg bw: all males died

Clinical signs:

Animals treated with 505 mg/kg bw showed sluggish behaviour. No clinical signs were observed in the other test groups.

Body weight:

Weight gain was observed in all surviving animals treated with 505 and 1010 mg/kg bw.

Gross pathology:

At gross pathology, congestion was observed in the lungs and the abdominal viscera.

Table 1: Acute oral toxicity

Dose [mg/kg bw]	Toxicological results*	Duration of clinical signs	Time of death	Mortality (%)
Males				
505	0/0/5	---	---	0
1010	4/0/5	---	Day 1	80
2020	5/5/5	---	Day 1	100
LD_{50} = 818 mg/kg bw				

*First number = number of dead animals
Second number = number of animals with clinical signs
Third number = number of animals used

Table 2: Body weight changes

Dose [mg/kg bw]	Weight Change*
505	+++++
1010	+
2020	N/A

*all surviving animals
+ = animals with weight gain
- = animals with weight loss

3.1.1.2 Acute oral toxicity - Study 2 (1980)

Study reference: *Rhône-Poulenc Inc., 1980c*

Test type

Equivalent to OECD guideline 401

Non GLP

Deviations: only dead animals were necropsied, no histopathology, prior to GLP.

Test substance

- 2-MEA
- Degree of purity: no data
- Batch number: no data

Test animals

- Sprague-Dawley
- Mae/female
- 5/sex/dose
- Age and weight at the study initiation: 200-300g, no data on age

Administration/exposure

- Duration of test/exposure period: single dose by oral gavage
- Doses:

  0.25 mL/kg bw corresponding to 252.5 mg/kg bw
  0.35 mL/kg bw corresponding to 353.5 mg/kg bw
  0.50 mL/kg bw corresponding to 505.0 mg/kg bw
  0.55 mL/kg bw corresponding to 555.5 mg/kg bw
  0.60 mL/kg bw corresponding to 606.0 mg/kg bw

  Based on the density of 2-MEA: 1.01 g/cm³

- Vehicle : water
- 14-days observation period following administration
- Clinical observations and mortalities were recorded.

Statistics: The defined oral LD_{50} was calculated by the Litchfield-Wilcoxin method of Probit Analysis (J. Pharmacology and Experimental Therapeutics 96: 99-115, 1949).

Results and discussion

Mortality:

252.5 mg/kg bw: no animals died
353.5 mg/kg bw: 2 males and 2 females died
505.0 mg/kg bw: 2 males and 3 females died
555.5 mg/kg bw: 4 males and 4 females died
606.0 mg/kg bw: 5 males and 5 females died

Clinical signs: No clinical signs were noted.

Body weight:

252.5 mg/kg bw: bw gains were within normal ranges.
353.5 mg/kg bw onwards: decreased bw gain was observed in males and females at study termination.

Gross pathology: Necropsy revealed pulmonary haemorrhages (only dead animals were necropsied).
Incidences:
353.5 mg/kg bw: 1 female
505.0 mg/kg bw: 1 male
555.5 mg/kg bw: 4 males, 2 females
606.0 mg/kg bw: 2 males, 1 females

Table 3: Acute oral toxicity

Dose [mg/kg bw]	Toxicological results*	Duration of clinical signs	Time of death	Mortality (%)
Males				
252.5	0/0/0	---	---	0
353.5	2/0/5	---	Day 2	40
505.0	2/0/5	---	Day 3 - Day 4	40
555.5	4/0/5	---	Day 2	80
606.0	5/0/5	---	Day 2	100
Females				
252.5	0/0/0	---	---	0
353.5	2/0/5	---	Day 3	40
505.0	3/0/5	---	Day 2 - Day 3	60
555.5	4/0/5	---	Day 2 - Day 3	80
606.0	5/0/5	---	Day 2- Day 4	100
LD_{50} = 404 mg/kg bw				

* First number = number of dead animals; second number = number of animals with clinical signs; third number = number of animals used

3.2 Acute toxicity - dermal route

Not evaluated.

3.3 Acute toxicity - inhalation route

Study reference: *Union Carbide corporation, 1968*

Test type

Equivalent to OECD guideline 403 (Acute inhalation toxicity)

Non GLP

Deviations: prior to GLP and OECD guideline, no details on analytical purity of the test substance; limited details on inhalation exposure as well as on test animals and environmental conditions.

Test substance

- 2-MEA
- Degree of purity: no data
- Batch number: no data

Test animals

- Wistar Rats
- 6 animals/dose, males
- Age and weight at the study initiation: not specified

Administration/exposure

- 4h whole body inhalation exposure
- Inhalation: vapour
- Vehicle: air
- Concentrations:
- pre-study:
  1 h: 1576 ppm corresponding to 8.5 mg/L
  30 min: 1810 ppm corresponding to 9.8 mg/L
  15 min: 1966 ppm corresponding to 10.6 mg/L

  main study:
  4 h: 1000 ppm corresponding to 5.4 mg/L
  4 h: 500 ppm corresponding to 2.7 mg/L
  4 h: 250 ppm corresponding to 1.4 mg/L

  Dose calculation was based on a molecular weight of 130.14 g/mol and a molar volume of 24.1 L/mol (at 20 °C).

Results and discussion

Mortality:

Pre-study:
1 h (8.5 mg/L): all animals died

30 min (9.8 mg/L): 1 animal died
15 min (10.6 mg/L): no animals died

main study:
4 h (5.4 mg/L): all animals died
4 h (2.7 mg/L): 3 animals died
4 h (1.4 mg/L): no animal died

Clinical signs:

Within 30 min exposure to 9.8 mg/L of the test substance, animals showed laboured breathing at 10 min. Animals exposed to 8.5 and 10.6 mg/L did not show clinical signs of toxicity during the 1 h or 15 min exposure period, respectively. After 4 h exposure to 1.4 and 2.7 mg/L, swollen abdomens were recognised in the animals. In addition, laboured breathing was observed after exposure to 1.4 mg/L for 4 h. Within the 4-h inhalation period, gasping was observed in animals exposed to 5.4 mg/L at 2 h.

Body weight:

All surviving animals showed a weight gain after exposure to the test substance at different concentrations.

Gross pathology:

After exposure to the test substance for 30 min and 1 h, rats dying prior to study termination had bright red livers and gas-filled intestinal tract. Rats surviving to study termination after 15 and 30 min exposure had no remarkable findings.
After 4-h exposure, rats dying prior to scheduled termination had slight hemorrhage of lungs and blood in intestines. For rats surviving until scheduled termination, 2 of 3 rats at 2.7 mg/L had areas of focal consolidation scattered throughout the lungs. All others showed nothing remarkable.

Other observations:
1 h (8.5 mg/L): irritation of eyes at 10 min; irritation of extremities at 35 min
30 min (9.8 mg/L): irritation of eyes at 5 min; irritation of extremities at 25 min
15 min (10.6 mg/L): irritation of eyes at 5 min; irritation of nose at 10 min

4 h (5.4 mg/L): irritation of eyes at 20 min; irritation of extremities at 25 min
4 h (2.7 mg/L): irritation of eyes at 5 min; irritation of extremities at 1-1/2 h
4 h (1.4 mg/L): irritation of eyes; irritation of extremities at 2-1/2 h

Exposure to 1.4 mg/L or approximately 10% of saturated vapour for a few minutes may cause ocular irritation. A longer exposure to this vapour concentration may cause skin irritation. Experimentation with rats indicates that prolonged exposure to this vapour could lead to irritation of the mucous membranes and skin and pulmonary injury.

3.4 Skin corrosion/irritation

3.4.1 Animal data

3.4.1.1 Skin irritation - Study 1 (1968)

Study reference: *Union Carbide corporation, 1968*

Detailed study summary and results:

Test type

Equivalent to OECD guideline 404

Non GLP

Deviations: substance was tested under open conditions; prolonged exposure period was used; scoring of skin reactions according to Draize was not performed

Test substance

- 2-MEA
- Degree of purity: no data
- Batch number: sample no 31-126

Test animals

- Rabbit, strain not specified
- 5 animal, sex not specified
- Age and weight at the study initiation: not specified

Administration/exposure

- Duration of test/exposure period: 24h
- Total dose: 0.01 mL applied on abdomen (open coverage)
- Post exposure observation period: not specified
- No vehicle
- Observation immediately after treatment.

Results and discussion

Immediately after exposure to the test substance, very slight to slight irritation was observed in 1/5 and 4/5 animals, respectively (no further details).

3.4.1.2 Skin irritation - Study 2 (1980a)

Study reference:

Rhône Poulenc Inc. (1980a). DOT Skin Corrosion. Report date: 1980-08-04

Detailed study summary and results:

Test type

Equivalent to OECD guideline 404

Other guideline: 16 CFR 1500.41 Method of testing primary irritant substances

Non GLP

Deviations: no single animal scores were documented; only two reading time points (24 h and 72 h); the study was terminated after 72 h; occlusive test conditions and prolonged exposure period (24 h).

Test substance

- 2-MEA
- Degree of purity: 99%
- Batch number: E169H8

Test animals

- New Zealand albino Rabbit
- 6 animals, sex not specified
- Age and weight at the study initiation: not specified

Administration/exposure

- Duration of test/exposure period: 24h
- Total dose: 0.5 mL applied on shaved and abraded skin,
- occlusive dressing
- Area of exposure: 2.5 cm^2. The treated skin was covered with a gauze patch, held in place with adhesive tape. The entire trunk was wrapped with a rubberised elastic cloth.
- No post exposure observation period
- No vehicle
- Observation immediately after treatment.
- Washing was performed after the end of exposure

Results and discussion

Irritation results on the abraded skin were similar compared to the intact skin.

Table 4: Results of skin irritation

Observation time	Mean out of all 6 rabbits	
	Erythema	Edema
24 h	3.0	3.00
48 h	No experimental data available	
72 h	3.17	2.50

3.4.1.3 Skin irritation - Study 3 (1980b)

Study reference:

Rhône Poulenc Inc. (1980b). Primary Skin Irritation. Report date: 1980-08-04.

Detailed study summary and results:

Test type

Equivalent to OECD guideline 404; similar to 49CFR 173.1200 Method testing corrosion to skin

Non GLP

Deviations: Basic data given. An application volume of 1 mL was used instead of 0.5 mL. Only 4 and 48 h readings were performed; the study was terminated after 48 h. No erythema or edema scores were given.

Test substance

- 2-MEA
- Degree of purity: 99%
- Batch number: E169H8

Test animals

- New Zealand white Rabbit
- 6 animals, sex not specified
- Age and weight at the study initiation not specified

Administration/exposure

- Duration of test/exposure period: 4h
- Total dose: 1 mL applied
- No post exposure observation period
- Area of exposure: 2.5 cm^2. The treated skin was covered with a gauze patch, held in place with adhesive tape. The entire trunk was wrapped with a rubberised elastic cloth
- Reading time point at 4 and 48 h after exposure
- No vehicle
- Observation immediately after treatment.
- Washing was performed after the end of exposure of 4h

Results and discussion

- At the 4 h reading time point, all six rabbits showed no corrosive effects on skin. At 48 h after exposure, skin corrosion was noted in 5 of 6 rabbits. Only one animal was free of skin corrosivity.

3.4.2 Human data

No data available.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

3.5.1.1 Eye irritation study in rabbits – Study 1 (1968)

Study reference:

Union carbide Corporation, 1968. Eye irritation study

Detailed study summary and results:

Test type

Equivalent to OECD guideline 405

Non GLP

Original report not available and documentation insufficient for assessment.

Test substance

- 2-MEA
- Degree of purity: no data
- Batch number: Sample No. 31-126

Test animals

- Albino rabbits, strain not specified
- 6 animals/dose
- Age and weight at the study initiation: not specified

Administration/exposure

- Duration of test/exposure period: 24h
- Amount(s) applied: 0.001, 0.005, 0.02, 0.1, and 0.5 mL of the undiluted test substance; 0.5 mL of varying dilutions of the test substance (40, 15, 5, 1, and 0.1%)
- Post exposure observation period: 24h
- Vehicle: none
- Reading time point: 24h

Results and discussion

At the 24 h reading, severe corneal injury was observed in 3 eyes treated with 0.02 mL of the undiluted test substance. Minor to moderate injury was observed in the eyes after treatment with 0.005 mL of the undiluted test substance (no further details).

3.5.1.2 Modified eye irritation study in rabbits - Study 2 (1980c)

Study reference:

Rhône-Poulenc Inc., 1980c. Modified eye irritation. Report date: 1980-06-24

Detailed study summary and results:

Test type

Equivalent to OECD guideline 405

Other guideline: EPA 40 CFR 163.81.4

Non GLP

Basic data given. The study period was terminated at day 7, therefore only limited information on reversibility.

Test substance

- 2-MEA
- Degree of purity: 99%
- Batch number: E169H8

Test animals

- New Zealand albino rabbits
- 6 animals: single application without washing
- 3animals: 30s + washing
- Age and weight at the study initiation: not specified

Administration/exposure

- Total dose: 0.1mL
- Post exposure observation period: 7-day
- Control group and treatment
- Vehicle: none
- Reading time point: 24, 48, 72h as well as 4 and 7 days
- The untreated eye served as control

Results and discussion

The test substance was found to be an eye irritant in the unwashed eyes of 6 rabbits. All animals showed effects on the cornea (scores 1-2) and conjunctivae (redness: score 1-3; chemosis: score 1-4). In 3 out of 6 animals conjunctival redness was fully reversible within 7 days. Conjunctival oedemas were persistent in all animals at any time during the test. Corneal opacity was only reversed in 1out of 6 animals within the observation period of 7 days. Effects on iris (scores of 1) were observed in 2 out of 6 animals and were fully reversible within 7 days. As none of the animals was completely free from signs of eye irritation within the observation period of 7 days and it is unknown whether these effects on the cornea and conjunctiva are transient or persistent within an observation period of 21 days, the test substance needs to be classified into Category 1.

The severity and duration of the irritation to the eyes was reduced in the washed eyes of 3 tested animals.

However, in one animal, effects on conjunctival redness and chemosis were not fully reversible within 7 days.

Table 5: Results of the unwashed eyes

Rabbit #	Time [h]	conjunctivae		iris	cornea		conjunctivae		iris	cornea
		redness	swelling				redness	swelling		
1										
	24	2	3	0	1					
	48	2	4	0	1					
	72	2	3	0	1					
	4 d	1	3	0	1					
	7 d	1	1	0	0					
	Average: 24+48+72h	2.0	3.3	0.0	1.0	Time to reversion	Not reversible within 7 d	Not reversible within 7 d	-	7 d
2										
	24	2	4	0	2					
	48	3	4	0	1					
	72	3	4	0	1					
	4 d	2	4	0	1					
	7 d	0	3	0	2					
	Average: 24+48+72h	2.3	4.0	0.0	1.3	Time to reversion	7 d	Not reversible within 7 d	-	Not reversible within 7 d
3										
	24	2	4	0	2					
	48	3	4	0	2					
	72	3	4	0	2					
	4 d	3	4	0	2					
	7 d	2	4	0	3					
	Average: 24+48+72h	2.3	4.0	0.0	2.0	Time to reversion	Not reversible within 7 d	Not reversible within 7 d	-	Not reversible within 7 d
4										
	24	2	4	0	2					
	48	2	4	0	2					
	72	2	4	0	2					
	4 d	1	3	0	2					
	7 d	0	2	0	2					
	Average: 24+48+72h	2.0	4.0	0.0	2.0	Time to reversion	7 d	Not reversible within 7 d	-	Not reversible within 7 d

5										
	24	2	4	1	2					
	48	2	4	0	2					
	72	2	4	0	2					
	4 d	2	4	0	2					
	7 d	0	3	0	1					
	Average: 24+48+72h	2.0	4.0	0.3	2.0	Time to reversion	7 d	Not reversible within 7 d	48 h	Not reversible within 7 d
6										
	24	3	4	1	2					
	48	3	4	1	2					
	72	3	4	1	2					
	4 d	2	4	1	3					
	7 d	1	4	0	2					
	Average: 24+48+72h	3.0	4.0	1.0	2.0	Time to reversion	Not reversible within 7 d	Not reversible within 7 d	7 d	Not reversible within 7 d
Mean out of all 6 rabbits	24+48+72h	2.67	3.88	0.2	1.7					

3.5.2 Human data

No data.

3.6 Respiratory sensitisation

No data available.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 Local lymph node assay

Study reference:

Local lymph node assay. Confidential report available in REACH registration IUCLID file, 2012a.

Detailed study summary and results:

Test type

OECD Guideline 429

GLP

No deviations

Test substance

- 2-MEA
- Degree of purity: 99%
- Batch number: C220424SP6

Test animals

- CBA/Ca (CBA/CaOlaHsd) female mice
- No. of animals: 4 in the main assay and 1 in the preliminary screening test.
- Age and weight at the study initiation: 8-12 weeks, 15-23g

Administration/exposure

- Vehicle: acetone/olive oil (4:1 v/v)
- Concentrations: 25 and 50 % (v/v), 100% undiluted
- Range finding test: In a preliminary screening test, the systemic toxicity/irritancy potential of the test item was assessed in each one mouse treated once daily with 25 µL of the undiluted test item (100%) and the test item at concentrations of 50%, 25% or 10% v/v in acetone/olive oil 4:1 on the dorsal surface of each ear for three consecutive days (Days 1, 2, 3). The mice were observed twice daily on Days 1, 2 and 3 and once daily on Days 4, 5 and 6. Local skin irritation was scored daily and any clinical signs of toxicity, if present, were also recorded. The bodyweight of each mouse was recorded on Day 1 (prior to dosing) and on Day 6. The thickness of each ear was measured using an Oditest micrometer (Dyer, PA), pre-dose on Day 1, post dose on Day 3 and on Day 6. A mean ear thickness increase of equal to or greater than 25% was considered to indicate excessive irritation and limited biological relevance to the endpoint of sensitisation.

  - Compound solubility: the test substance at 50% (v/v) in acetone/olive oil 4:1 was well soluble, and thus suitable for dosing.

  - Irritation: no irritation indicated by an equal to or greater than 25% increase in mean ear thickness was noted.
- Main study:

ANIMAL ASSIGNMENT AND TREATMENT

- Name of test method: ^{3}H-methyl thymidine (^{3}HTdR) incorporation determined by β-scintillation
- Criteria used to consider a positive response: the test item will be regarded as a sensitiser if at least one concentration of the test item results in a 3-fold or greater increase in ^{3}HTdR incorporation compared to control values. Any test item failing to produce a 3-fold or greater increase in ^{3}HTdR incorporation will be classified as a "non-sensitiser".

TREATMENT PREPARATION AND ADMINISTRATION: based on the preliminary screening test,

groups of 4 mice were treated with the undiluted test item (100%) or the test item at concentrations of 50% or 25% v/v in acetone/olive oil 4:1. The test substance (25 µL) was daily applied to the dorsal surface of each ear for three consecutive days (Days 1, 2, 3) using an automatic micropipette and spread over the dorsal surface of the ear using the tip of the pipette. A further group of four mice received the vehicle alone in the same manner. Five days following the first topical application of the test item or vehicle (on Day 6) all mice were injected via the tail vein with 250 µL of phosphate buffered saline (PBS) containing ^{3}HTdR at 80 µCi/mL (specific activity 2.0 Ci/mmoL, ARC UK Ltd), giving a total of 20 µCi to each mouse. Five hours later, draining auricular lymph nodes were excised and pooled for each experimental group. A single cell suspension of pooled lymph node cells was prepared by gentle mechanical disaggregation through 200-mesh stainless steel gauze. The lymph node cells per group were each rinsed through the gauze with 4 mL of PBS into a petri dish and then transferred to the respective centrifuge tube. The petri dish was washed with an additional 5 mL of PBS to remove all remaining lymph node cells and these were added to the centrifuge tube. The pooled lymph node cells were pelleted by centrifugation and the obtained pellet was resuspended in 10 mL of PBS and re-pelleted. Then, the pellet was resuspended in 3 mL of 5% trichloroacetic acid (TCA) and precipitated at 4 °C for a period of approx. 18 h. After centrifugation, the pellet was resuspended in 5% TCA and ^{3}HTdR incorporation was measured by β-scintillation counting.

- Positive control substance: hexyl cinnamic aldehyde (CAS No 101-86-0) 25% (v/v) in acetone/olive oil 4:1

Results and discussion

Preliminary study:

Very slight erythema was noted in animals treated with the test item at concentrations of 25% and 10% v/v in acetone/olive oil 4:1 during Days 3-5. No visual local skin irritation was noted in animals treated with the undiluted test item or the test item at a concentration of 50% v/v in acetone/olive oil 4:1.
- Clinical signs: no signs of systemic toxicity were observed.

Based on this information, the undiluted test item and the test item at concentrations of 50% and 25% v/v in acetone/olive oil 4:1 were selected for the main test.

Positive control results

The current positive control substance α-hexylcinnamaldehyde at 25% v/v in acetone/olive oil 4:1 produced a stimulation index (SI) of 5.76, thus fulfilling the reliability criteria for the LLNA (SI > 3).

Main study:

The mean DPM/node per test group were 14860.45, 20735.73 and 18646.09 after treatment with 25, 50 and 100% of the test substance, respectively, compared to a mean value of 1614.78 in the control group.

Table 6: Results of the LLNA

Concentration (% v/v)	dpm	dpm/Node[a]	Stimulation Index [b]	Result
Vehicle	12918.23	1614.78	na	na
25	11883.60	14860.45	9.20	positive
50	165885.80	20735.73	12.84	positive
100	149168.70	18646.09	11.55	positive

dpm = Disintegrations per minute ; a = Disintegrations per minute/node obtained by dividing the disintegrations per minute value by 8 (total number of lymph nodes) ; b = Stimulation Index of 3.0 or greater indicates a positive result ; na = Not applicable

CLINICAL OBSERVATIONS
No mortalities and no signs of systemic toxicity were observed during the study.

BODYWEIGHTS
Bodyweight changes of the test animals between Day 1 and Day 6 were comparable to those observed in the corresponding control group animals over the same period.

3.7.2 Human data

No data available.

3.8 Germ cell mutagenicity

3.8.1 *In vitro* data

3.8.1.1 Gene mutation study in bacteria (Study 1)

Study reference: Confidential report available in REACH registration IUCLID file, 1991

Test type

- Similar to OECD guideline 471 (bacterial Reverse Mutation Assay)
- GLP: no
- Deviations: Only 4 strains tested instead of 5 recommended; Basic data given: limited details on test system and conditions; no analytical purity; positive controls not specified; dose rationale not justifed;
- Strain: *S. Typhimurium* TA1535, TA 97, TA 98, TA 100 ;
- Main assay:

TA 100, preincubation method, without S9 mix: 10, 33, 50, 100, 333, 1000 µg/plate
TA 100, preincubation method, with 30% HLI (induced male Syrian hamster liver S9): 33, 100, 333, 1000, 3333 µg/plate
TA 100, preincubation method, with 30% RLI (induced male Sprague Dawley rat liver): 33, 100, 333, 1000, 3333 µg/plate

TA 100, plate test, without S9 mix: 0.5, 1, 5, 10, 25, 50 mL/chamber
TA 100, plate test, with 10% HLI: 0.5, 1, 5, 10, 25, 50 mL/chamber
TA 100, plate test, with 30% HLI: 1, 5, 10, 25 mL/chamber
TA 100, plate test, with 10% RLI: 0.5, 1, 5, 10, 25 mL/chamber
TA 100, plate test, with 30% RLI: 1, 5, 10, 25, 50 mL/chamber
TA 1535, plate test, with and without 10% HLI, 30% HLI, 10% RLI, 30% RLI: 0.5, 1, 5, 10, 25 mL/chamber
TA 97, plate test, with and without 10% HLI, 30% HLI, 10% RLI, 30% RLI: 0.5, 1, 5, 10, 25 mL/chamber
TA 98, preincubation method, without S9 mix: 10, 33, 100, 333, 1000 µg/plate
TA 98, preincubation method, with 30% HLI (induced male Syrian hamster liver S9): 33, 100, 333, 1000, 3333 µg/plate
TA 98, preincubation method, with 30% RLI (induced male Sprague Dawley rat liver): 33, 100, 333, 1000, 3333 µg/plate
TA 98, plate test, without S9 mix: 0.5, 1, 5, 10, 25 mL/chamber
TA 98, plate test, with 10% HLI: 0.5, 1, 5, 10, 25 mL/chamber
TA 98, plate test, with 30% HLI: 1, 5, 10, 25, 50 mL/chamber
TA 98, plate test, with 10% RLI: 0.5, 1, 5, 10, 25 mL/chamber
TA 98, plate test, with 30% RLI: 1, 5, 10, 25, 50 mL/chamber Method of application :

- preincubation method or plate test with vapour from the test liquid
- Vehicle: Water

Test substance

- 2-MEA (CAS no. 3121-61-7)

Results and discussion

Table 9: Test results of TA 100 (preincubation test and plate test – vapour from liquid)

With or without S9-Mix	**Test substance concentration (µg/plate for the preincubation method; mL/chamber for the plate test)**	**Mean number of revertant colonies per plate (average of plates ± Standard deviation)**		
		Preincubation	**Plate test- vapour from liquid: test I**	**Plate test-vapour from liquid: test II**
–	0	143 ± 11.9	153 ± 5.5	112 ± 6.1
–	0.5	-	-	117 ± 6.3
–	1	-	127 ± 1.5	121 ± 11.5
–	5	-	104 ± 4.3	98 ± 5
–	10	144 ± 5	104 ± 7.1	84 ± 4.3
–	25	-	56 ± 12.3	48 ± 10.2
–	33	166 ± 10.8	-	-
–	50	-	62 ± 2.4	-
–	100	151 ± 13.4	-	-
–	333	151 ± 8.5	-	-
–	1000	118 ± 17.8	-	-
Positive	**Name**	**SA**	**SA**	**SA**

controls, -S9	Mean No. of colonies/plate (average of plates ± SD)	312 ± 8.7	675 ± 45.5	515 ± 44.2
+ 10% HLI	0	-	130 ± 3.8	-
+ 10% HLI	0.5	-	131 ± 2	-
+ 10% HLI	1	-	113 ± 7.1	-
+ 10% HLI	5	-	122 ± 12.8	-
+ 10% HLI	10	-	100 ± 7.8	-
+ 10% HLI	25	-	72 ± 3.8	-
Positive controls, +S9	**Name**	**-**	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of plates ± SD)	-	312 ± 7.7	-
+ 30% HLI	0	162 ± 7.9	156 ± 1.8	-
+ 30% HLI	0.5	-	-	-
+ 30% HLI	1	-	142 ± 7.3	-
+ 30% HLI	5	-	136 ± 14.4	-
+ 30% HLI	10	-	133 ± 14.5	-
+ 30% HLI	25	-	101 ± 8.3	-
+ 30% HLI	33	172 ± 9	-	-
+ 30% HLI	50	-	113 ± 11.7	-
+ 30% HLI	100	164 ± 6.2	-	-
+ 30% HLI	333	172 ± 14.8	-	-
+ 30% HLI	1000	183 ± 13.2	-	-
+ 30% HLI	3333	130 ± 11.7	-	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of plates ± SD)	510 ± 18.2	1019 ± 65.8	-
+ 10% RLI	0	-	117 ± 7.5	-
+ 10% RLI	0.5	-	116 ± 8.7	-
+ 10% RLI	1	-	140 ± 0.6	-
+ 10% RLI	5	-	118 ± 9	-
+ 10% RLI	10	-	112 ± 8	-
+ 10% RLI	25	-	77 ± 5.2	-
Positive controls, +S9	**Name**	**-**	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of plates ± SD)	-	369 ± 11.5	-
+ 30% RLI	0	167 ± 3.7	153 ± 18.1	-
+ 30% RLI	0.5	-	-	-
+ 30% RLI	1	-	158 ± 3.3	-

+ 30% RLI	5	-	146 ± 5.9	-
+ 30% RLI	10	-	134 ± 13.2	-
+ 30% RLI	25	-	117 ± 3.8	-
+ 30% RLI	33	166 ± 9.4	-	-
+ 30% RLI	50	-	122 ± 2.4	
+ 30% RLI	100	185 ± 8.3	-	-
+ 30% RLI	333	174 ± 11.5	-	-
+ 30% RLI	1000	170 ± 5.6	-	-
+ 30% RLI	3333	174 ± 7.6	-	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of 3 ± SD)	381 ± 23.1	734 ± 79.4	-

SA = sodium azide
HLI = induced male Syrian hamster liver S9
RLI = induced male Sprague Dawley rat liver S9
* = not further specified

Table 10: Test results of TA 1535 (plate test – vapour from liquid)

With or without S9-Mix	**Test substance concentration (mL/chamber)**	**Mean number of revertant colonies per plate (average of plates ± Standard deviation)**	
		Plate test- vapour from liquid: test I	**Plate test- vapour from liquid: test II**
–	0	7 ± 0.9	6 ± 0.7
–	0.5	8 ± 1.2	9 ± 0.9
–	1	11 ± 2.2	8 ± 0.7
–	5	9 ± 1.8	7 ± 0.6
–	10	9 ± 1.2	6 ± 1
–	25	4 ± 0.3	7 ± 1.2
Positive controls, –S9	**Name**	**SA**	**SA**
	Mean No. of colonies/plate (average of plates ± SD)	**243 ± 4.9**	**211 ± 11.6**
+ 10% HLI	0	11 ± 1.9	-
+ 10% HLI	0.5	9 ± 1.2	-
+ 10% HLI	1	8 ± 1.2	-
+ 10% HLI	5	7 ± 0.3	-
+ 10% HLI	10	5 ± 0.7	-
+ 10% HLI	25	5 ± 1.5 s	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of plates ± SD)	74 ± 5.2	-
+ 30% HLI	0	10 ± 2	-

+ 30% HLI	0.5	6 ± 1	-
+ 30% HLI	1	7 ± 1.8	-
+ 30% HLI	5	8 ± 0.6	-
+ 30% HLI	10	9 ± 0.9	-
+ 30% HLI	25	11 ± 2.5	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	-
	Mean No. of colonies/plate (average of plates ± SD)	153 ± 12.5	-
+ 10% RLI	0	7 ± 1.2	-
+ 10% RLI	0.5	10 ± 1.2	-
+ 10% RLI	1	10 ± 2.2	-
+ 10% RLI	5	7 ± 0.9	-
+ 10% RLI	10	7 ± 1.3	-
+ 10% RLI	25	4 ± 0.9	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	-
	Mean No. of colonies/plate (average of plates ± SD)	85 ± 3.7	-
+ 30% RLI	0	13 ± 1.7	-
+ 30% RLI	0.5	12 ± 2.2	-
+ 30% RLI	1	14 ± 0.6	-
+ 30% RLI	5	10 ± 3.1	-
+ 30% RLI	10	12 ± 1.5	-
+ 30% RLI	25	10 ± 0.9	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	-
	Mean No. of colonies/plate (average of plates ± SD)	259 ± 10.8	-

SA = sodium azide
HLI = induced male Syrian hamster liver S9
RLI = induced male Sprague Dawley rat liver S9
s= slight toxicity
* = not further specified

Table 11: Test results of TA 97 (plate test – vapour from liquid)

With or without S9-Mix	**Test substance concentration (mL/chamber)**	**Mean number of revertant colonies per plate (average of plates ± Standard deviation)**	
		Plate test- vapour from liquid: test I	**Plate test- vapour from liquid: test II**
–	0	215 ± 10.5	198 ± 11.4
–	0.5	225 ± 6.8	209 ± 5.5
–	1	192 ± 26.6	206 ± 13.7
–	5	212 ± 12.6	177 ± 5.8
–	10	95 ± 26.1	107 ± 9.9
–	25	48 ± 21.2 s	171 ± 11.4

Positive controls, –S9	**Name**	**9-AA**	**9-AA**
	Mean No. of colonies/plate (average of plates ± SD)	464 ± 26	447 ± 18.5
+ 10% HLI	0	181 ± 4.3	-
+ 10% HLI	0.5	171 ± 8	-
+ 10% HLI	1	192 ± 11.3	-
+ 10% HLI	5	185 ± 11.1	-
+ 10% HLI	10	161 ± 24.5	-
+ 10% HLI	25	152 ± 6.4	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	-
	Mean No. of colonies/plate (average of plates ± SD)	541 ± 14.5	-
+ 30% HLI	0	184 ± 5.2	-
+ 30% HLI	0.5	193 ± 4.7	-
+ 30% HLI	1	162 ± 1.8	-
+ 30% HLI	5	157 ± 7.1	-
+ 30% HLI	10	142 ± 8.8	-
+ 30% HLI	25	145 ± 7.7	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	-
	Mean No. of colonies/plate (average of plates ± SD)	737 ± 45.8	-
+ 10% RLI	0	201 ± 6.5	-
+ 10% RLI	0.5	204 ± 9.3	-
+ 10% RLI	1	206 ± 14.3	-
+ 10% RLI	5	195 ± 4.7	-
+ 10% RLI	10	188 ± 7.4	-
+ 10% RLI	25	174 ± 18.2	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	-
	Mean No. of colonies/plate (average of plates ± SD)	380 ± 8.3	-
+ 30% RLI	0	191 ± 11.8	-
+ 30%	0.5	221 ± 8.1	-

RLI			
+ 30% RLI	1	213 ± 0.7	-
+ 30% RLI	5	201 ± 5	-
+ 30% RLI	10	160 ± 18	-
+ 30% RLI	25	172 ± 14.3	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	-
	Mean No. of colonies/plate (average of plates ± SD)	391 ± 11.6	-

9-AA = 9-aminoacridine
HLI = induced male Syrian hamster liver S9
RLI = induced male Sprague Dawley rat liver S9
s= slight toxicity
* = not further specified

Table 12: Test results of TA 98 (preincubation test and plate test – vapour from liquid)

With or without S9-Mix	**Test substance concentration (µg/plate for the preincubation method; mL/chamber for the plate test)**	**Mean number of revertant colonies per plate (average of plates ± Standard deviation)**		
		Preincubation	**Plate test- vapour from liquid: test I**	**Plate test- vapour from liquid: test II**
–	0	27 ± 1.2	16 ± 0.6	15 ± 2.5
–	0.5	-	-	17 ± 2
–	1	-	18 ± 3.8	21 ± 2.5
–	5	-	16 ± 1.2	14 ± 3.6
–	10	23 ± 2.7	15 ± 1.2	7 ± 1
–	25	-	12 ± 4.6 s	1 ± 0.6 s
–	33	21 ± 4.2	-	-
–	50	-	t	-
–	100	18 ± 6.1	-	-
–	333	17 ± 2.1	-	-
–	1000	11 ± 1.9	-	-
Positive controls, –S9	**Name**	**2-nitrofluorene or 4-nitro-o-phenylenediamine***	**2-nitrofluorene or 4-nitro-o-phenylenediamine***	**2-nitrofluorene or 4-nitro-o-phenylenediamine***
	Mean No. of colonies/plate (average of plates ± SD)	387 ± 24.9	671 ± 60.8	849 ± 22.4
+ 10% HLI	0	-	24 ± 3.8	-
+ 10% HLI	0.5	-	23 ± 1.3	-
+ 10% HLI	1	-	20 ± 1.5	-
+ 10%	5	-	21 ± 2.5	-

HLI				
+ 10% HLI	10	-	18 ± 0.3	-
+ 10% HLI	25	-	14 ± 2.6	-
Positive controls, +S9	**Name**	**-**	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of plates ± SD)	-	522 ± 10.7	-
+ 30% HLI	0	25 ± 3.2	29 ± 3.2	-
+ 30% HLI	0.5	-	-	-
+ 30% HLI	1	-	30 ± 2.2	-
+ 30% HLI	5	-	24 ± 1.5	-
+ 30% HLI	10	-	17 ± 1.9	-
+ 30% HLI	25	-	15 ± 1 s	-
+ 30% HLI	33	29 ± 1.2	-	-
+ 30% HLI	50	-	6 ± 3.6 s	-
+ 30% HLI	100	34 ± 2.2	-	-
+ 30% HLI	333	23 ± 1.8	-	-
+ 30% HLI	1000	22 ± 3	-	-
+ 30% HLI	3333	16 ± 2	-	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of plates ± SD)	290 ± 20.6	817 ± 21	-
+ 10% RLI	0	-	17 ± 2	-
+ 10% RLI	0.5	-	18 ± 1.3	-
+ 10% RLI	1	-	24 ± 2.5	-
+ 10% RLI	5	-	20 ± 2.9	-
+ 10% RLI	10	-	16 ± 1.9	-

+ 10% RLI	25	-	12 ± 3.1	-
Positive controls, +S9	**Name**	**-**	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of 3 ± SD)	-	344 ± 10.7	-
+ 30% RLI	0	29 ± 3.5	22 ± 3.8	-
+ 30% RLI	0.5	-	-	-
+ 30% RLI	1	-	28 ± 1.9	-
+ 30% RLI	5	-	25 ± 2.4	-
+ 30% RLI	10	-	23 ± 2.2	-
+ 30% RLI	25	-	22 ± 0.9	-
+ 30% RLI	33	25 ± 0.3	-	-
+ 30% RLI	50	-	10 ± 2.9 s	
+ 30% RLI	100	21 ± 2.9	-	-
+ 30% RLI	333	23 ± 4.5	-	-
+ 30% RLI	1000	27 ± 4.5	-	-
+ 30% RLI	3333	23 ± 3.7	-	-
Positive controls, +S9	**Name**	**2-aminoanthracene or sterigmatocystin***	**2-aminoanthracene or sterigmatocystin***	**-**
	Mean No. of colonies/plate (average of plates ± SD)	73 ± 0.7	170 ± 2.3	-

HLI = induced male Syrian hamster liver S9
RLI = induced male Sprague Dawley rat liver S9
s = slight toxicity
t = toxic
*not further specified

3.8.1.2 Gene mutation study in bacteria (study 2)

Study reference: Confidential report available in REACH registration IUCLID file, 2012

Test type

- According to OECD guideline 471 (bacterial Reverse Mutation Assay)
- No deviations

- GLP
- Strain: *S. Typhimurium* TA1535, TA 1537, TA 98, TA 100, *E.coli* WP2 uvr A
- Preliminary cytotoxicity test (TA 100 and WP2 uvrA): 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate, with and without S9 mix
- Main assay: Experiment I and II:
  Salmonella strains: 5, 15, 50, 150, 500, 1500, 5000 µg/plate, with and without S9 mix
  E.coli strain: 50, 150, 500, 1500, 5000 µg/plate, with and without S9 mix
- Method of application : experiment I and preliminary cytotoxicity test: in agar (plate incorporation); experiment II: preincubation method

Test substance

- 2-MEA (CAS no. 3121-61-7)

Results and discussion

RANGE-FINDING/SCREENING STUDIES: in a preliminary cytotoxicity test, *S. typhimurium* strain TA 100 and *E. coli* WP2 uvrA were treated with concentrations ranging from 5 to 5000 µg/plate in the presence and absence of metabolic activation. The test item was initially toxic to TA 100 at 1500 µg/plate, but non-toxic to WP2uvrA as indicated by the number of mean revertants per plate and the inspection of bacterial background lawn. The test item formulation and S9-mix used in this experiment were both shown to be sterile.

COMPARISON WITH HISTORICAL CONTROL DATA: vehicle and positive control values were within the expected historical ranges of each tester strain.

ADDITIONAL INFORMATION ON CYTOTOXICITY: the test item caused a visible reduction in the growth of the bacterial background lawns and/or a substantial reduction in the frequency of revertant colonies of all of the *Salmonella* tester strains, initially from 1500 µg/plate in the absence and presence of S9-mix. The sensitivity of the bacterial tester strains to the toxicity of the test item varied slightly between strain type, exposures with or without S9-mix and experimental methodology (plate incorporation or preincubation). No toxicity was noted in *E. coli* strain WP2 uvrA at any test item concentration in either the absence or presence of S9-mix.

Table 7: Test results of experiment I

Bacterial Reverse Mutation Assay, mean revertant colonies/plate (n=3 ± SD)					
EXPERIMENT I (plate incorporation)					
S9-Mix	**Without**				
Concentration (per plate)	**TA 100**	**TA 1535**	**WP2 uvrA**	**TA 98**	**TA 1537**
SC (water)	124 ± 9	25 ± 10	30 ± 3	21 ± 4	20 ± 5
Test item					

5 µg	120 ± 8	29 ± 10	N/T	19 ± 2	13 ± 1
15 µg	131 ± 5	26 ± 4	N/T	23 ± 6	13 ± 9
50 µg	107 ± 10	23 ± 6	23 ± 2	17 ± 6	11 ± 3
150 µg	119 ± 19	23 ± 8	32 ± 5	17 ± 0	10 ± 1
500 µg	107 ± 6	16 ± 6	28 ± 10	17 ± 12	13 ± 2
1500 µg	86 ± 10	15 ± 8	36 ± 8	2 ± 2	7 ± 2
5000 µg	25 ± 11	2 ± 1	20 ± 2	0 ± 0#	4 ± 1#
PC					
ENNG (3 µg)	506 ± 42	-	-	-	-
ENNG (5 µg)	-	288 ± 29	-	-	-
ENNG (2 µg)	-	-	530 ± 36	-	-
4NQO (0.2 µg)	-	-	-	141 ± 19	-
9AA (80 µg)	-	-	-	-	473 ± 103
S9-Mix	**With**				
Concentration (per plate)	**TA 100**	**TA 1535**	**WP2 uvrA**	**TA 98**	**TA 1537**
SC (water)	128 ± 6	14 ± 2	36 ± 7	25 ± 3	11 ± 6
Test item					
5 µg	122 ± 7	16 ± 1	N/T	21 ± 2	9 ± 6
15 µg	109 ± 15	12 ± 7	N/T	23 ± 6	8 ± 1
50 µg	121 ± 5	12 ± 1	36 ± 10	25 ± 5	10 ± 2
150 µg	108 ± 8	12 ± 1	33 ± 3	21 ± 2	14 ± 2
500 µg	123 ± 3	9 ± 2	41 ± 2	18 ± 3	8 ± 2
1500 µg	95 ± 4	9 ± 2	31 ± 7	25 ± 4	9 ± 7
5000 µg	38 ± 16	0 ± 0	39 ± 1	6 ± 2#	2 ± 1#
PC					
2-AA (1 µg)	1413 ± 75	-	-	-	-
2-AA (2 µg)	-	292 ± 30	-	-	313 ± 19
2-AA (10 µg)	-	-	429 ± 42	-	-
BP (5 µg)	-	-	-	222 ± 31	-

SC = Solvent control; PC = Positive control substances; SD = standard deviation;
ENNG: N-ethyl-N-nitro-N-nitrosoguanidine; 9AA: 9-aminoacridine; 4NQO: 4-nitroquinoline-N-oxide;
2AA: 2-aminoanthracene; BP: benzo(a)pyrene
partial absence of bacterial background lawn
N/T: not tested at this dose level

Table 8: Test results of experiment II

Bacterial Reverse Mutation Assay, mean revertant colonies/plate (n=3 ± SD)					
EXPERIMENT II (preincubation)					
S9-Mix	**Without**				
Concentration (per plate)	**TA 100**	**TA 1535**	**WP2 uvrA**	**TA 98**	**TA 1537**
SC (water)	112 ± 17	24 ± 4	35 ± 2	26 ± 3	9 ± 2
Test item					
5 µg	85 ± 8	19 ± 5	N/T	18 ± 3	9 ± 3
15 µg	95 ± 5	23 ± 1	N/T	24 ± 5	8 ± 1
50 µg	135 ± 1	22 ± 8	37 ± 5	25 ± 6	10 ± 2
150 µg	91 ± 18	20 ± 2	30 ± 2	30 ± 5	8 ± 3

500 µg	90 ± 6	14 ± 6	29 ± 13	19 ± 6	10 ± 1
1500 µg	58 ± 8	8 ± 1	43 ± 3	15 ± 3	5 ± 4#
5000 µg	14 ± 11	0 ± 0	34 ± 9	2 ± 2#	1 ± 1#
PC					
ENNG (3 µg)	488 ± 56	-	-	-	-
ENNG (5 µg)	-	335 ± 39	-	-	-
ENNG (2 µg)	-	-	774 ± 80	-	-
4NQO (0.2 µg)	-	-	-	135 ± 8	-
9AA (80 µg)	-	-	-	-	572 ± 24
S9-Mix			**With**		
Concentration (per plate)	**TA 100**	**TA 1535**	**WP2 uvrA**	**TA 98**	**TA 1537**
SC (water)	148 ± 21	14 ± 2	34 ± 3	26 ± 6	12 ± 1
Test item					
5 µg	112 ± 3	12 ± 4	N/T	26 ± 3	10 ± 2
15 µg	105 ± 6	11 ± 6	N/T	27 ± 6	11 ± 2
50 µg	124 ± 8	13 ± 6	30 ± 2	25 ± 2	11 ± 2
150 µg	101 ± 17	11 ± 6	35 ± 9	23 ± 2	11 ± 2
500 µg	109 ± 9	14 ± 2	41 ± 6	29 ± 4	13 ± 2
1500 µg	100 ± 20	1 ± 1	33 ± 2	12 ± 4	5 ± 3#
5000 µg	75 ± 12	2 ± 1	32 ± 13	5 ± 5#	2 ± 3#
PC					
2-AA (1 µg)	1897 ± 62	-	-	-	-
2-AA (2 µg)	-	374 ± 34	-	-	509 ± 48
2-AA (10 µg)	-	-	672 ± 76	-	-
BP (5 µg)	-	-	-	288 ± 13	-

SC = Solvent control; PC = Positive control substances; SD = standard deviation;
ENNG: N-ethyl-N-nitro-N-nitrosoguanidine; 9AA: 9-aminoacridine; 4NQO: 4-nitroquinoline-N-oxide; 2AA: 2-aminoanthracene; BP: benzo(a)pyrene
partial absence of bacterial background lawn
N/T: not tested at this dose level

3.8.1.3 Gene mutation in mammalian cells test

Study reference: Confidential report available in REACH registration IUCLID file, 2013

Test type

- According to OECD guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test);
- GLP: yes;
- Deviations: None;
- Species/strain: Cultured peripheral human lymphocytes ;
- With and without rat metabolic activation; Cofactor supplemented post-mitochondrial fraction (S9 mix), prepared from the livers of rats daily treated with oral doses of a mixture of phenobarbitone (80 mg/kg bw) and β-naphthoflavone (100 mg/kg bw) for 3 consecutive days prior to sacrifice.
- Method of application: in medium
- Duration

- Exposure duration: 4 h (± S9 mix)

- Expression time (cells in growth medium): 2 days after the end of treatment, cells were plated for determination of cell viability and the mutation frequency in 96-well microtitre plates containing TFT-selective medium.

- Selection time (if incubation with a selection agent): 10-14 days

- Fixation time (start of exposure up to fixation or harvest of cells): 12-16 days

- Selection agent (mutation assays): 4 µg/mL 5-trifluorothymidine (TFT)
- Number of replications: duplicate cultures in one experiment in 96-well microtitre plates
- Determination of cytotoxicity: relative total growth; other: relative suspension growth and viability
- Other examinations: Small and large colonies were differentiated, as small colonies are capable to indicate chromosomal aberrations.
- Preliminary cytotoxicity test:
  4 h treatment (-S9 mix): 0.64, 1.27, 2.54, 5.08, 10.16, 20.31, 40.63, 81.25 and 162.5 µg/mL
  4 h treatment (+S9 mix): 10.16, 20.31, 40.63, 81.25, 162.5, 325, 650, 975 and 1300 µg/mL
  24 h treatment (-S9 mix): 0.16, 0.32, 0.64, 1.27, 2.54, 5.08, 10.16, 20.31 and 40.63 µg/mL

  Main experiment:
  4 h treatment (-S9 mix): 0.63, 1.25, 2.5, 5, 10, 20, 30, 40 µg/mL
  4 h treatment (+S9 mix): 20.25Password94, 40.5, 81, 162, 324, 432, 540 and 648 µg/mL
- Vehicle: none

Test substance

- 2-MEA (CAS no. 3121-61-7)

Results and discussion

RANGE-FINDING/SCREENING STUDIES: based on the toxicity observed in a previous chromosome aberration test, the concentration range used in the preliminary toxicity test was 0.64 to 162.5 µg/mL for the 4-h exposure in the absence of metabolic activation, 10.16 to 1300 µg/mL (corresponding to approx. 10 mM) for the 4-h exposure in the presence of metabolic activation, and 0.16 to 40.63 µg/mL for the 24-h exposure in the absence of metabolic activation. In all three exposure groups, there were marked dose-related reductions in the Relative Suspension Growth (%RSG) of cells treated with the test item when compared to the concurrent vehicle controls. After 4-h treatment, cytotoxicity was observed at concentrations ≥ 5.08 µg/mL without S9 mix and ≥ 162.5 µg/mL with S9 mix. After 24-h exposure in the absence of S9 mix, cytotoxicity was noted at ≥ 2.54 µg/mL (RSG: 72%). Due to the steep nature of the toxicity curve at the higher concentrations (RSG: ≤ 10), it was difficult to achieve the optimum toxicity for all exposure conditions.

COMPARISON WITH HISTORICAL CONTROL DATA: the frequency of chromosomal aberrations in the negative and positive control was within the historical ranges.

ADDITIONAL INFORMATION ON CYTOTOXICITY: in the main experiment, there was evidence of marked concentration-related toxicity following exposure to the test item in both the absence and presence of metabolic activation, as indicated by the reduction in %RSG and RTG (relative total growth) values. Toxicity occurred at concentrations ≥ 10 µg/mL in the absence of S9 mix and at concentrations ≥ 324 µg/mL in the presence of S9 mix. Optimum levels of toxicity were achieved in both the absence and presence of metabolic activation. The toxicity observed at 40 µg/mL in the absence of metabolic activation, and at 648 µg/mL in the presence of metabolic activation exceeded the upper acceptable limit of 90%. Therefore, these concentrations were excluded from the statistical analysis.

Table 15: Experiment I - 4 h exposure - Without Metabolic Activation

Concentration [mg/mL]	**Relative suspension growth [%]**	**Relative Total Growth**	**Mutants per 10^{-6} surviving cells**	**Mutation factor**	**Mutants per 10^{-6} surviving cells**		**Proportion small colony mutants**
					Small colonies	**Large colonies**	
0 (Medium)	100	1	143.78	1	47.8	88.3	0.36
0.63	100	NA	NA	NA	NA	NA	NA
1.25	92	NA	NA	NA	NA	NA	NA
2.5	87	0.76	196.49	1.37	66.9	116.7	0.37
5	85	0.78	193.76	1.35	70.2	110.3	0.40
10	77	0.62	227.23*	1.58	100.7	109.9	0.48
20	49	0.47	240.24*	1.67	90.4	128.3	0.42
30	29	0.13	407.18*	2.83	239.7	132.2	0.63
40#	13	0.04	974.72	6.78	568.0	295.7	0.64
Linear trend			***				
EMS, 400	59	0.34	1237.59	8.61	373.3	524.0	0.43

EMS = ethylmethanesulphonate; NA = not analysed; # = excluded from statistical analysis due to overt toxicity

*p < 0.05; ***p < 0.001

Table 16: Experiment I - 4 h exposure - With Metabolic Activation

Concentration [mg/mL]	**Relative suspension growth [%]**	**Relative Total Growth**	**Mutants per 10^{-6} surviving cells**	**Mutation factor**	**Mutants per 10^{-6} surviving cells**		**Proportion small colony mutants**
					Small colonies	**Large colonies**	
0 (Medium)	100	1	173.24	1	68.0	94.0	0.42
20.25	102	NA	NA	NA	NA	NA	NA
40.5	99	NA	NA	NA	NA	NA	NA
81	93	1.02	167.72	0.97	88.7	67.5	0.56
162	84	0.78	228.559	1.32	123.2	87.2	0.58
324	68	0.76	199.2	1.15	107.1	75.4	0.58
432	43	0.37	297.77**	1.72	141.2	127.2	0.52
540	20	0.11	444.21**	2.56	238.9	163.9	0.59
648#	9	0.03	768.32	4.44	568.0	147.7	0.78
Linear trend			***				
CP, 2.0	63	0.35	1328.84	7.67	883.6	193.0	0.78

CP = cyclophosphamide; NA = not analysed; # = excluded from statistical analysis due to overt toxicity
*p < 0.05; ***p < 0.001

DIFFERENTIATION OF LARGE AND SMALL COLONIES

The increases in mutant frequency observed in the absence and presence of metabolic activation were mainly due to small colony formation indicating a clastogenic response (see tables 15 and 16).

3.8.1.4 Chromosome aberration Test

Study reference: Confidential report available in REACH registration IUCLID file, 2013

Test type

- According to OECD guideline 473 (*In vitro* mammalian Chromosome Aberration Test);
- GLP: yes;
- Limitations: No long term treatment without S9
- Species/strain: Cultured peripheral human lymphocytes ;
- With and without rat metabolic activation;
- Preliminary cytotoxicity test:
  4 and 24 h treatment (±S9 mix): 5.08, 10.16, 20.31, 40.63, 81.25, 162.5, 325, 650 and 1300 µg/mL
- Main experiment:
  4 h treatment (-S9 mix): 10*, 20*, 30, 40*, 60 and 80 µg/mL
  4 h treatment (+S9 mix): 80, 160, 320*, 480*, 640* and 960 µg/mL
  *These concentrations were used for chromosomal analysis (960 µg/mL was too toxic for metaphase analysis).
- Vehicle: DMSO (for positive control)

Test substance

- 2-MEA (CAS no. 3121-61-7)

Results and discussion

Range-finding/screening studies: a preliminary cytotoxicity test with concentrations ranging from 5.08 to 1300 µg/mL (corresponding to approx. 10 mM) was performed in lymphocyte cultures for an exposure period of 4 h with and without S9 mix and for 24 h with S9 mix, respectively. After 4 h exposure, a significant decrease in mitotic index (MI < 50% compared to control) was observed at 81.25 µg/mL (MI = 13%) in the absence of S9 mix and at 650 µg/mL (MI = 29%) in the presence of S9 mix. The optimum toxicity (MI < 50% compare to control) after 20-h treatment was achieved at a concentration of 20.31 µg/mL. Based on these cytotoxicity data, the selection of the maximum concentration for the 4 h treatment in the main experiment was 80 and 960 µg/mL in the absence and presence of S9 mix, respectively.

Comparison with historical control data: the frequency of chromosomal aberrations in the negative and positive control was within the historical ranges.

Additional information on cytotoxicity: in the main experiment, a dose-related inhibition of mitotic index was observed after 4 h treatment with the test substance. A 49% mitotic inhibition was achieved at 40 µg/mL in the absence of S9 mix, whereas in the presence of S9 mix the toxicity curve was relatively steep with 35% mitotic inhibition at 640 µg/mL and 96% mitotic inhibition at 960 µg/mL. The maximum concentration selected for metaphase analysis was 40 µg/mL in the absence of S9, where optimum toxicity was achieved. In the presence of S9, the maximum concentration selected for metaphase analysis was 640 µg/mL as the concentration above (960 µg/mL) was considered too toxic for metaphase analysis.

There was no significant change in pH when the test item was dosed into media and the osmolality did not increase by more than 50 mOsm (see Table 13).

Table 13: Results of pH and osmolality measurements

Parameter	**Concentrations [µg/mL]**									
	0	**5.08**	**10.16**	**20.31**	**40.63**	**81.25**	**162.5**	**325**	**650**	**1300**
pH	7.38	7.38	7.35	7.34	7.42	7.43	7.38	7.39	7.39	7.43
Osmolality [mOsm]	281	280	-	-	279	280	-	-	-	295

Table 14: Test results of experiment I

Test item	**Concentration in µg/mL**	**Mitotic index in %**	**Aberrant cells in %**		**Polyploid cells in %**
			with gaps	**without gaps**	
Exposure period 4 h, fixation time 24 h, without S9 mix (2%)					
NC	0	100	0	0	0
MMC	0.4	77	1.5	14**	0
Test item	10	82	0	0	0
	20	83	0	0	0.5
	40	51	0.5	1	0
Exposure period 4 h, fixation time 24 h, with S9 mix (2%)					
NC	0	100	1	1	0
CP	5	50	16	35**	1
Test item	320	103	0	0.5	0
	480	71	4.5*	1	1.5
	640	65	4	7.5**	0.5

MMC: Mitomycin C; CP: Cyclophosphamide (positive controls); NC: Negative control; * = $P < 0.05$; ** = $P < 0.001$

3.8.2 Animal data

3.8.2.1 *In vivo* mammalian cell study

Study reference:

Confidential report available in REACH registration IUCLID file, 2016.

Detailed study summary and results:

Test type

- According to OECD 489 (*In vivo* Mammalian Alkaline comet Assay)
- GLP: yes
- Limitations; negative controls were below historical control data;

Test substance

- 2-methoxyethyl acrylate
- Purity: confidential
- Batch: confidential

Test animals

- Wistar male rats
- No. of animals per sex per dose: 7 males (negative and test item groups), 5 males for positive control
- Age and weight at the study initiation : 150-200g, 8-10 weeks

Administration/exposure

- Route of administration: oral gavage;
- 2 single treatments within 24 hours;
- Animals were sacrified 4 hours after final treatment;
- Vehicle*:* Phosphate buffered saline (PBS);
- Dose concentrations: 120, 240, 590 mg/kg bw/d ;
- Treatment and sampling times: Animals were treated two times at 0 and 24 hours. 28 hours after first treatment, animals were sacrificed and tissues were prepared. Sub-samples of the liver, glandular and non-glandular stomach were taken from the vehicle control animals and the dose group animals and preserved in 10% buffered formalin for possible histopathological examination;
- Details of the slide preparation: Tissue samples were processed to provide single cell suspensions. Cell suspensions were mixed using low melting point agarose and placed onto pre-coated slides. Four slides/animal for each tissues were prepared. After cell lysis the slides were transferred to electrophoresis to allow the DNA to unwind for 20 minutes. Electrophoresis was conducted at approximately 0.7 V/cm, 300 mA for 20 minutes. Slides were fixed in 100% methanol after electrophoresis and stained with propidium iodide (20 µg/mL);
- Method of analysis: Two slides for each tissue per animal were analyzed using a fluorescence microscope combined with a CCD camera attached to a PC-based image analysis program (Comet

IV version 4.3.1.) and a maximum of 200 cells per tissue and animal were scored primary for relative tail intensity. Each slide was assessed for the incidence of hedgehog to give an indication of cell integrity. Due to statistical increase on percentage tail intensity in glandular and non-glandular stomach tissue, additional histopathological examinations of the non-glandular and glandular stomach were conducted. Therefore tissue samples were processed to paraffin wax, sectioned and stained with hematoxylin and eosin;

- Positive control: N-methyl-N-nitrosourea (25 mg/kg bw);
- Tissue and slide preparation: Liver, non-glandular and glandular stomach;
- Statistics: Students t-test on transformed data using a transformation √(x+1);

Results and discussion

RESULTS OF RANGE-FINDING STUDY

A range-finding test was performed to find suitable dose levels of the test item following a double oral administration at zero and 24 hours and to select the most appropriate sex for use in the main test. The upper dose level selected should ideally be the maximum tolerated dose level or that which produces some evidence of toxicity up to a maximum recommended dose of 2000 mg/kg bw.

- Dose range: 400, 480, 500, 600 mg/kg bw
- Clinical signs of toxicity in test animals: mortality was observed in males (2/3 animals, one animal was dosed only once) at 600 mg/kg and females at 500 (1/1 animal) and 600 mg/kg bw (1/1 animal), hunched posture was observed at 480-600 mg/kg bw
- Rationale for exposure: starting dose based on oral LD50 in rats (404 mg/kg bw)

Table 17: Mortality data in the range-finding study

Dose Level (mg/kg)	Sex (M/F)	No. of animals treated	Total deaths
400	M	2	0
	F	2	0
480	M	2	0
500	M	1	0
	F	1	1
600	M	3	2
	F	1	1

RESULTS OF DEFINITIVE STUDY

In the 480 mg/kg dose group one animal died prematurely prior to the second dosing, although it was unclear whether or not this was treatment related. The remaining animals in this group were seen to have hunched posture approximately one hour after each dosing. No clinical signs were observed in animals dosed with the test item at 240 mg/kg and 120 mg/kg. The presence of clinical signs indicated that systemic absorption had occurred.

Percentage tail intensity: No significant increase in percentage tail intensity was observed in liver tissue of treatment groups compared with the negative control.

A significant increase in the mean of median percentage tail intensity in the glandular stomach tissue was noted in all dose groups, and in the mean percentage tail intensity in the mid- and high dose group, compared with the control group respectively (1.4 - 3.6 fold compared with mean of controls) (Table 18). However, the increase fell within the historical negative control values (2.67 - 12.74% mean % tail intensity).
A significant increase in the percentage tail intensity was also observed in the non-glandular stomach tissue of the mid- and high-dose group, compared with the control group (1.7 - 2.3 fold compared to mean of controls, for mean percentage tail intensity and mean of median percentage tail intensity, Table 2). The individual variation in each dose group and in the control groups was quite high in stomach tissues.

Table 18: Summary Table Comet Assay – Glandular Stomach

Dose Level	Group Mean % Hedgehogs	Group Mean % Tail Intensity	Group Mean of Mean of Median % Tail Intensity per Animal
Vehicle	3.84 ± 1.44	2.05 ± 0.62	0.69 ± 0.42
480 mg/kg bw	2.65 ± 1.03	3.98 ± 1.71[a]	2.52 ± 1.61[b]
240 mg/kg bw	4.18 ± 1.33	2.92 ± 0.79[b]	1.22 ± 0.63[c]
120 mg/kg bw	4.00 ± 1.40	2.72 ± 0.99	1.18 ± 0.64[c]
Positive (MNU)	6.04 ± 1.01	21.09 ± 1.81[a]	19.28 ± 1.88[a]

[a]= P < 0.001
[b]= P < 0.01
[c]= P < 0.05

Table 19: Summary Table Comet Assay – Non-Glandular Stomach

Dose Level	Group Mean % Hedgehogs	Group Mean % Tail Intensity	Group Mean of Mean of Median % Tail Intensity per Animal
Vehicle	6.12 ± 2.32	6.68 ± 1.88	4.35 ± 1.74
480 mg/kg bw	6.41 ± 1.07	11.42 ± 3.16[a]	9.30 ± 3.87[a]
240 mg/kg bw	3.93 ± 1.04	11.92 ± 3.58[a]	10.29 ± 3.97[a]
120 mg/kg bw	4.83 ± 1.28	7.92 ± 2.42	5.92 ± 2.42
Positive (MNU)	7.78 ± 1.71	41.68 ± 3.60[a]	41.90 ± 4.21[a]

[a]= P < 0.001

Table 20: Summary Table Comet Assay – Liver

Dose Level	Group Mean % Hedgehogs	Group Mean % Tail Intensity	Group Mean of Mean of Median % Tail Intensity per Animal
Vehicle	1.16 ± 0.71	0.34 ± 0.06	0.01 ± 0.01

480 mg/kg bw	0.25 ± 0.27	0.34 ± 0.08	0.01 ± 0.01
240 mg/kg bw	0.21 ± 0.26	0.32 ± 0.08	0.00 ± 0.00
120 mg/kg bw	0.69 ± 0.26	0.41 ± 0.23	0.01 ± 0.00
Positive (MNU)	1.04 ± 1.01	16.08 ± 3.85[a]	15.56 ± 4.07[a]

[a]= P < 0.001

Histopathological findings:

- Glandular stomach: Erosion of the glandular epithelium observed in males treated with 240 mg/kg bw (4/7 animals) or 480 mg/kg bw (6/6 animals), inflammation of submucosa observed in males treated with 240 mg/kg bw (3/7 animals) and 480 mg/kg bw (6/6 animals), ulceration in 1 male treated with 480 mg/kg bw, myofiber degeneration observed in males treated with 480 mg/kg bw (4/6 animals)
- Non-glandular stomach: Ulceration observed in males (3/6 animals) treated with 480 mg/kg bw, erosion observed in males (2/6 animals) treated with 480 mg/kg bw, vacuolation of the non-glandular epithelium in males treated with 480 mg/kg bw (3/6 animals) and in 1 male treated with 240 mg/kg bw; Ulceration of the limiting ridge in males treated with 240 mg/kg bw(2/7 animals) and 480 mg/kg bw (4/6 animals), mucosal necrosis in males treated with 480 mg/kg bw (2/6 animals), vacuolation of the epithelium at the limiting ridge in males treated with 120 mg/kg bw (3/7 animals) or 240 mg/kg bw (3/7 animals) , epithelial hyperplasia in 1 male treated with 240 mg/kg bw, inflammation observed treated with 240 mg/kg bw (1/7 animals) and 480 mg/kg bw (6/6 animals), myofiber degeneration in males treated with 480 mg/kg bw (6/6 animals)
- Statistical evaluation: Significant increase in percentage tail intensity (mean percentage tail intensity and mean of median percentage tail intensity) was observed in the glandular- and non-glandular stomach tissues at 240 and 480 mg/kg bw, and at 120 mg/kg bw in the glandular stomach tissue (mean of median percentage tail intensity).

Table 21: Histopathological results of glandular and non glandular stomach (No affected animals/total No. examined)

Organ	**Effect**	**control**	**480 mg/kg bw**	**240 mg/kg bw**	**120 m/kg bw**
Glandular stomach	Erosion - minimal - slight	 0/7 0/7	 2/6 4/6	 4/7 0/7	 0/7 0/7
	Ulceration - slight	 0/7	 1/6	 0/7	 0/7
	Inflammation submucosa - minimal - slight	 0/7 0/7	 6/6 0/6	 3/7 0/7	 0/7 0/7
	Myofiber degeneration - minimal - slight	 0/7 0/7	 3/6 1/6	 0/7 0/7	 0/7 0/7

Non-glandular stomach	Ulceration - slight - marked	0/7 0/7	1/6 2/6	0/7 0/7	0/7 0/7
	Erosion - slight - moderate	0/7 0/7	1/6 1/6	0/7 0/7	0/7 0/7
	Vascuolation - minimal - slight	0/7 0/7	3/6 0/6	0/7 1/7	0/7 0/7
	Vacuolation limiting ridge - minimal - slight	0/7 0/7	0/6 0/6	1/7 2/7	3/7 0/7
	Epithelial Hyperplasia - slight	0/7	0/6	1/7	0/7
	Ulceration, Limiting Ridge - minimal - slight	0/7 0/7	2/6 2/6	1/7 1/7	0/7 0/7
	Mucosal necrosis, Limiting ridge - slight	0/7	2/6	0/7	0/7
	Inflammation submucosa - minimal - slight	0/7 0/7	0/6 6/6	0/7 1/7	0/7 0/7
	Myofiber Degeneration - minimal - slight - moderate	0/7 0/7 0/7	2/6 2/6 2/6	0/7 0/7 0/7	0/7 0/7 0/7

Study report's conclusion:

The test item did not induce any increases in the percentage tail intensity values in liver and the test item was considered to be non-genotoxic to the rat liver tissue investigated *in vivo*.

The glandular stomach and non-glandular stomach demonstrated modest but statistically significant increases in percentage tail intensity and median percentage tail intensity. However, the increases seen in the glandular stomach were within the historical control range for a vehicle and were therefore considered to be of no biological relevance. The results of the histological examination of the glandular stomach and non-glandular stomach confirmed the cytotoxicity of the test item and increases seen in the non-glandular stomach were considered to be as a result of this. The authors of the report concluded that, the test item 2-MEA was considered to be non-genotoxic in the liver and glandular stomach and thatthe effects observed in the non-glandular stomach were judged to be a secondary effect due to cytotoxicity of the compound.

Dossier submitter's comment:

This conclusion is only the interpretation of the authors of the study. Dossier submitter considered that the observed effect in non-glandular stomach are related to genotoxic effects as discussed in the CLH report.

3.9 Carcinogenicity

Not evaluated. No data available in the dossier.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test

Study reference: Study report, 2012b. Combined repeated dose toxicity study with reproduction/developmental toxicity screening test of 2-methoxyethyl acrylate in rats by oral gavage according to OECD 422.

Detailed study summary and results:

Test type:

- OECD guideline 422
- GLP compliant

Test substance:

- 2-methoxyethyl acrylate
- Degree of purity: ≥ 99%
- Batch number: C211114SP6

Test animals:

- Crl:WI(Han) rats
- 10 animals/sex/dose
- Age at study initiation: 11 weeks
- Age at mating: ca. 13 weeks
- Weight at study initiation: (P) Males: 255-301 g; Females: 179-205 g

Administration/exposure:

- Oral: gavage
- Once daily, 7 days/week
- Duration of treatment/exposure:
  P (Males): 31-35 days i.e. 2 weeks prior to mating, during mating, and up to termination;
  P (Females): 42-56 days, i.e. during 2 weeks prior to mating, during mating, during post-coitum, and during at least 4 days of lactation

- Acutal Doses: 40, 100 and 250/150 mg/kg bw/day (250 mg/kg bw/day: from Day 1 to 11; 150 mg/kg bw/day: from Day 12 to study termination).
- Controls: concurrent vehicle
- Dose selection rationale: dose levels were based on a preliminary range finding study, in which 3 female rats per dose group received the test substance at 100, 200 and 400 mg/kg bw/day. Animals treated with 100 and 200 mg/kg bw/day received the test substance for a period of 10 days, whereas animals of the 400 mg/kg bw/day group were only treated for 2 days. On Day 2 of the study, one animal died and piloerection was observed in one animal at 400 mg/kg bw/day. At necropsy, abnormalities of the stomach, lungs and thymus were found in the animals of this dose group. No mortalities occurred at 100 and 200 mg/kg bw/day. At both dose levels, salivation was observed in all animals during the first days of the study (Day 1-3). No adverse changes in body weights and food consumption were observed and macroscopic examination did not reveal any abnormal findings, except for a reduced thymus size in all animals treated with 200 mg/kg bw/day. Based on the results of this range finding study, dose levels for the main study were 40, 100 and 250 mg/kg bw/day. From Day 12 of study onwards, the dose level was lowered to 150 mg/kg bw/day for both sexes due to severe toxicity noted at 250 mg/kg bw/day.
- Vehicle: propylene glycol

Description of test design:

- M/F ratio per cage: 1/1
- Length of cohabitation: up to 14 days
- Proof of pregnancy: vaginal plug / sperm in vaginal smear referred to as day 0 of pregnancy
- After 14 days of unsuccessful pairing replacement of first male by another male with proven fertility.
- Further matings after two unsuccessful attempts: no
- After successful mating each pregnant female was caged: individually in plastic cages (MIII type, height 18 cm).

Examination:

- Parental animals: cage side observations, clinical observations, body weight and food consumption. Sacrifice of all surviving male animals after completion of the mating period. Sacrifice of all surviving maternal animals which delivered, on lactation Days 5-7
  Females which did not deliver were sacrificed on post-coitum Days 25-27 (females with evidence of mating) or approximately 21 days after the last day of the mating period (females without evidence of mating). Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera. The tissues in the tables below were prepared for microscopic examination and weighed, respectively.

A) for 5 selected animals/sex/group and all animals that died spontaneously or were killed in extremis

Adrenal glands	Duodenum
Ovaries	Sciatic nerve
(Aorta)	Epididymides
(Pancreas)	Seminal vesicles
Brain - cerebellum, mid-brain, cortex	Eyes (with optic nerve (if detectable) and
Peyer's patches [jejunum, ileum] if detectable	Harderian gland)
Caecum	Skeletal muscle
Pituitary gland	Female mammary gland area
Cervix	(Skin)
Preputial gland	Femur including joint
Clitoral gland	Spinal cord -cervical, midthoracic, lumbar
Prostate gland	Heart
Colon	Spleen
Rectum	Ileum
Coagulation gland	Sternum with bone marrow
(Salivary glands - mandibular, sublingual)	Jejunum
Thyroid including parathyroid if detectable	Stomach
Liver	Kidneys
(Tongue)	Testes
Lung, infused with formalin	(Lacrimal gland, exorbital)
Trachea	Thymus
Lymph nodes - mandibular, mesenteric	(Larynx)
Urinary bladder	Uterus
(Nasopharynx)	(Esophagus)
	Vagina
B) for all remaining animals	
Cervix	Epididymides
Prostate gland	Uterus
Clitoral gland	Mammary gland area
Seminal vesicles	Vagina
Coagulation gland	Ovaries
Testes	all tissues/organs showing gross lesions
Preputial gland	

Tissues/organs mentioned in parentheses were not examined by the pathologist, since no signs of toxicity were noted at macroscopic examination.

Organ weights determined in 5 selected animals/sex/group	
glands	Uterus (including cervix)
Spleen	Kidneys
Brain	Prostate
Testes*	Liver
Epididymides*	Seminal vesicles including coagulating glands
Thymus	Ovaries
Heart	Thyroid including parathyroid

* these organs were also examined in all remaining males

- Staging of spermatogenesis in testes was performed.
- Sperm parameters: examined in P male parental generations: testis weight, epididymis weight, other: weight of seminal vesicles including coagulating glands
- Litter observations: number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, body weight

Statistics:

- If the variables could be assumed to follow a normal distribution, the Dunnett-test (many-to-one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex.
- The Steel-test (many-to-one rank test) was applied if the data could not be assumed to follow a normal distribution.
- The Fisher Exact-test was applied to frequency data.
- Motor activity data was subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences followed by the Wilcoxon test to compare the treated groups to the control group.

All tests were two-sided and in all cases $p < 0.05$ was accepted as the lowest level of significance. Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables. Test statistics were calculated on the basis of exact values for means and pooled variances.

Results and discussion

For P and F1 adults (per dose):

Mortality and Clinical signs

At 250 mg/kg bw/day: 2 males died on Day 2 (no cause of death could be determined), 1 male was killed on Day 8 (showed ulcerative inflammation in the stomach with resultant peritonitis);

At 100 mg/kg bw/day: 1 female killed in extremis on day 21 post-coitum;

Clinical signs in males and females found dead at both dose levels included hunched posture, piloerection, pale and lean appearance.

Scheduled deaths: treatment-related clinical signs were noted at 250 mg/kg bw/day, and consisted of hunched posture (18 animals 1-5 days), piloerection (1 female 2 days), salivation (3 animals 1 day), and rales (1 female 1 day). These findings disappeared during dosing at 150 mg/kg bw/day. Red vagina or bleeding from the vagina was noted for three females treated at 100 and 250/150 mg/kg bw/day on Days 14, 19 and 23 post-coitum, respectively. This was probably bleeding caused by loss of fetuses and considered treatment-related. These females showed total litter loss or implantation sites only. One female treated at 150 mg/kg bw/day showed hunched posture and piloerection on Days 19 to 21 post-coitum. As this was not noted for the other females at this dose, and recovered during further treatment, it was not considered toxicologically significant.
Incidental findings that were noted included salivation, alopecia, scales and scabs. These findings occurred within the range of background findings to be expected for rats of this age and strain and were thus considered to be of no toxicological relevance.

Body weight and food consumption (No tabulated data in the Endpoint study record of the dossier)
At 250 mg/kg bw/day, most male animals and a few female animals showed a body weight loss, which slightly recovered during treatment at 150 mg/kg bw/day. Reduced body weight gains were also noted for males at 100 mg/kg bw/day. At 250/150 mg/kg bw/day, reduced body weight gains were observed during the first two weeks of post-coitum and a body weight loss during the last week. A body weight loss was also noted at the end of postcoitum for females treated with 100 mg/kg bw/day. The reduced body weight gains for females of the 100 mg/kg bw/day dose group during the first two weeks of post-coitum was considered a cause of their pregnancy status (i.e. implantation sites only instead of live fetuses) and not considered toxicologically relevant. No changes in body weights were observed at 40 mg/kg bw/day. Since no litters were born at the 100 and 250/150 mg/kg bw/day, no body weight gain of these animals during lactation could be determined.

Reduced food consumption was noted for the first two weeks of treatment at 250 mg/kg bw/day for both sexes. This recovered during treatment at 150 mg/kg bw/day. At the end of post-coitum, a dose related decrease in food consumption was noted for females treated at 100 mg/kg bw/day (Days 17-20) and 250/150 mg/kg bw/day (Days 14-20) when compared to the concurrent control group. The lower food consumption levels at 100 mg/kg bw/day (Days 7 to 17 post-coitum) and 250/150 mg/kg bw/day (Days 7-14 post-coitum) were considered a cause of their pregnancy status (i.e. implantation sites only or not pregnant), and therefore not considered toxicologically relevant. During lactation, females treated at 40 mg/kg bw/day showed reduced food consumption when compared to the concurrent control animals. However, this was considered due to the reduced number of live pups in these litters. No data on food consumption during lactation was obtained for 100 and 250/150 mg/kg bw/day as no litters were born in these groups. All other statistical significant changes were not considered toxicologically relevant as the values were within normal limits.

Estrous cycle

Estrous cyclicity was not determined in females. However, there was evidence of cyclic changes in the reproductive tracts of non-pregnant females treated with 250/150 mg/kg bw/day.

Sperm measures

Staging of spermatogenesis in testes provided evidence of test article related impairment to the spermatogenetic cycle at all dose levels in a dose related manner. The most subtle changes were those of enlarged spermatagonia with finely granular cytoplasm and asynchronous tubules in which normal cell associations were absent. Individual cell necrosis, spermatidic giant cells, and reduced layers of spermatagonia were common observations. At the highest 250/150 mg/kg bw/day dose level there were no recognizable stages. Tubules were often lined by a single layer of cells with uncertain identity (Sertoli cells, primary spermatagonia or both). Mitotic figures were occasionally present and appeared abnormal.

Reproductive effects:

Male and female reproduction was affected at all dose levels. Precoital time of females treated at 40 and 250/150 mg/kg bw/day was increased. Two females of the high dose group did not mate in the first pairing period of 15 days. Out of these, one female mated with her second male, whereas the other did not. Fertility and conception indices were decreased at 250/150 mg/kg bw/day, as out of the nine mated females only two were pregnant. In addition, a dose related decrease was noted for number of corpora lutea and implantation sites at 100 and 250/150 mg/kg bw/day. At 40 mg/kg bw/day, an increased duration of gestation was noted.

Table 22: Reproduction data

Parameter	**Dose [mg/kg bw/day]**			
	0	**40**	**100**	**250/150**
Mating index [%]	100	100	100	90
No. of females mated	10/10	10/10	10/10	9/10
Fertility index [%]	100	100	90	20
No. of implantation sites#	11.1 ± 2.0	9.8 ± 1.4	7.8 ± 4.3	3.5 ± 3.5
No. of *corpea lutea*#	14.5 ± 4.3	11.3 ± 1.8	9.9 ± 4.5	1.1 ± 3.0**
Duration of gestation [d]#	21.4 ± 0.5	23.1 ± 0.6	n.a.	n.a.
Conception index [%]	100	100	90	22.2
No. of pregnant females	10/10	10/10	9/10	2/10
No. of non-pregnant females	0/10	0/10	1/10	8/10
No. of females with live pups (Day 1)	10/10	7/10	0/10	0/10
Gestation index [%]	100	70	0	0
Litter size	10	9	n.a.	n.a.

*/** Steel−test significant at 5% (*) or 1% (**) level, n.a. = not applicable; # mean value ± standard deviation

Organ weight

In males treated at 100 and 250/150 mg/kg bw/day, reduced absolute and/or relative weights of the thymus, testes, prostate, epididymides and seminal vesicles were noted (see table below). No toxicologically relevant findings were noted for the females. All other statistically significant changes were considered not to be a sign of toxicity as they occurred in the absence of a treatment-related distribution, were due to the lower terminal body weight or were considered due to the pregnancy/lactation status of the females (i.e. for the liver, adrenals, spleen, ovaries).

Table 23: Statistically significant changes in reproduction organ weights of males

Organ weights	**Dose [mg/kg bw/day]**			
	0	**40**	**100**	**250/150**
Thymus, absolute [g]	0.260 ± 0.017	0.258 ± 0.033	0.141 ± 0.014**	0.116 ± 0.022**
Thymus, relative [%]	0.079 ± 0.006	0.083 ± 0.011	0.048 ± 0.006**	0.040 ± 0.007**
Testis, absolute [g]	3.31 ± 0.18	3.26 ± 0.31	1.86 ± 0.30**	1.46 ± 0.11**
Testis, relative [%]	1.02 ± 0.12	1.03 ± 0.07	0.62 ± 0.10**	0.51 ± 0.05**
Epididymides, absolute [g]	1.133 ± 0.106	1.104 ± 0.077	0.801 ± 0.091**	0.645 ± 0.068**
Epididymides, relative [%]	0.348 ± 0.043	0.349 ± 0.027	0.268 ± 0.040**	0.225 ± 0.027**
Seminal vesicles, absolute [g]	1.778 ± 0.222	1.460 ± 0.138	1.377 ± 0.201*	1.420 ± 0.213*
Seminal vesicles, relative [%]	0.543 ± 0.067	0.468 ± 0.049	0.471 ± 0.065	0.482 ± 0.065
Prostate, absolute [g]	0.662 ± 0.138	0.549 ± 0.111	0.471 ± 0.075*	0.413 ± 0.042*
Prostate, relative [%]	0.201 ± 0.038	0.176 ± 0.035	0.161 ± 0.025	

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

Gross pathology

Testes of reduced size (with or without flaccidity) were present in 8/10 males at 100 mg/kg bw/day and 8/10 males at 250/150 mg/kg bw/day. Epididymides of reduced size (with or without flaccidity) were present in 7/10 males at 100 mg/kg bw/day and 7/10 males at 250/150 mg/kg bw/day. Forestomachs (non-glandular portion) had irregular surfaces in 7/10 males and 5/10 females at 250/150 mg/kg bw/day. This observation was also noted in the glandular stomach of 3/10 males at 250/150 mg/kg bw/day. One male and one female at 100 mg/kg bw/day and two males at 250/150 mg/kg bw/day had reduced thymus size. The male animals that did not survive until planned necropsy showed additionally several abnormalities in the following organs: lungs, stomach, liver, seminal vesicles, spleen, mesenteric and mandibular lymph nodes, lacrimal glands, diaphragm, and body cavities. In addition, beginning autolysis was noted for the two animals that were found dead. Macroscopic examination of the female animal that was killed in extremis revealed findings in the uterus, cervix, spleen, mandibular lymph nodes, upper jaw, and abdominal cavity. The incidence of other incidental findings among control and treated animals was within the background range of findings that are encountered among rats of this age and strain, and did not show a dose-related

incidence trend. These necropsy observations included findings in the lungs (foci), liver (reduced in size), kidneys (pelvic dilation), epididymides (nodule), mandibular lymph nodes (enlarged, discolouration), uterus (enlarged, thickened, contains fluid), clitoral glands (discolouration), skin (alopecia), and spleen (nodule, enlarged).

Histopathology

Abnormal findings in the **stomach** included inflammation, haemorrhage, hyperkeratosis and hyperplasia of non-glandular epithelium, and degeneration of glandular epithelium..

In the testes, degeneration of seminiferous tubular epithelium, increased severity of edema, chronic active inflammation, and enlarged amphophilic cells were observed. Incidences of pathological findings in the testes are summarised in table below. Staging of spermatogenesis in testes provided evidence of test article-related impairment to the spermatogenetic cycle at all dose levels in a dose related manner. The most subtle changes were those of enlarged spermatagonia with finely granular cytoplasm and asynchronous tubules in which normal cell associations were absent. Individual cell necrosis, spermatidic giant cells, and reduced layers of spermatagonia were common observations. At the 250/150 mg/kg bw/day dose level, there were no recognizable stages. Tubules were lined often by a single layer of cells with uncertain identity (Sertoli cells, primary spermatagonia or both). Mitotic figures were occasionally present and appeared abnormal. Epididymal observations included degenerate sperm, hypospermia, atrophy and inflammation.

Incidences of pathological findings in the **epididymides** are summarised in table below.

In the **spleen,** reduced (but not dose-related) numbers of hematopoietic cells were noted.

Minimal or slight hepatocellular necrosis was observed in the **liver** of two males and one female at 250/150 mg/kg bw/day.

In the **uterus**, implantation sites were observed in 5/5, 6/6, 3/8 and 1/6 females at 0, 40, 100 and 250/150 mg/kg bw/day, respectively. Another three females of the 100 mg/kg bw/day group had placental trophoblasts and necrotic debris in the uterine lumen as evidence of litter loss. There was evidence of cyclic changes in the reproductive tracts of non-pregnant 250/150 mg/kg bw/day females. No pregnancy associated hypertrophy of the cortical adrenal was present in the females of all dose groups.

In the **mammary gland**, observations consisted of reduced incidences and severities of normally expected pregnancy-related hyperplasia of the mammary gland. The incidences of hyperplasia were 5/5, 4/4, 4/5 and 0/5 in the respective 0, 40, 100 and 250/150 mg/kg bw/day dose groups with progressive decreases (3.0, 2.3, 1.5 and 0) in average severity.

In the **thymus**, lymphoid cortical atrophy was present in 1/4 males at 100 mg/kg bw/day and 4/10 males 250/150 mg/kg bw/day. In females, these effects occurred in 1/5, 2/6 and 1/5 females at 40, 100 and 250 mg/kg bw/day, respectively. The unscheduled deaths at 100 and 250 mg/kg bw/day showed marked atrophy of the thymus. In the remaining animals, the incidence of the effect was only minimal or slight.

Table 24: Histopathological findings in reproduction organs/endocrine organs of males and females

Histopathological findings in reproduction organs/endocrine organs	**Dose [mg/kg bw/day]**			
	0	**40**	**100**	**250/150**
Number of animals per group	10	10	10	10
Primary effects				
TESTES (males)	5	5	9	10
- Enlarged cells	-	-	-	2
- Degeneration of seminiferous tubular epithelium	1	2	9	8
- Edema	4	3	7	7
- Chronic active inflammation	-	-	-	2
- dilated rete	-	1	1	-
TESTES, PAS STAGING (males)	5	5	5	8
- All stages missing	-	-	1	5
- Enlarged cells	-	-	-	2
- Most stages missing	-	-	4	1
- Some stages missing	-	-	-	1
- Multiple acrosomes	-	-	-	1
- Asynchronous tubules	-	2	4	1
- Individual cell necrosis	-	3	3	-
- Reduced spermatagonia	-	1	5	6
- Spermatidic giant cells	-	1	3	1
- Vacuolation basilar	-	-	1	-
EPIDIDYMIDES (males)	5	5	8	10
- Sperm granuloma	-	1	1	-
- Sperm degeneration	-	-	8	8
- Hypospermia	-	-	1	8
- Atrophy	-	-	7	8
- Chronic active inflammation	-	-	1	4
Secondary effects				
UTERUS (female)	5	6	8	6
- Stromal hyperplasia	-	-	-	1
- Dilation cyclic	-	-	-	3
- Haemorrhage	2	6	3	-

- Inflammation supp	-	-	1	-
- Necrotic debris/neut	-	-	1	-
- Implant sites	5	6	3	1
- Throphoblasts/Necro	-	-	3	-
ADRENALS(females)	5	-	1	5
- Hypertrophy cortex	5	-	-	-
- Extra cortical nodule	1	-	-	-
- Extramed haematopoies	-	-	1	-
MAMMARY GLAND AREA(females)	5	4	5	5
- Hyperplasia	5	4	4	-
- Inactive gland	-	-	1	5
- Active gland	-	1	1	-
- Infiltrate lymphoid	-	1	-	-
THYMUS (females)	5	5	6	5
- Increased apoptosis	-	1	-	-
- Haemorrhage/Congestion	-	1	1	-
- Athrophy lymphoid	-	1	2	1
- Hyperplasia duct	-	1	-	-
THYMUS (males)	5	5	4	8
- Increased apoptosis	-	1	2	-
- Haemorrhage/Congestion	-	1	1	1
- Athrophy lymphoid	-	-	1	4

*/** Fisher's Exact test significant at 5% (*) or 1% (**) level; +/++ Steel−test significant at 5% (+) or 1% (++) level; n.a. = not applicable; # mean values ± standard deviation

Hematology (No tabulated data in the Endpoint study record of the dossier)

Several haematological parameters were statistically significantly affected by treatment with the test substance. Decreased levels of haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets were noted for males treated at 250/150 mg/kg bw/day. For females, decreased levels of haemoglobin, mean corpuscular volume (MCV), MCH, MCHC and increased prothrombin time were noted at 100 and 250/150 mg/kg bw/day. In addition, levels of MCV and MCH were also statistically significantly decreased for females treated at 40 mg/kg bw/day.

For pups/litters:

Viability and clinical signs

A clear dose-related effect on development was noted at 40, 100 and 250/150 mg/kg bw/day. Implantation sites were only noted for nine females at 100 mg/kg bw/day and two females at 250/150 mg/kg bw/day. The remaining females were non pregnant or did not mate. Therefore, no pups could be examined for postnatal development. Out of the nine litters at 40 mg/kg bw/day, only six had live pups at first litter check. The number of pups per litter was decreased when compared to the control group. In addition, most of these pups did not survive the first days of lactation; only four litters had some live pups at planned necropsy.

At 40 mg/kg bw/day, lean and pale appearance were seen in the surviving pups.

Body weights

Body weights of the pups at 40 mg/kg bw/day were slightly, but not statistically significantly decreased when compared to the control group.

Gross pathology

Macroscopic findings involved absence of milk in the stomach and blue discolouration of the snout. In addition, autolysis was noted for pups found dead.

Table 25: Developmental data

	Dose [mg/kg bw/day]			
	0	**40**	**100**	**250/150**
Pub weight [g]#	6.0 ± 0.7	5.6 ± 0.5	n.a.	n.a.
Sex ratio [% males]	42	43	n.a.	n.a.
Viability index [%]	99	66.7**	n.a.	n.a.
Litter size	10	9	n.a.	n.a.
Dead pups at first litter check				
- Litters affected	0/10	6/10**	n.a.	n.a.
- Total	0	13	n.a.	n.a.
Living pups at first litter check	103	30	n.a.	n.a.
- Mean per litter	10.3 ± 2.4	3.3 ± 3.2++	n.a.	n.a.
Postnatal loss				
- Litters affected	1/10	1/9	n.a.	n.a.
- Total	1	10**	n.a.	n.a.
- % of living pups	1.0	33	n.a.	n.a.

*/** Fisher's Exact test significant at 5% (*) or 1% (**) level; +/++ Steel−test significant at 5% (+) or 1% (++) level; n.a. = not applicable; # mean values ± standard deviation

3.10.1.2 Pre/postnatal developmental toxicity study in mouse

Hardin et al., 1987. Evaluation of 60 chemical in a preliminary developmental toxicity Test. Teratog Carcinog mutagen. 7:29-48.

Test type

- Published data
- No guideline was followed
- Non GLP
- In this screening developmental toxicity test with a total of 60 chemicals, pregnant mice were dosed with the respective test substance during Days 6-13 of pregnancy and then allowed to deliver litters. The number of liveborn pups, their birth weight, and their growth and survival until Day 3 of age were used as indices of potential developmental toxicity.

Test substance

- Glycol, ethylene, monomethyl ether acrylate;
- Analytical purity: no data;
- Batch: no data;

Test animals

- Female CD-1 mouse (Charles River laboratory);
- 50 females/doses;
- Age at study initiation: 6-8 weeks;

Administration/exposure

- Oral: gavage;
- daily, 7 days/week;
- Day 6-13 of gestation;
- vehicle: distilled water at 10 mL/kg bw;
- Actual dose ingested: 650 mg/kg bw;
- Control : concurrent vehicle;
- Dose selection rationale: dose levels was based on a preliminary dose-finding study, in which 10 virgin females per group received the test substance once daily at 5 different dose levels for 8 consecutive days. The animals were observed for mortalities and clinical signs of toxicity during a period of 8 days post-treatment. Based on the outcome of the study, the predicted LC_{10} value (650 mg/kg bw) was selected as dose level for treatment in the developmental toxicity screening test
- Statistical methods: The Statistical Analysis System (SAS Institute Inc., Cary, NC, USA) was used to analyse data of individual parameters. Body weights on GD 6 were analysed by two-tail ANOVA to verify that there were no group differences in the initial body weight. Mortality (excluding deaths attributed to doing errors) was contrasted between pregnant and nonpregnant mice by two-tail Fisher's exact test. Nonpregant mice were excluded from all subsequent analysis. One-tail Fisher's exact test was used to compare the proportion of pregnant survivors with viable litters (at least 1 liveborn pup) to the concurrent vehicle control. For mice that delivered a viable litter, maternal body weight change from GD 6 to PD (postnatal day) 3, the number of liveborn pups per litter. Percent

neonatal survival to PD 3, average pub weight at birth, and average pup weight gain by PD 3 were analysed by pairwise multiple comparisons of control and treated groups using a two-tail Mann-Whitney U-test.

- Experimental procedures were carried out by personnel without knowledge of the specific dose or treatment groups.

Examination

Cage side observations: Time schedule: animals were observed for clinical signs of toxicity and mortality twice daily during treatment (GD 6-13) and once daily on GD 14-17. Beginning on GD 18, mice were observed twice daily for signs of parturition.

Post-mortem examinations: Sacrifice on Day 3 post-partum; Organs examined: the uteri of animals, which failed to deliver, were examined for the presence of implantation sites as evidence of early termination of pregnancy.

Results and discussion

Maternal examination

The test substance induced deaths in 15/50 dams, corresponding to a mortality rate of 30%.

Developmental effects

The number of litters was reduced in treated dams (14 litters) compared to control dams (28 litters). No liveborn pups were observed in any of the 14 litters of the treated dams. This effect was significantly different from the control group, which had 25 viable litters compared to a total of 28 litters. Overall, the test substance adversely affected all measures of reproductive success, since no liveborn pups were recorded.

Table 26: Test results (mean ± SD)

	Dose (mg/kg bw)	Maternal response variables			Neonatal response variables			
		No dead/treated	Weigh change (g)	Viable litters	Liveborn per litter	Percentage survival	Birth weight (g)	Weight gain (g)
Control	water	0/50	6.9±3	25/28	10±2.9	96±10	1.5±0.2	0.4±0.3
2-methoxyethyl acrylate	650	15/50	na	0/14*	0	na	na	na

*p<0.05 relative to concurrent control;
na: not available

3.10.2 Human data

No data.

3.10.3 Other data (e.g. studies on mechanism of action)

No data.

3.11 Specific target organ toxicity – single exposure

Not evaluated.

3.12 Specific target organ toxicity – repeated exposure

No specific study available.

3.13 Aspiration hazard

Not evaluated.

4 ENVIRONMENTAL HAZARDS

Not evaluated.