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:

α,α'-propylenedinitrilodi-o-cresol

EC Number: **202-374-2**

CAS Number: **94-91-7**

Index Number: **604-RST-VW-Y**

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Evaluation not performed for this substance.

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Evaluation not performed for this substance.

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Evaluation not performed for this substance.

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Evaluation not performed for this substance.

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Evaluation not performed for this substance.

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Study reference:

Study report, 2012 reported from ECHA Dissemination (2021); Study: 001, key

Detailed study summary and results:

Test type

Study according to OECD Guideline 471, GLP compliant (incl. QA statement)

Test substance

- α,α'-propylenedinitrilodi-o-cresol
- Purity: >99 corr. area %
- BASF Test Item No.: 11/0600-1
- Batch Number: 11000129U0
- Expiration Date: February 04, 2013
- Physical state, appearance: Liquid, highly viscous, yellowish
- Storage conditions: Room temperature

Administration/exposure

- Strains: *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100; *E. coli* WP2 uvr A
- Target gene: HIS/TRP
- Metabolic activation system:
    - S9-mix:
        - The S9 fraction was prepared at BASF SE in an AAALAC-approved laboratory in accordance with the German Animal Welfare Act and the effective European Council Directive.
        - At least 5 male Wistar rats [Crl:WI(Han)] (200 - 300 g; Charles River Laboratories Germany GmbH) received 80 mg/kg bw phenobarbital i.p. and β-naphthoflavone orally (both supplied by Sigma-Aldrich, 82024 Taufkirchen, Germany) each on three consecutive days. During this time, the animals were housed in Makrolon cages: central air conditioning with a fixed range of temperature of 20 - 24°C and a relative humidity of 30 - 70%. The day/night rhythm was 12 hours.
        - Standardized pelleted feed and tap water from bottles were available ad libitum.
        - 24 hours after the last administration, the rats were sacrificed, and the livers were prepared using sterile solvents and glassware at a temperature of +4°C. The livers were weighed and washed in a weight-equivalent volume of a 150 mM KCl solution, then cut into small pieces and homogenized in three volumes of KCl

solution. After centrifugation of the homogenate at 9000 x g for 10 min at +4°C, 5 mL portions of the supernatant (so-called S9 fraction) were stored at -70°C to -80°C.

- The preparation date: 01 Sep 2011
- Content of protein: 28.7 g/L

- induced or not induced: induced
- co-factors used:
  - 8 mM $MgCl_2$
  - 33 mM KCl
  - 5 mM glucose-6-phosphate
  - 4 mM NADP
  - 15 mM phosphate buffer (pH 7.4)

- Test concentrations:
  - 1 µg - 5000 µg/plate (SPT, *Salmonella* strains)
  - 33 µg - 5000 µg/plate (SPT, *E. coli*)
  - 1 µg - 333 µg/plate (PIT, *Salmonella* strains)
  - 10 µg - 2500 µg/plate (PIT, *E. coli*)

  - 1. Experiment:
    0; 33; 100; 333; 1000; 2500 and 5000 µg/plate, Standard plate test (SPT) with all strains with and without S9 mix
  - 2. Experiment:
    0; 1; 3.3; 10; 33; 100 and 333 µg/plate, SPT with *Salmonella* strains with and without S9 mix; Reason: bacteriotoxicity was observed in the standard plate test
  - 3. Experiment:
    0; 1; 3.3; 10; 33; 100 and 333 µg/plate (*Salmonella* strains), 0; 10; 33; 100; 333; 1000 and 2500 µg/plate (*E. coli* WP2uvrA), Preincubation test (PIT) with and without S9 mix; Reason: no mutagenicity was observed in the standard plate test
  - Number of plates: 3 per dose or control
- Vehicle: DMSO
  DMSO was used due to the limited solubility of the test substance in water. DMSO had been demonstrated to be suitable in bacterial reverse mutation tests; historical control data are available
- Controls:
  - Sterility controls: yes
  - Vehicle controls: yes
  - Positive controls: yes
  - Positive control substance:

- With S9 mix:
  - 2-aminoanthracene (2-AA):
    - 2.5 µg/plate, dissolved in DMSO; strains: TA 1535, TA 100, TA 1537, TA 98
    - 60 µg/plate, dissolved in DMSO; strain: *E. coli* WP2 uvrA
- Without S9 mix:
  - N-methyl-N'-nitro-N-nitrosoguanidine (MNNG):
    - 5 µg/plate, dissolved in DMSO; strains: TA 1535, TA 100
  - 4-nitro-o-phenylenediamine (NOPD):
    - 10 µg/plate, dissolved in DMSO; strain: TA 98
  - 9-aminoacridine (AAC):
    - 100 µg/plate, dissolved in DMSO; strain: TA 1537
  - 4-nitroquinoline-N-oxide (4-NQO):
    - 5 µg/plate, dissolved in DMSO; strain: *E. coli* WP2 uvrA

- Method of application:
  - in agar (plate incubation, SPT)
  - preincubation (PIT)
  - Incubation period: 20 min preincubation in medium (PIT) and incubation in agar at 37°C for 48 - 72 hours (PIT and SPT)
- The test substance is considered positive in this assay if the following criteria are met:
  - A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.
- A test substance is generally considered non-mutagenic in this test if:
  - The number of revertants for all tester strains are within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.
- Statistical methods: not applicable

Results and discussion

- In agreement with the recommendations of current guidelines 5 mg/plate or 5 µL/plate are generally selected as maximum test dose at least in the first experiment.
- Cytotoxicity:
  - A strong bacteriotoxic effect (reduced his- background growth, decrease in the number of his+ revertants, reduction in the titer) was observed in the standard plate test depending on the strain and test conditions from about 100 µg/plate onward.

- In the standard plate test with *E.coli* WP2uvrA a bacteriotoxic effect (reduced trp- background growth, decrease in the number of trp+ revertants, reduction in the titer) was observed from about 1000 µg/mL onward.
- In the preincubation assay bacteriotoxicity (reduced his- or trp- background growth, decrease in the number of his+ or trp+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 33 µg/plate onward.

- Genotoxicity: negative (results of experiment 1 and 3 are shown in Table 2 and Table 3)
  - The test substance did not lead to a relevant increase in the number of revertant colonies either without S9 mix or after adding a metabolizing system in three experiments carried out independently of each other (standard plate test and preincubation assay).
- Negative control data: With and without S9 mix, the number of revertant colonies in the negative controls was within or nearby the range of the historical negative control data for each tester strain.
- Positive control data: The positive control substances both with and without S9 mix induced a significant increase in the number of revertant colonies within the range of the historical positive control data or above.
- No test substance precipitation was found with and without S9 mix.

Table 1: Results of bacterial reverse mutation test (experiment 1; standard plate test) of α,α'-propylenedinitrilodi-o-cresol

With or without S9 mix	Concentration of test substance (µg/plate)	Number of revertants (number of colonies/plate in each of 3 plates, mean ± S.D.)				
		TA1535	TA100	TA1537	TA98	WP2uvrA
Without S9-mix	DMSO	12 13 15 (13 ± 2)	73 81 76 (77 ± 4)	7 8 6 (7 ± 1)	19 17 26 (26 ± 5)	48 48 36 44 ± 7)
	33	12 15 11 (13 ± 2)	70 73 79 (74 ± 5)	5 5 9 (6 ± 2)	19 24 20 (21 ± 3)	58 41 37 (45 ± 11)
	100	10 13 17 (13 ± 4)	74 75 71 (73 ± 2)	7 5 11 (8 ± 3)	18 17 21 (19 ± 2)	46 38 38 (41 ± 5)
	333	9 11 5 (8 ± 3)	14 31 16 (20 ± 09)	4 4 4 (4 ± 0)	16 10 12 (13 ± 3)	48 52 44 (48 ± 4)
	1000	1B 3B 1B (2 ± 1)	0B 0B 0B	0B 0B 0B	3B 3B 2B (3 ± 1)	47 30 31 (36 ± 10)
	2500	0B 0B 0B	0B 0B 0B	0B 0B 0B	0B 0B 0B	26 27 23 (25 ± 2)
	5000	0B 0B 0B	0B 0B 0B	0B 0B 0B	0B 0B 0B	0B 0B 0B
With S9-mix	DMSO	14 16 16 (15 ± 1)	93 92 88 (91 ± 3)	9 5 11 (8 ± 3)	25 21 26 (24 ± 3)	49 49 51 (50 ± 1)
	33	17 12 17 (15 ± 3)	91 75 92 (86 ± 10)	7 7 9 (8 ± 1)	19 24 30 (24 ± 6)	57 41 50 (49 ± 8)
	100	15 14 17 (15 ± 2)	93 73 93 (45 ± 17)	6 7 9 (7 ± 2)	17 20 24 (20 ± 4)	62 50 40 (51 ± 11)
	333	12 10 10 (11 ± 1)	57 26 53 (45 ± 17)	2 4 2 (3 ± 1)	11 13 11 (12 ± 1)	50 51 31 (44 ± 11)
	1000	2B 2B 1B (2 ± 1)	0B 0B 0B	0B 0B 0B	2B 1B 1B (1 ± 1)	32 34 22 (29 ± 6)
	2500	0B 0B 0B	0B 0B 0B	0B 0B 0B	0B 0B 0B	12 9 12

With or without S9 mix	Concentration of test substance (µg/plate)	Number of revertants (number of colonies/plate in each of 3 plates, mean ± S.D.)				
		TA1535	TA100	TA1537	TA98	WP2uvrA
						(11 ± 2)
	5000	0B 0B 0B	0B 0B 0B	0B 0B 0B	0B 0B 0B	0B 0B 0B
Without S9-mix	MNG (5 µg)	917 882 983 (927 ± 51)	786 902 906 (865 ± 68)	274 328 333 (312 ± 33)	688 611 523 (607 ± 83)	836 811 935 (884 ± 50)
With S9-mix	2-AA (2.5 µg)	157 126 178 (154 ± 26)	912 936 1014 (954 ± 53)	139 193 141 (158 ± 31)	694 622 700 (672 ± 43)	217 280 285 (261 ± 38)

B: reduced background growth

Table 2: Results of bacterial reverse mutation test (experiment 2; standard plate test) of α,α'-propylenedinitrilodi-o-cresol

With or without S9 mix	Concentration of test substance (µg/plate)	Number of revertants (number of colonies/plate in each of 3 plates, mean ± S.D.)			
		TA1535	TA100	TA1537	TA98
Without S9-mix	DMSO	10 12 12 (11 ± 1)	81 70 72 (74 ± 6)	6 7 6 (6 ± 1)	15 16 20 (17 ± 3)
	1	7 15 12 (11 ± 4)	85 79 85 (83 ± 3)	6 6 7 (6 ± 1)	18 17 14 (16 ± 2
	3.3	12 10 13 (12 ± 2)	75 73 73 (74 ± 1)	5 7 5 (6 ± 1)	17 14 21 (17 ± 4)
	10	10 14 16 (13 ± 3)	74 70 72 (72 ± 2)	8 4 8 (7 ± 2)	18 16 17 (17 ± 1)
	33	9 14 15 (13 ± 3)	61 77 59 (66 ± 10)	4 7 6 (6 ± 2)	21 16 16 (18 ± 3)
	100	10 13 7 (10 ± 3)	78 73 65 (72 ± 7)	4 4 4 (4 ± 0)	15 10 8 (11 ± 4)
	333	7 5 2 (5 ± 3)	36 26 19 (27 ± 9)	1 1 2 (1 ± 1)	5 3 6 (5 ± 2)
With S9-mix	DMSO	15 12 16 (14 ± 2)	73 90 84 (82 ± 9)	8 5 6 (6 ± 2)	24 22 27 (24 ± 3)
	1	13 10 14 (12 ± 2)	79 80 86 (82 ± 4)	6 7 10 (8 ± 2)	20 21 29 (23 ± 5)
	3.3	17 16 11 (15 ± 3)	76 88 87 (84 ± 7)	7 6 7 (7 ± 1)	24 21 20 (22 ± 2)
	10	13 18 10 (14 ± 4)	65 96 84 (82 ± 16)	5 7 8 (7 ± 2)	29 28 27 (28 ± 1)
	33	11 13 10 (11 ± 2)	72 90 75 (79 ± 10)	6 7 4 (6 ± 2)	26 20 17 (22 ± 4)
	100	14 15 14 (14 ± 1)	62 61 50 (58 ± 7)	5 6 5 (5 ± 1)	22 22 17 (20 ± 3)
	333	10 8 6 (8 ± 2)	22 42 30 (31 ± 10)	3 3 1 (2 ± 1)	10 13 10 (11 ± 2)
Without S9-mix	MNG (5 µg)	833 772 802 (802 ± 31)	832 807 869 (836 ± 31)	442 378 365 (395 ± 41)	558 632 671 (587 ± 40)
With S9-mix	2-AA (2.5 µg)	165 178 124 (156 ± 28)	721 667 765 (718 ± 49)	135 128 149 (137 ± 11)	655 580 613 (616 ± 38)

Table 3: Results of bacterial reverse mutation test (experiment 3; preincubation test) of α,α'-propylenedinitrilodi-o-cresol

With or without S9 mix	Concentration of test substance (µg/plate)	Number of revertants (number of colonies/plate in each of 3 plates, mean ± S.D.)				
		TA1535	TA100	TA1537	TA98	WP2uvrA
Without S9-mix	DMSO	10 11 14 (12 ± 2)	87 74 70 (77 ± 9)	5 6 8 (6 ± 2)	18 21 17 (19 ± 2)	45 50 41 (45 ± 5)
	1	9 13 12 (11 ± 2)	68 74 80 (74 ± 6)	5 6 7 (6 ± 1)	17 19 22 (19 ± 3)	-
	3.3	14 12 15 (14 ± 2)	90 72 81 (81 ± 9)	6 7 6 (6 ± 1)	16 18 21 (18 ± 3)	-
	10	11 11 11 (11 ± 0)	75 93 85 (84 ± 9)	5 7 8 (7 ± 2)	14 23 19 (19 ± 5)	39 45 45 (43 ± 3)
	33	9 12 10 (10 ± 2)	75 62 69 (69 ± 7)	4 8 6 (6 ± 2)	24 22 14 (20 ± 5)	41 42 40 (41 ± 1)
	100	7B 5B 5B (6 ± 1)	25B 31B 26B (27 ± 3)	5B 4B 4B (4 ± 1)	10B 8B 8B (9 ± 1)	36 17 34 (29 ± 10)
	333	0B 0B 0B	0B 0B 0B	0B 0B 0B	0B 0B 0B	15 23 12 (17 ± 6)
	1000	-	-	-	-	7 5 8 (7 ± 2)
	2500	-	-	-	-	0B 0B 0B
With S9-mix	DMSO	13 12 15 (13 ± 2)	99 105 91 (98 ± 7)	5 8 8 (7 ± 2)	25 21 24 (23 ± 2)	44 52 49 (48 ± 4)
	1	14 16 12 (14 ± 2)	96 132 78 (102 ± 27)	5 6 9 (7 ± 2)	19 28 22 (23 ± 5)	-
	3.3	14 14 10 (13 ± 2)	101 88 121 (103 ± 17)	10 3 8 (7 ± 4)	23 19 31 (24 ± 6)	-
	10	14 13 9 (12 ± 3)	80 86 90 (85 ± 5)	7 7 8 (7 ± 1)	21 15 25 (20 ± 5)	43 63 42 (49 ± 12)
	33	10 12 7 (10 ± 3)	77 86 88 84 ± 6)	3 6 6 (5 ± 2)	17 21 20 (19 ± 2)	42 42 46 (43 ± 2)
	100	7B 10B 8B (8 ± 2)	63B 62B 60B (62 ± 2)	6B 3B 1B (4 ± 2)	12B 14B 10B (12 ± 2)	34 40 33 (36 ± 4)
	333	2B 3B 4B (3 ± 1)	30B 24B 36B (30 ± 6)	2B 4B 1B (2 ± 2)	2B 5B 5B (4 ± 2)	30 33 30 (31 ± 2)
	1000	-	-	-	-	8 11 4 (8 ± 4)
	2500	-	-	-	-	0B 0B 0B
Without S9-mix	MNG (5 µg)	621 594 637 (617 ± 22)	884 731 766 (794 ± 80)	-	-	-
	AAC (100 µg)	-	-	335 411 354 (367 ± 40)	-	-
	NOPD (10 µg)	-	-	-	558 731 774 (688 ± 114)	-
	NQO (5.0µg)	-	-	-	-	641 687 663 (664 ± 23)
With S9-mix	2-AA (2.5 µg)	774 731 652 (719 ± 62)	884 831 907 (874 ± 39)	147 119 133 (133 ± 14)	774 651 625 (683 ± 80)	-
	2-AA (60 µg)	-	-	-	-	255 237 266 (253 ± 15)

B: reduced background growth

3.8.1.2 Study 2

Study reference:

Study report, 2012 reported from ECHA Dissemination (2021); Study: 002, key

Detailed study summary and results:

Test type

Study according to OECD Guideline 476, GLP compliant (incl. QA statement)

Test substance

- α,α'-propylenedinitrilodi-o-cresol
- Purity: >99 corr. area %
- BASF Test Item No.: 11/0600-1
- Batch Number: 11000129U0
- Expiration Date: February 04, 2013
- Physical state, appearance: Liquid, highly viscous, yellowish
- Storage conditions: Room temperature

Administration/exposure

- Strains:
    - Chinese hamster lung fibroblasts (V79)
    - Before freezing, the level of spontaneous mutants was depressed by treatment with HAT-medium. Each batch is screened for mycoplasm contamination and checked for karyotype stability and spontaneous mutant frequency.
- Target gene: HPRT (hypoxanthine-guanine phosphoribosyl transferase)
- Metabolic activation system: with and without
    - Phenobarbital/β-naphthoflavone induced rat liver S9 mix:
        - Preparation: The S9 were prepared from 8 - 12 weeks old male Wistar rats (Hsd Cpb: WU, weight approx. 220 – 320 g, Harlan Laboratories B.V., 5960 AD Horst, The Netherlands) induced by intraperitoneal applications of 80 mg/kg bw phenobarbital (Desitin, 22335 Hamburg, Germany) and by peroral administrations of 80 mg/kg bw β-naphthoflavone (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 x g. Aliquots of the supernatant were frozen and stored in ampoules at -80 °C. Small

numbers of the ampoules were kept at -20 °C for up to one week. Each batch of S9 mix was routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

- The protein concentration of the S9 preparation was 22.5 mg/mL (Lot. No.: 020212) in the pre-experiment and 27.3 mg/mL (Lot. No.: 050412) in experiment I and 35.0 mg/mL (Lot No.: 110512) in experiment II.
- An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 supernatant to reach following concentrations in the S9 mix:
  - 8 mM $MgCl_2$
  - 33 mM KCl
  - 5 mM glucose-6-phosphate
  - 4 mM NADP
  - in 100 mM sodium-phosphate-buffer, pH 7.4.

- Test concentrations:
  - Pre-experiment:
    - Without S9 mix: 22.0; 44.0; 88.0; 132.0; 176.0; 352.5; 705.0; 1410.0; 2820.0 µg/mL
    - With S9 mix: 22.0; 44.0; 88.0; 132.0; 176.0; 352.5; 705.0; 1410.0; 2820.0 µg/mL
  - 1. Experiment:
    - Without S9 mix: 0.0; 11.0; 22.0; 44.0; 88.0; 132.0; 176.0 µg/mL
    - With S9 mix: 0.0; 11.0; 22.0; 44.0; 88.0; 176.0; 264.0 µg/mL
  - 2. Experiment:
    - Without S9 mix: 0.0; 1.4; 2.8; 5.5; 11.0; 22.0; 33.0 µg/mL
    - With S9 mix: 0.0; 22.0; 44.0; 88.0; 176.0; 264.0; 352.0 µg/mL
  - In experiment I with metabolic activation and in experiment II with and without metabolic activation the cultures at the lowest concentrations were not continued as a minimum of only four analysable concentrations is required by the guidelines. In experiment I without metabolic activation the cultures at the two highest concentrations were not continued due to exceedingly severe cytotoxicity.
- Vehicle: DMSO; the final concentration of DMSO in the culture medium was 0.5% v/v.
- Controls:
  - Untreateted negative controls: no
  - Negative solvent / vehicle controls: yes
  - True negative controls: no
  - Positive controls: yes
  - Positive control substance:

- with metabolic activation: 7,12-dimethylbenz(a)anthracene; final concentration: 1.1 µg/mL = 4.3 µM
- without metabolic activation: Ethylmethanesulphonate; final concentration: 0.15 mg/mL = 1.2 mM

- Method of application:
  - in medium
  - Preincubation period: 24 hours
  - Exposure duration:
    - In the first experiment the treatment period was 4 h with and without metabolic activation. The second experiment was performed with a treatment time of 4 h with and 24 h without metabolic activation.
    - Expression/fixation time: Three or four days after treatment $1.5x10^6$ cells per experimental point were sub-cultivated in 175 cm² flasks containing 30 mL medium. Following the expression time of 7 days five 80 cm² cell culture flasks were seeded with about 3 - $5x10^5$ cells each in medium containing 6-thioguanine (6-TG). Two additional 25 cm² flasks were seeded with approx. 500 cells each in non-selective medium to determine the viability. The cultures were incubated at 37 °C in a humidified atmosphere with 1.5% CO_2 for about 8 days. The colonies were stained with 10% methylene blue in 0.01% KOH solution. The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope.
  - Number of replicants: two independent experiments with identical experimental procedures
  - Determination of cytotoxicity: Toxicity of the test item was indicated by a reduction of the cloning efficiency (CE)
- Evaluation criteria:
  - The assay is considered acceptable if it meets the following criteria: The numbers of mutant colonies per 10^6 cells found in the solvent controls falls within the laboratory historical control data.
  - Positive control: The positive control substances should produce a significant increase in mutant colony frequencies.
  - The cloning efficiency II (absolute value) of the solvent controls should exceed 50%.
    → The data of this study comply with the above mentioned criteria.
  - A test item is classified as positive if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points. A test item producing neither a concentration-related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.

- A positive response is described as follows:
  - A test item is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
  - The test item is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed. However, in a case by case evaluation this decision depends on the level of the corresponding solvent control data. If there is by chance a low spontaneous mutation rate within the laboratory´s historical control data range, a concentration-related increase of the mutations within this range has to be discussed. The variability of the mutation rates of solvent controls within all experiments of this study was also taken into consideration.

- Statistics: A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. The number of mutant colonies obtained for the groups treated with the test item were compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological and statistical significance was considered together.

Results and discussion

- Range finding pre-experiment: The range finding pre-experiment was performed using a concentration range of 22.1 to 2820 µg/mL (≈10 mM) to evaluate toxicity in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation.
- Cytotoxicity:
  - Pre-experiment: Relevant cytotoxic effects indicated by a relative suspension growth below 50 were noted at 176.3 µg/mL and above in the presence of metabolic activation following 4 h treatment. Strong toxic effects indicated by a completely inhibited cell growth occurred in the absence of metabolic activation at 176.3 µg/mL and above following 4 h treatment and at 44.1 µg/mL and above following 24 h treatment.
  - Main experiment: Relevant cytotoxic effects indicated by a relative cloning efficiency I or cell density below 50% in both parallel cultures occurred in the first experiment at 88.0 µg/mL without metabolic activation and at 264 µg/mL with metabolic activation. In the second experiment cytotoxic effects as described above occurred at 176 µg/mL and above with metabolic activation. The recommended cytotoxic range of approximately 10-20%

relative cloning efficiency I or relative cell density was covered with and without metabolic activation.

- Precipitation:
  - Pre-experiment: The test medium was checked for precipitation or phase separation at the end of each treatment period (4 or 24 h). Precipitation occurred at 1410 µg/mL and above with and without metabolic activation following 4 and 24 h treatment.
  - Main experiment: Precipitation of the test item visible to the naked eye was noted at 88.0 µg/mL and above in experiment I with metabolic activation. However, the precipitate was probably denatured protein rather than test item per se as there was no precipitation in the second experiment at comparable or even higher concentrations.
- Osmolarity: There was no relevant shift of the osmolarity of the medium even at the maximum concentration of the test item measured in the pre-experiment.
- Genotoxicity:
  - Negative
  - No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximum concentration (see
  -
  -
  - Table 4, Table 5, Table 6, Table 7). The mutant frequency generally did not exceed the historical range of solvent controls. A single increase of the induction factor exceeding the threshold of three times the mutation frequency of the corresponding solvent control was observed in the first culture of the second experiment without metabolic activation at 2.8 µg/mL (24 h treatment). However, the increase was based on a rather low mutation frequency of the solvent control of just 4.8 colonies per 10^6 cells. Furthermore, the effect was not reproduced in the parallel culture under identical experimental conditions. Therefore, the increase of the induction factor was judged as biologically irrelevant fluctuation.
  - Statistics: A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. A significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was solely detected in the second culture of the second experiment without metabolic activation. This trend however, was judged as irrelevant since it was not reproduced in the parallel culture under identical experimental conditions and the threshold described above was not exceeded.

Table 4: Mutagenicity data (mutation rates), experiment I, culture I

Test group	**Conc. µg/mL**	**P**	**S9 mix**	**Number of mutant colonies per flask found after plating in 6-TG medium**							**Mutant Colonies per 10^6 cells**	**Induction factor**
				I	II	III	IV	V	mean	SD		
Column	1	2	3	4	5	6	7	8	9	10	11	12
Solvent control with DMSO			-	3	3	4	4	4	3.6	0.5	12.4	1.0
Positive control with EMS	150.0		-	54	49	38	41	39	44.2	7.0	130.8	10.6
Test item	11.0		-	1	1	2	2	1	1.4	0.5	5.2	0.4
Test item	22.0		-	5	5	6	6	7	5.8	0.8	17.7	1.4
Test item	44.0		-	1	1	2	5	5	2.8	2.0	10.4	0.8
Test item	88.0		-	3	3	1	4	1	2.4	1.3	8.9	0.7
Test item	132.0		-	culture was not continued[#]								
Test item	176.0		-	culture was not continued[#]								
Solvent control with DMSO			+	2	1	2	4	1	2.0	1.2	9.4	1.0
Positive control with DMBA	1.1		+	215	202	195	211	223	209.2	11.0	742.8	78.8
Test item	11.0		+	culture was not continued[##]								
Test item	22.0		+	2	2	5	6	3	3.6	1.8	11.8	1.3
Test item	44.0		+	5	5	6	7	8	6.2	1.3	17.9	1.9
Test item	88.0	P	+	3	1	2	4	3	2.6	1.1	4.8	0.5
Test item	176.0	P	+	4	2	2	0	1	1.8	1.5	3.8	0.4
Test item	264.0	P	+	2	1	2	5	1	2.2	1.6	7.1	0.7

[#] culture was not continued due to strong toxic effects

[##] culture was not continued since a minimum of only four analysable concentrations is required

P precipitation

Table 5: Mutagenicity data (mutation rates), experiment I, culture II

Test group	Conc. µg/mL	P	S9 mix	Number of mutant colonies per flask found after plating in 6-TG medium							Mutant Colonies per 10^6 cells	Induction factor
				I	II	III	IV	V	mean	SD		
Column	1	2	3	4	5	6	7	8	9	10	11	12
Solvent control with DMSO			-	1	2	1	3	1	1.6	0.9	5.4	1.0
Positive control with EMS	150.0		-	35	39	40	35	37	37.2	2.3	91.4	16.8
Test item	11.0		-	1	1	1	0	1	0.8	0.4	2.1	0.4
Test item	22.0		-	1	2	1	1	0	1.0	0.7	2.6	0.5
Test item	44.0		-	1	3	3	4	1	2.4	1.3	7.1	**1.3**
Test item	88.0		-	1	3	1	1	4	2.0	1.4	6.4	1.2
Test item	132.0		-	culture was not continued[#]								
Test item	176.0		-	culture was not continued[#]								
Solvent control with DMSO			+	2	3	4	5	6	4.0	1.6	16.6	1.0
Positive control with DMBA	1.1		+	239	221	207	200	211	215.6	15.1	1057.2	63.5
Test item	11.0		+	culture was not continued[##]								
Test item	22.0		+	1	2	4	7	4	3.6	2.3	12.2	0.7
Test item	44.0		+	4	1	2	2	4	2.6	1.3	11.5	0.7
Test item	88.0	P	+	2	3	3	7	4	3.8	1.9	15.1	0.9
Test item	176.0	P	+	2	1	1	2	1	1.4	0.5	5.3	0.3
Test item	264.0	P	+	4	3	2	1	1	2.2	1.3	9.7	0.6

[#] culture was not continued due to strong toxic effects

[##] culture was not continued since a minimum of only four analysable concentrations is required

P precipitation

Table 6: Mutagenicity data (mutation rates), experiment II, culture I

Test group	Conc. µg/mL	S9 mix	Number of mutant colonies per flask found after plating in 6-TG medium							Mutant Colonies per 10^6 cells	Induction factor
			I	II	III	IV	V	mean	SD		
Column	1	2	3	4	5	6	7	8	9	10	11
Solvent control with DMSO		-	1	2	2	2	1	1.6	0.5	4.8	1.0
Positive control with EMS	150.0	-	83	90	99	92	91	91.0	5.7	244.4	51.2
Test item	1.4	-	culture was not continued[#]								
Test item	2.8	-	4	6	6	4	6	5.2	1.1	17.4	3.7
Test item	5.5	-	2	1	3	6	2	2.8	1.9	10.2	2.1
Test item	11.0	-	4	5	2	3	4	3.6	1.1	12.4	2.6
Test item	22.0	-	1	5	5	2	2	3.0	1.9	10.7	2.2
Test item	33.0	-	3	1	1	2	3	2.0	1.0	7.0	1.5
Solvent control with DMSO		+	4	8	5	3	10	6.0	2.9	18.8	1.0
Positive control with DMBA	1.1	+	112	126	110	102	99	109.8	10.5	304.2	16.2
Test item	22.0	+	culture was not continued[#]								
Test item	44.0	+	3	4	3	2	3	3.0	0.7	7.5	0.4
Test item	88.0	+	6	7	5	5	7	6.0	1.0	18.1	1.0
Test item	176.0	+	4	2	1	4	7	3.6	2.3	11.5	0.6
Test item	264.0	+	10	4	8	3	5	6.0	2.9	19.3	1.0
Test item	352.0	+	6	2	2	6	8	4.8	2.7	13.2	0.7

[#] culture was not continued since a minimum of only four analysable concentrations is required

Table 7: Mutagenicity data (mutation rates), experiment II, culture II

Test group	Conc. µg/mL	S9 mix	Number of mutant colonies per flask found after plating in 6-TG medium							Mutant Colonies per 10^6 cells	Induction factor
			I	II	III	IV	V	mean	SD		
Column	1	2	3	4	5	6	7	8	9	10	11
Solvent control with DMSO		-	2	2	1	2	1	1.6	0.5	4.3	1.0
Positive control with EMS	150.0	-	30	32	29	35	37	32.6	3.4	129.5	30.1
Test item	1.4	-	culture was not continued[#]								
Test item	2.8	-	4	1	2	3	3	2.6	1.1	7.4	1.7
Test item	5.5	-	1	2	1	1	2	1.4	0.5	5.1	1.2
Test item	11.0	-	2	1	1	2	1	1.4	0.5	4.6	1.1
Test item	22.0	-	2	1	2	2	3	2.0	0.7	7.4	1.7
Test item	33.0	-	6	6	5	4	1	4.4	2.1	12.6	2.9
Solvent control with DMSO		+	8	8	3	5	6	6.0	2.1	23.6	1.0
Positive control with DMBA	1.1	+	104	104	114	98	103	104.6	5.8	348.6	14.8
Test item	22.0	+	culture was not continued[#]								
Test item	44.0	+	3	8	7	10	3	6.2	3.1	19.0	0.8
Test item	88.0	+	8	3	3	3	3	4.0	2.2	12.6	0.5
Test item	176.0	+	2	6	6	4	5	4.6	1.7	14.6	0.6
Test item	264.0	+	9	5	4	6	2	5.2	2.6	16.7	0.7
Test item	352.0	+	13	11	12	13	12	12.2	0.8	38.7	1.6

[#] culture was not continued since a minimum of only four analysable concentrations is required

3.8.1.3 Study 3

Study reference:

Study report, 2012 reported from ECHA Dissemination (2021); Study: 004, supp.

Detailed study summary and results:

Test type

Study according to OECD Guideline 473, GLP compliant (incl. QA statement)

Test substance

- α,α'-propylenedinitrilodi-o-cresol
- Purity: >99 corr. area %
- BASF Test Item No.: 11/0600-1
- Batch Number: 11000129U0
- Expiration Date: February 04, 2013
- Physical state, appearance: Liquid, highly viscous, yellowish
- Storage conditions: Room temperature
- Stability under test conditions: Not indicated by the sponsor; test item was always prepared freshly before use

Administration/exposure

- Strains: Chinese hamster lung fibroblasts (V79)
- Handling of cell type:
    - Type and identity of media: MEM (minimal essential medium) containing Hank's salts, glutamine and Hepes (25 mM)
    - Properly maintained: yes
    - Periodically checked for Mycoplasma contamination: yes
    - Periodically checked for karyotype stability: yes
- Metabolic activation system: with and without
    - Phenobarbital/β-naphthoflavone induced S9 mix:
        - Phenobarbital/ β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from 8 - 12 weeks old male Wistar rats (Hsd Cpb: WU, Harlan Laboratories B.V., 5960 AD Horst, The Netherlands) weight approx. 220 - 320 g induced by intraperitoneal applications of 80 mg/kg bw phenobarbital (Desitin, 22335 Hamburg, Germany) and by orally administrations of 80 mg/kg bw β-naphthoflavone (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) each, on three consecutive days. The livers were prepared 24 h after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 x g. Aliquots of the supernatant were frozen and stored in ampoules at -80 °C. Small numbers of the ampoules were kept at -20 °C for up to one week. Each batch of S9 mix was routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.
        - The protein concentration was 29.8 mg/mL (Lot no. 120811).
    - chemicals used for induction: phenobarbital
    - co-factors used:

        - 8 mM $MgCl_2$
        - 33 mM KCl
        - 5 mM glucose-6-phosphate
        - 4 mM NADP
        - 100 mM sodium-ortho-phosphate-buffer (pH 7.4)
- Pre-experiment/test concentrations:
    - maximum concentration 2820 µg/mL (due to molecular weight of the test item)
    - Preliminary test (concludingly used as main test): 11, 22, 44, 88, 176, 352, 705, 1410, and 2820 µg/mL (with and without S9-mix)
- Vehicle:
    - DMSO
    - Justification for choice of solvent/vehicle: The solvent was chosen due to its solubility properties and its relative non-toxicity to the cell cultures.
- Controls:
    - Untreateted negative controls: no
    - Negative solvent / vehicle controls: yes, with DMSO
    - True negative controls: no
    - Positive controls: yes
    - Positive control substance:
        - without metabolic activation: ethylmethanesulphonate (EMS)
        - with metabolic activation:; cyclophosphamide (CPA)
- Experimental conditions:
    - Method of application: in medium
    - Duration:
        - Preincubation period: 54 h
        - Exposure duration: 4 h
        - Expression time (cells in growth medium): 14 h
        - Preparation interval: 18 h
        - Fixation time (start of exposure up to fixation or harvest of cells): approx. 22 h
    - Spindle inhibitor (cytogenetic assays): Colcemid
    - Staining (for cytogenetic assays): Giemsa
    - Number of cells evaluated: 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides
- Number of replications: two parallel cultures
- Evaluation of cytotoxicity: mitotic index
- Determination of polyploidy: yes

- Determination of endoreplication: yes
- Evaluation criteria:
    - The chromosome aberration test is considered acceptable, if
        - The number of structural aberrations found in the solvent controls falls within the range of the laboratory historical control data.
        - The positive control substances produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratory historical control data.
    - A test item is classified as non-clastogenic if: the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory historical control data and no significant increase of the number of structural chromosome aberrations is observed.
    - A test item is classified as clastogenic if: the number of induced structural chromosome aberrations is not in the range of the laboratory historical control data and either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.
    - If the criteria mentioned above for the test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed. Although the inclusion of the structural chromosome aberrations is the purpose of this study, it is important to include the polyploids and endoreduplications.
    - The following criterion is valid: A test item can be classified as aneugenic if: the number of induced numerical aberrations is not in the range of the laboratory historical control data.
- Statistical methods: Statistical significance was confirmed by means of the Fisher's exact test ($p < 0.05$). However, both biological and statistical significance should be considered together.

Results and discussion

- Validity of untreted negative controls: valid
- Validity of vehicle controls: valid
- Validity of positive controls: valid; induction of statistically significant increases in cells with structural chromosome aberrations.
- Cytotoxicity:
    - Reduced mitotic indices could be observed in the main experiment at 352.5 µg/mL and above in the absence of S9 mix and at 176.3 µg/mL and above in the presence of S9 mix.
    - In the absence of S9 mix concentrations showing clear cytotoxicity were not scorable for cytogenetic damage.

- Genotoxicity:
    - Positive
    - Clastogenicity (see Table 8):
        - without S9 mix after treatment with 22.0, 44.1 and 88.1 µg/mL: 13.5, 12.5, 14.0% aberrant cells, excluding gaps
        - with S9 mix after treatment with 22.0, 44.1, 88.1 and 176.3 µg/mL: 10.5, 7.0, 10.0 and 20.5% aberrant cells, excluding gaps
        - clearly exceeding the range of the historical control data of 0.0 - 4.0% aberrant cells, excluding gaps.
    - increase in the rate of polyploid metaphases was found after treatment with the test item (2.1 - 3.6%) as compared to the rates of the solvent controls (2.5 and 3.1%).
    - No relevant increase in endomitotic metaphases was found after treatment with the test item as compared to the frequencies of the control cultures.
    - A slight increase in the rate of endomitotic metaphases was found in the presence of S9 mix after treatment with the test item (0.0 - 0.9%) as compared to the rates of the solvent controls (0.0 - 0.3%). However, these increases occurred at the two lowest evaluated concentrations and no dose-dependency could be observed.
- Test-specific confounding factors:
    - Effects of pH: no, pH: 7.5
    - Effects of osmolality: mOsm (solvent control) : 384 and mOsm (test item) : 357
    - Precipitation: yes, at 352.5 µg/mL and above
- Range-finding studies: Yes, also used as main study
- Comparison with historical control data: yes

Table 8: Summary of results of the chromosomal aberration study

Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Endomitotic cells in %	Cell numbers in % of control	Mitotic indices in % of control	Incl. gaps* (%)	Aberrant cells in % excl. gaps *	With exchanges (%)
Exposure period 4 h without S9 mix								
18 h	Solvent control[1]	2.5	0.0	100.0	100.0	1.5	1.0	0.0
	Positive control[2]	n.d.	n.d.	n.d.	73.8	19.5	19.5[S]	11.0
	11.0	2.9	0.0	95.5	122.4	3.5	3.0	1.0
	22.0	2.2	0.0	102.3	120.5	14.0	13.5[S]	9.0
	44.1	3.1	0.0	94.1	115.7	12.5	12.5[S]	8.0
	88.1	2.1	0.0	104.9	84.3	15.0	14.0[S]	9.0
	176.3	2.9	0.0	111.0	75.2	2.0	1.5	0.5
Exposure period 4 h with S9 mix								
18 h	Solvent control[1]	3.1	0.3	100.0	100.0	1.5	1.5	0.5
	Positive control[3]	n.d.	n.d.	n.d.	52.3	9.5	7.5[S]	3.0
	11.0	3.6	0.9	100.0	108.5	1.5	1.0	0.5
	22.0	3.5	0.9	82.3	120.6	11.0	10.5[S]	6.5
	44.1	2.6	0.0	84.8	124.9	7.0	7.0[S]	4.0
	88.1	2.4	0.0	78.2	95.7	10.0	10.0[S]	4.0
	176.3	2.1	0.0	83.3	46.6	21.0	20.5[S]	9.0

* Inclusive cells carrying exchanges

n.d. Not determined

[S] Aberration frequency statistically significant higher than corresponding control values

[1] DMSO 0.5% (v/v)

[2] EMS 900.0 µg/mL

[3] CPA 1.4 µg/mL

3.8.2 Animal data

3.8.2.1 Study 1

Study reference:

Study report, 2013 reported from ECHA Dissemination (2021)

Detailed study summary and results:

Test type

Study according to OECD Guideline 474, GLP compliant

Test substance

- α,α'-propylenedinitrilodi-o-cresol
- Purity: >99 corr. area %
- BASF Test Item No.: 11/0600-1
- Batch Number: 11000129U0
- Expiration Date: February 04, 2013
- Physical state, appearance: Liquid, highly viscous, yellowish
- Storage conditions: Room temperature

Test animals

- Male NMRI mice
- Source: Charles River Germany
- Age at study initiation: 8 - 9 weeks
- Total number of animals: 43 males
- Acclimation: minimum 5 days
- Weight at study initiation: mean value 35.0 g (SD ± 1.8 g)
- Randomly assigned to test groups
- Housing: single
- Diet: ad libitum
- Water: ad libitum
- Environmental conditions:
    - Temperature (°C): 22 ± 2°C
    - Humidity (%):45 - 65% (> 95% for few hours; during the acclimation phase of the animals used for the pre-experiments the relative humidity was > 95% for a few hours. This deviation, however, does not affect the validity of the study.)
    - Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- No. of animals per sex per dose: 7 males for the test substance, 5 males for controls (vehicle and positive control)
- Oral via gavage

- Vehicle: polyethylene glycol (PEG) 400, the vehicle was chosen due to its relative non-toxicity for the animals. The administered volume was 10 mL/kg bw including test substance.
- Doses:
    - 24 h preparation interval: 500, 1000, and 2000 mg/kg bw
    - 48 h preparation interval: 2000 mg/kg bw
    - Criteria for dose selection:
        - A preliminary study on acute toxicity was performed with two animals per sex under identical conditions as in the mutagenicity study concerning: animal strain, vehicle, route, frequency, and volume of administration.
        - The animals were treated orally with the test item and examined for acute toxic symptoms at intervals of approximately 1 h, 2-4 h, 6 h, 24 h, 30 h, and 48 h after administration of the test item.
- Control animals: yes, concurrent vehicle
- Positive control:
    - cyclophosphamide
    - Justification for choice: Potent inducer of micronuclei for this route of administration
    - Doses / concentrations: 40 mg/kg bw
- Preparation of dosing solutions: On the day of the experiment, the test item was dissolved in PEG 400.
- Duration of treatment: one single oral application
- Post exposure period: 24 or 48 h
- Tissues and cell types examined: Tissues and cell types examined: Per animal 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei. To investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.
- Methods of animal handling and slide preparation
    - At the beginning of the treatment the animals (including the controls) were weighed and the individual volume to be administered was adjusted to the animal’s body weight.
    - The animals of all dose groups, except the positive control were examined for acute clinical signs at intervals of around 1 h, 2 - 4 h, 6 h, 24 h, and/or 48 h after administration of the test item and vehicles.
    - Sampling of the bone marrow was done 24 and 48 hours after treatment, respectively.
    - The animals were sacrificed using CO_2 followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out with foetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 min and the supernatant was discarded. A small drop of the resuspended cell pellet was spread on a slide.

The smear was air-dried and then stained with May-Grünwald (Merck, 64293 Darmstadt, Germany)/Giemsa (Merck, 64293 Darmstadt, Germany). Cover slips were mounted with EUKITT (Kindler, 79110 Freiburg, Germany). At least one slide was made from each bone marrow sample.

- Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. Per animal 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei. To investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides. All animals per sex and test group were evaluated as described.

- Evaluation criteria:
  - The study is considered valid if the following criteria are met:
    - at least 5 animals per test group can be evaluated.
    - PCE to erythrocyte ratio should not be less than 20% of the vehicle control.
    - the positive control shows a statistically significant and biological relevant increase of micronucleated PCEs compared to the vehicle control.
  - A test item is classified as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group.
  - Statistical methods (nonparametric Mann-Whitney test (8)) are used as an aid in evaluating the results, if necessary. However, the primary point of consideration is the biological relevance of the results.
  - A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered non-mutagenic in this system.
- Statistics: Statistical methods (nonparametric Mann-Whitney test are used as an aid in evaluating the results, if necessary.

Results and discussion

- Genotoxicity: negative
- Toxicity: yes, mortality in one top dose amimal
- Validity of vehicle controls: valid
- Validity of negative controls: valid
- Validity of positive controls: valid
- Range finding study:
  - Two animals of each sex treated in the pre-experiments received the test item dissolved in PEG 400 once orally. The volume administered was 10 mL/kg bw

- Pre-experiment: In the first pre-experiment a dose of 1500 mg/kg bw and in the second pre-experiment a dose of 2000 mg/kg bw was tested in males and females. In both pre-experiments no clinical signs of toxicity were observed.
- On the basis of these data 2000 mg/kg bw were estimated to be suitable as highest dose. No sex specific differences were observed with regard to clinical signs. In accordance with the test guidelines the main study was performed using males only.

- Definitive study:
  - For each test item dose group 7 males received once orally administrations of α,α'-propylenedinitrilodi-o-cresol dissolved in PEG 400. The volume administered was 10 mL/kg bw
  - Clinical signs of toxicity (reduction of spontaneous activity, abdominal position, eyelid closure and ruffled fur): only observed in one high dose animal that died within 24 hours. All other dosed animals were free of clinical signs.
  - Negative control (PEG 400): no clinical signs.
  - After treatment with the test item at 48 h preparation interval the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that α,α'-propylenedinitrilodi-o-cresol did not induce cytotoxic effects in the bone marrow.
  - In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item (see Table 9). The mean values of micronuclei observed after treatment were below or near to the value of the vehicle control group. Additionally, no dose dependent increase in the frequency of detected micronuclei was observed with increasing dosages and all values in dose groups were very well within the laboratory's historical vehicle control data.
  - Positive control: 40 mg/kg bw cyclophosphamide administered orally was used as positive control which showed a statistically significant increase of induced micronucleus frequency.
  - The following dose levels of the test item were investigated (seven animals per group; except for the group with 2000 mg/kg b.w., because one animal died approximately 24 h after administration):
    - 24 h preparation interval: 500, 1000, and 2000 mg/kg bw
    - 48 h preparation interval: 2000 mg/kg bw
  - The study was considered valid as all validity criteria were met.

Table 9: *in vivo* micronucleus test results

Test group	Dose (mg/kg b.w.)	Sampling time (h)	PCEs with micronuclei (%)	Range (absolut value per animal)*	PCE per 2000 erythrocytes
Vehicle control	0	24	0.160	1 - 5	1251
Test item	500	24	0.093	0 - 6	1254
Test item	1000	24	0.107	0 - 6	1305
Test item	2000	24	0.067	1 - 3	1206
Positive control	40	24	1.600	20 - 41	1240
Vehicle control	0	48	0.060	0 - 2	1185
Test item	2000	48	0.093	0 - 4	1216

* The range of the absolut numbers of PCEs with micronuclei per 2000 PCEs per animal is given here

3.9 Carcinogenicity

Evaluation not performed for this substance.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1

Study reference:

Study report, 2013 reported from ECHA Dissemination (2021); Study: 001, key

Detailed study summary and results:

Test type

Study according to OECD Guideline 422, GLP compliant

Test substance

- α,α'-propylenedinitrilodi-o-cresol
- Purity: >99 corr. area %
- No information on impurities
- Batch Number: 11000129U0
- BASF Test Item No.: 11/0600-1
- Expiration Date: February 04, 2013
- Physical state, appearance: liquid, highly viscous, yellowish
- Storage conditions: room temperature

Test animals

- Male and female Wistar rats (Crl:WI(Han))
- Source: Charles River Germany
- 10 animals/sex/dose
- Age at study initiation: approximately 11 weeks
- Weight at study initiation: not quantitatively specified, animals of comparable size and weight
- Housing: 5 animals/sex and cage pre-mating, one male and one female during mating, females individually after mating, males group-housed
- Diet and water ad libitum
- Acclimation period: At least 5 days prior to start of treatment
- Environmental conditions:
    - Temperature (°C): 18 to 24°C
    - Humidity (%): 40 to 70%
    - Air changes (per hr): 15 room air changes/hour
    - Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- Oral via gavage
- Exposure:
    - Frequency of treatment: daily
    - Males: exposed for 29 days, i.e. 2 weeks prior to mating, during mating, and up to termination.
    - Females: exposed for 42-45 days, i.e. during 2 weeks prior to mating, during mating, during post coitum, and during 4 days of lactation.
    - Animal assignment by computer-generated random algorithm according to body weight, with all animals within ± 20% of the sex mean.
- 25, 75 and 250 mg/kg bw/day (actual dose received); Doses based on a 14-days dose range finding study.
- Control animals: yes, concurrent vehicle
- Positive control: N/A
- No historical control data provided
- Vehicle: polyethylene glycol (PEG); vehicle was chosen based on trial formulations performed at WIL Research Europe
- Preparation of dosing solutions:
    - Formulations (w/w) were prepared daily within 6 hours prior to dosing

- Analytical verification of doses: Yes. Analyses were conducted on a single occasion during the treatment phase. Samples of formulations were analyzed for homogeneity (highest and lowest concentration) and accuracy of preparation (all concentrations). Stability in vehicle over 6 hours at room temperature was also determined (highest and lowest concentration).

Description of test design:

- In the context of the repeated dose part of the study, haematological and neurobehavioral examinations were performed and clinical chemnistry parameters compiled.
- Mating procedure:
  - Following a minimum of 14 days of exposure for the males and females, one female was cohabitated with one male of the same treatment group, avoiding sibling mating.
  - Detection of mating: by evidence of sperm in the vaginal lavage or by the appearance of an intravaginal copulatory plug. This day was designated Day 0 post-coitum.
  - After detection of mating, the males and females were separated.
  - A maximum of 14 days was allowed for mating, after which females who had not shown evidence of mating were separated from their males.
- Observations and tests of live animals P0:
  - Daily cage side observations for mortality: at least twice a day
  - Detailed clinical observations outside home cage in a standard arena
    - Once prior the start of treatment
    - Daily at least immediately after dosing
    - Weekly during treatment period
  - Body weight: Males and females weighed on the first day of exposure and weekly thereafter. Mated females were weighed on Days 0, 4, 7, 11, 14, 17 and 20 post-coitum and during lactation on Days 1 and 4.
  - Food consumption: Weekly, except for males and females which were housed together for mating and for females without evidence of mating. Food consumption of mated females was measured on Days 0, 4, 7, 11, 14, 17 and 20 post-coitum and on Days 1 and 4 of lactation.
  - Water consumption: Subjective appraisal during the study, no quantitative investigation
- Post mortem examinations for P0:
  - Prior to planned necropsy, all males and the selected 5 females/group were deprived of food overnight (with a maximum of 24 hours). Water was provided. Non-selected females were not deprived of food.
  - Animals surviving to scheduled necropsy were deeply anaesthetized using isoflurane (Abbott B.V., Hoofddorp, The Netherlands) and subsequently exsanguinated.

- Macroscopic examination: all animals were subject to macroscopic examination of the cranial, thoracic and abdominal tissues and organs, with special attention being paid to the reproductive organs.
- Samples of tissues and organs collected from all animals and fixed in 10% buffered formalin (neutral phosphate buffered 4% formaldehyde solution, Klinipath, Duiven, The Netherlands)
- The numbers of former implantation sites and corpora lutea recorded for all paired females.
- Histopathological examination:
  - All samples were embedded and cut at a thickness of 2-4 micrometers and stained with haematoxylin and eosin (Klinipath, Duiven, The Netherlands).
  - From the selected 5 males of the control and high dose group, and all males suspected to be infertile, additional slides of the testes were prepared to examine staging of spermatogenesis. The testes were processed, sectioned at 3-4 micrometers, and stained with PAS/haematoxylin (Klinipath, Duiven, The Netherlands).
  - A peer review on the histopathology data was performed by a second pathologist.
  - Slides examined by a pathologist:
    - Slides of the preserved organs and tissues of the selected 5 animals/sex of Groups 1 and 4.
    - The additional slides of the testes of all males of Groups 1 and 4 and all males suspected to be infertile to examine staging of spermatogenesis.
    - Slides of the preserved organs and tissues of the animals of all dose groups which died spontaneously.
    - Slides of all gross lesions of all animals (all dose groups).
    - The reproductive organs of all animals of Groups 1 and 4, and of males 31 and 36 (Group 4) and females 71 and 763 (Group 4); both females had a total litter loss.
- Organ weights: The following organ and terminal body weight were recorded on the scheduled day of necroscopy from the selected 5 animals/sex/group. Adrenal glands, Testes, Epididymides, Uterus (including cervix), Prostate, Seminal vesicles including coagulating glands, Ovaries, Thyroid including parathyroid

- Oestrous cycle: N/A
- Observations of live animals F1:
  - each litter observed for
    - mortality / viability: The numbers of live and dead pups on Day 1 of lactation and daily thereafter were determined. If possible, defects or cause of death were evaluated.

        - detailed clinical observations: at least one per day; external abnormalities recorded
        - body weight: Live pups weighed on Days 1 and 4 of lactation
        - sex: determined for all pups on Days 1 and 4 of lactation
        - the presence of milk in the stomach
    - necropsy pups: Pups surviving until planned termination were killed by decapitation on Days 5-6 of lactation.
    - If possible, defects or cause of death were evaluated.
- Postmortem examination for F1:
    - Pups surviving to planned termination were killed by decapitation on Days 5-6 of lactation.
    - All pups were sexed and descriptions of all external abnormalities were recorded. The stomach was examined for the presence of milk. If possible, defects or cause of death were evaluated.
- Statistics:
    - Dunnett-test (many-to-one t-test) for assumably normally distributed data
    - The Steel-test (many-to-one rank test) for data not assumed to follow a normal distribution
    - Fisher Exact-test applied to frequency data
    - All tests were two-sided and in all cases $p < 0.05$ was accepted as the lowest level of significance.
    - Group means calculated for continuous data and
    - Medians calculated for discrete data (scores)
    - Individual values, means and standard deviations may have been rounded off before printing.
- Reproductive indices assessed for each group:
    - Mating index (%)
    - Fertility index (%)
    - Conception index (%)
    - Gestation index (%)
    - Duration of gestation
- Offspring viability indices assessed for each group:
    - Percentage live males at First Litter Check
    - Percentage live females at First Litter Check
    - Percentage of postnatal loss Days 0-4 of lactation
    - Viability index

Results and discussion

- Parental generation (P0) General toxicity:
    - No clinical signs of toxicity were noted during the observation period.

- Salivation seen after dosing among animals of the 25, 75 and 250 mg/kg bw/day dose group was considered to be a physiological response rather than a sign of systemic toxicity considering the nature and minor severity of the effect and its time of occurrence (i.e. after dosing). This sign may be related to irritancy/taste of the test substance.
- Incidental findings that were noted included regurgitation together with rales at a slight degree, chromodacryorrhoea (snout) and scabbing on different parts of the body. These findings occurred within the range of background findings to be expected for rats of this age and strain which are housed and treated under the conditions in this study. At the incidence observed, these were considered signs of no toxicological relevance.

- Mortality:
  - One female at 75 mg/kg bw/day and one female at 250 mg/kg bw/day died after dosing on Day 9 of the pre-mating period. Before death, the high dose female showed severe restlessness together with breathing difficulties (laboured respiration and gasping).

    Gross findings at necropsy included perforation of the oesophagus (granulation tissue and an inflammatory process at microscopic examination), discoloration of the lungs (reddish or dark-red) and Harderian glands (pale) and/or (yellowish) watery-cloudy fluid in the thoracic cavity for both females. These findings were suggestive of a gavage accident as cause of death. In this context it is noteworthy that animals treated with the test substance (Groups 2-4) were more restless during dosing than controls.
  - Two females at 250 mg/kg bw/day had to be euthanized after total litter loss on Day 1 of lactation.
- Body weight and weight changes:
  - Body weights and body weight gains were statistically significantly lower in males at 75 and 250 mg/kg bw/day on Day 8 of the pre-mating period and during the mating period (mating days 1, 8, and 15). However, the differences to controls were slight, and values remained within the range considered normal for rats of this age and strain (normal range: 5-95% confidence interval body weight gain on mating Day 15: 11-30%); see Table 10. Therefore, these differences were considered not to be toxicologically relevant. Body weights of females were not affected.

Table 10: Body weight of male and female animals [g]

		Sex	Control	25 mg/kg bw/d	75 mg/kg bw/d	250 mg/kg/bw/d
PRE MATING						
DAY 1 WEEK 1	MEAN	male	321	316	311	317
	ST.DEV	male	10.9	9.4	9.6	11.9
	N	male	10	10	10	10
DAY 8 WEEK 2	MEAN	male	341	332	322 *	327
	ST.DEV	male	15.2	12.2	10.2	16.4
	N	male	10	10	10	10
MATING PERIOD						
DAY 1 WEEK 1	MEAN	male	361	347	335 **	339 *
	ST.DEV	male	16.4	15.3	14.8	19.2
	N	male	10	10	10	10
DAY 8 WEEK 2	MEAN	male	369	356	344 **	347 *
	ST.DEV	male	19.1	12.6	16.8	20.5
	N	male	10	10	10	10
DAY 15 WEEK 3	MEAN	male	379	366	353 *	352 **
	ST.DEV	male	20.4	15.0	17.9	22.8
	N	male	10	10	10	10
PRE MATING						
DAY 1 WEEK 1	MEAN	female	209	206	209	206
	ST.DEV	female	5.1	7.7	4.1	8.9
	N	female	10	10	10	10
DAY 8 WEEK 2	MEAN	female	215	211	215	213
	ST.DEV	female	7.4	11.3	9.1	9.6
	N	female	10	10	10	10
POST COITUM						
DAY 0	MEAN	female	221	214	218	218
	ST.DEV	female	4.6	9.8	4.7	8.5
	N	female	9	10	9	9
DAY 7	MEAN	female	243	239	242	240
	ST.DEV	female	5.8	9.9	5.6	9.9
	N	female	9	10	9	9
DAY 14	MEAN	female	270	261	265	264
	ST.DEV	female	8.4	14.5	7.8	10.9
	N	female	9	10	9	9
DAY 20	MEAN	female	328	317	328	308
	ST.DEV	female	16.7	27.7	11.8	18.0

		Sex	Control	25 mg/kg bw/d	75 mg/kg bw/d	250 mg/kg/bw/d
	N	female	9	10	9	9
LACTATION						
DAY 1	MEAN	female	253	248	250	245
	ST.DEV	female	11.8	11.9	8.3	10.7
	N	female	10	10	9	9
DAY 4	MEAN	female	264	259	261	256
	ST.DEV	female	12.0	16.8	10.3	14.5
	N	female	10	10	9	7

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

- Food consumption and compound intake:
  - Food consumption was within the normal range. No toxicologically relevant changes in food consumption before or after correction for body weight were noted.
  - The slightly, but statistically significantly lower food intake (absolute and/or relative to body weight) noted for females at 75 mg/kg bw/day from Days 7-11 post-coitum and for females at 250 mg/kg bw/day from Days 0-4 and 7-11 post-coitum was considered to be of no toxicological relevance, as values remained within the range considered normal for rats of this age and strain and/or changes occurred in the absence of a treatment-related distribution.
  - At the individual level, both absolute and relative food intake was lower for three females at 250 mg/kg bw/day (nos. 73, 75 and 77) during lactation. This was at least for females 73 and 75 in part due to the relatively small litter size (5 and 4 pups, respectively).
- Haematological parameters:

  A higher relative numbers of reticulocytes at 75 and 250 mg/kg bw/d in males, and a slightly lower prothrombin time (PT) and activated partial thromboplastin time (APTT) in males at 25 mg/kg bw/day were considered to be of no toxicological relevance as they occurred in the absence of a treatment-related distribution and/or remained within the range considered normal for rats of this age and strain.
- Clinical biochemistry

  For both males and females, a lower concentration of bilirubin was noted at 250 mg/kg bw/d. Mean values were at the lower end of the historical range of data (1.6 µmol/L for males and 1.7 µmol/L for females in this study versus a historical control (5-95% confidence interval: 1.7-2.60 µmol/L for males and 1.6-3.0 µmol/L for females). However, in the absence of corroborative changes on other parameters, this finding was considered of no toxicological relevance.

Any other statistically significant changes were considered to be of no toxicological relevance as they occurred in the absence of a treatment-related distribution and/or remained within the range considered normal for rats of this age and strain.

- Hearing ability, pupillary reflex, static righting reflex and grip strength were normal in all animals. Locomotor activity was unaffected up to 250 mg/kg bw/d in both sexes.
- Organ weight findings including organ / body weight ratios:
  - A trend towards lower thymus organ weights (absolute and relative to body weight) was noted at 250 mg/kg bw/day (both sexes) compared to controls. This change was relatively slight and reached statistical significance for the mean value of absolute thymus organ weight in females only. Study authors considered these changes most likely secondary to stress caused by the local effects of the test substance on the stomach.
- Histopathological findings:
  - Stomach (both sexes):
    - An increased incidence and severity of hyperplasia of the squamous epithelium with hyperkeratosis was recorded at 250 mg kg bw/day in 6/6 males (moderate) and in 5/5 terminal females (2: minimal, 2: slight, 1: moderate).
    - A lymphogranulocytic inflammation of the forestomach at a minimal degree was recorded at 250 mg/kg bw/day in 6/6 males and 2/5 terminal females.
  - Thymus (females):
    - A slightly increased incidence and severity of lymphoid atrophy was recorded in 3/5 terminal females (1: minimal, 2: slight) at 250 mg/kg bw/day.
    - Minimal lymphoid atrophy of the thymus was also recorded in a single female of each the control and 75 mg/kg bw/day group and a single male at 250 mg/kg bw/day.
    - The minimal lymphoid atrophy of the thymus in single animals and all remaining microscopic findings recorded were considered to be within the normal range of background pathology encountered in Wistar-Han rats of this age and strain.
  - Examination of the reproductive organs of the animals (incl. assessment of the integrity of the spermatogenetic cycle for males) did not reveal any abnormalities up to 250 mg/kg bw/day.
- Reproductive function:
  - Two females at 250 mg/kg bw/day had a total litter loss on Day 1 of lactation, resulting in a gestation index of only 77.8% for the highest dose group as compared

to 100% for the remaining groups. The reason for the total litter loss could not be established as part of this study. Based on the in-life data there were no indications for a poor condition of these two females, and examination of the reproductive organs of the animals that failed to deliver healthy offspring (male 31, 36, female 71, 76) did not reveal any abnormalities.

- No treatment-related toxicologically significant changes were noted in any of the remaining reproductive parameters investigated in this study (i.e. mating, fertility and conception indices, precoital time, and numbers of corpora lutea and implantation sites); see Table 11, Table 12, Table 13 and Table 14.
- There was a trend towards slightly lower numbers of corpora lutea and implantation sites at 250 mg/kg bw/day. This was mainly attributable to females 75 and 79 that had 7 corpora lutea each and 5 and 7 implantation sites, respectively. Since lower numbers were also seen for control female 42 (8 corpora lutea and 8 implantation sites), this finding was considered of no toxicological relevance.
- The assessment of the integrity of the spermatogenetic cycle did not provide any evidence of impaired spermatogenesis.

Table 11: Reproduction data summary

	Control	25 mg/kg bw/d	75 mg/kg bw/d	250 mg/kg bw/d
Females paired	10	10	9	9
Females mated	10	10	9	9
Pregnant females	10	10	9	9
Females with living pups on Day 1	10	10	9	7
Mating index (%) (Females mated / Females paired) * 100	100.0	100.0	100.0	100.0
Fertility index (%) (Pregnant females / Females paired) * 100	100.0	100.0	100.0	100.0
Conception index (%) (Pregnant females / Females mated) * 100	100.0	100.0	100.0	100.0
Gestation index (%) (Females with living pups on Day 1 / Pregnant females) * 100	100.0	100.0	100.0	77.8

Table 12: Precoital time – P0 generation

Day of the pairing period	Control	25 mg/kg bw/d	75 mg/kg bw/d	250 mg/kg bw/d
NUMBER OF FEMALES MATED				
1	–	2	–	1
2	1	1	–	2
3	5	6	6	2
4	4	1	3	4
MEDIAN PRECOITAL TIME (days)	3	3	3	3
MEAN PRECOITAL TIME (days)	3.3	2.6	3.3	3.0
N	10	10	9	9

Table 13: Summary of corpora lutea and implantation sites at necropsy

		Control	25 mg/kg bw/d	75 mg/kg bw/d	250 mg/kg bw/d
Corpora Lutea (per female)	MEAN	12.6	14.0	14.1	11.1
	ST.DEV	2.7	4.5	3.1	2.8
	N	10	10	9	9
Implantations (per female)	MEAN	11.4	12.0	12.7	10.0
	ST.DEV	1.6	3.1	1.7	2.7
	N	10	10	9	9

Table 14: Developmental data: P0 generation - lactation

	Control	25 mg/kg bw/d	75 mg/kg bw/d	250 mg/kg bw/d
LITTERS TOTAL	10	10	9	9
DURATION OF GESTATION				
MEAN (+)	21.2	21.3	21.1	22.0
ST.DEV.	0.4	0.5	0.3	0.7
N	10	10	9	9
DEAD PUPS AT FIRST LITTER CHECK				
LITTERS AFFECTED (#)	1	1	0	4
TOTAL	1	2	0	15
MEAN (+)	0.1	0.2	0.0	1.7
ST.DEV.	0.3	0.6	0.0	3.6
N	10	10	9	9
LIVING PUPS AT FIRST LITTER CHECK				
% OF MALES / FEMALES (#)	45 / 55	45 / 55	57 / 43	45 / 55
TOTAL	110	102	112	58
MEAN (+)	11.0	10.2	12.4	6.4 +
ST.DEV.	1.7	3.6	1.9	4.7
N	10	10	9	9
POSTNATAL LOSS (after first litter check)				
% OF LIVING PUPS	0.9	0.0	0.0	0.0
LITTERS AFFECTED (#)	1	0	0	0
TOTAL (#)	1	0	0	0
MEAN (+)	0.1	0.0	0.0	0.0
ST.DEV.	0.3	0.0	0.0	0.0
N	10	10	9	9
VIABILITY INDEX (#)	99.1	100.0	100.0	100.0

Viability index = (Number of alive pups before planned necropsy / Number of pups born alive) * 100

+/++ Steel−test significant at 5% (+) or 1% (++) level

/ ## Fisher's Exact test significant at 5% (#) or 1% (##) level

- Gestation: Gestation index was decreased at 250 mg/kg bw/day, but 100% for the remaining groups, including controls. Duration of gestation was comparable for all groups.
- No signs of difficult or prolonged parturition were noted among the pregnant females.
- Examination of cage debris of pregnant females revealed no signs of abortion or premature birth.
- No deficiencies in maternal care were observed.
- Total litter loss was noted for two dams at 250 mg/kg bw/day at first litter check, leading to a decreased gestation index of 77.8%. In addition, excluding these two dams, mean live litter size was slightly lower in this group (control: 11.0; 25 mg/kg bw/day: 10.2; 75 mg/kg

bw/day: 12.4; 250 mg/kg bw/day: 8.3). No effects on the number of live and dead pups were observed at the lower dose levels.

- F1 generation
  - Clinical signs:
    - Incidental clinical symptoms of pups consisted of no milk in the stomach, missing tail apex, and wound and scabbing on the head. The nature and incidence of these clinical signs remained within the range considered normal for pups of this age, and they were therefore considered to be of no toxicological relevance.
  - Mortalilty: (see Table 15)
    - The mean number of living pups at first litter check was significantly lower at 250 mg/kg bw/day compared to controls. A total of 15 dead pups were recorded for 4 litters in the high dose group compared to 1 pup/1 litter, 2 pups/1 litter and 0 pups at 0, 25 and 75 mg/kg bw/day, respectively. Two out of these four females at 250 mg/kg bw/day (nos. 71 and 76) had a total litter loss on Day 1 of lactation with 11 and 1 dead pup, respectively.
    - There were no treatment-related effects at the lower dose levels of 25 and 75 mg/kg bw/day.
    - One control pup went missing on Day 3 of lactation. It was most likely cannibalised.

Table 15: Mortality, clinical signs and macroscopy of pups

Litter No	Number of pups without clinical symptoms	No of pups with clinical symptoms	Findings	
			Observation	Information
Control				
41	13	-		
42	6	2	FLC	No milk, missing on day 3
			FLC	Dead
43	9	1	FLC Day 2 Day 3 Day 4 LLC Macro	Missing tail apex Missing tail apex Missing tail apex Missing tail apex Missing tail apex Missing tail apex
44	10	-		
45	11	-		
46	12	-		
47	12	-		
48	12	-		
49	12	-		
50	11	-		
25 mg/kg bw/d				
51	2	-		
52	10	-		
53	13	-		
54	14	-		
55	7	-		
56	11	-		
57	8	-		
58	13	2	FLC Macro	Dead No findings
			FLC Macro	Dead No findings
59	12	-		
60	12	-		
75 mg/kg bw/d				
61	12	-		
62	13	-		
63	12	-		
64	16	-		

Litter No	Number of pups without clinical symptoms	No of pups with clinical symptoms	Findings	
			Observation	Information
66	12	-		
67	14	-		
68	13	-		
69	10	-		
70	9	1	FLC Day 2 Day 3 Day 4 LLC Macro	Wound head Scab No findings No findings No findings No findings
250 mg/kg bw/d				
71	-	11	FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
72	9	-		
73	5	2	FLC Macro	Dead No Milk
			FLC Macro	Dead No Milk
74	14	-		
75	4	-		

Litter No	Number of pups without clinical symptoms	No of pups with clinical symptoms	Findings	
			Observation	Information
76	-	1	FLC Macro	Dead Beginning autolysis, no milk
77	8	1	FLC Macro	Dead No Milk
78	11	-		
79	7	-		

FLC – first litter check, LLC – last litter check, Macro – macroscopic findings, pups without clinical symptoms were sacrificed on day 5 post partum.

- Body weight: No effects

Table 16: Pups' body weights [g]

DAY	SEX		Control	25 mg/kg bw/d	75 mg/kg bw/d	250 mg/kg bw/d
1	M	MEAN	6.1	6.2	5.8	5.9
		ST.DEV.	0.7	0.8	0.4	0.9
		N	10	9	9	7
	F	MEAN	5.8	5.9	5.4	5.8
		ST.DEV.	0.7	0.6	0.2	1.3
		N	10	10	9	7
	M+F	MEAN	5.9	6.0	5.6	5.9
		ST.DEV.	0.7	0.7	0.3	1.1
		N	10	10	9	7
4	M	MEAN	9.4	9.4	8.7	8.5
		ST.DEV.	1.4	1.0	0.5	2.2
		N	10	9	9	7
	F	MEAN	9.0	8.9	8.2	8.4
		ST.DEV.	1.4	0.8	0.3	2.4
		N	10	10	9	7
	M+F	MEAN	9.2	9.1	8.5	8.5
		ST.DEV.	1.4	0.9	0.4	2.3
		N	10	10	9	7

- Gross pathological findings:
  - Incidental macroscopic findings of pups that were found dead included beginning autolysis and/or no milk in the stomach. The only macroscopic finding among surviving pups was a missing tail apex for one control pup.

- There were no adverse effects on the duration of pregnancy, parturition, maternal care, sex ratio of the pups and pup development after first litter check regarding clinical signs, body weight and macroscopy up to 250 mg/kg bw/day.

3.10.1.2 Study 2

Study reference:

Study report, 2014 reported from ECHA Dissemination (2021); Study: 002, key

Detailed study summary and results:

Test type

Study similar to OECD Guideline 421, GLP compliant, deviations from the guideline: only one dose level tested with additional parameters of an OECD 416 study

Test substance

- α,α'-propylenedinitrilodi-o-cresol
- Analytical purity: >99.0 corr. area-%
- Lot/batch No.: 11000129U0
- Expiration date of the lot/batch: 04 Feb 2014
- Stability under test conditions: The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor, and the sponsor holds this responsibility.

Test animals

- Male and female Wistar rats (Crl:WI(Han))
- Source: Charles River Germany
- 10 animals/sex/dose
- Age at study initiation: 11-13 weeks
- Wheight at study initiation: males: 322.3 g - 353.5 g; females: 191.9 g - 213.2 g
- Housing: During the study period, the rats were housed individually in Makrolon type M III cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area of about 800 cm^2), with the following exceptions:
  - During overnight matings, male and female mating partners were housed together in Makrolon type M III cages.
  - Pregnant animals and their litters were housed together until PND 4.
- Diet and water ad libitum
- Acclimation period: yes
- Environmental conditions:

- Temperature (°C): 20 to 24°C
- Humidity (%): 30 to 70%
- Air changes (per hr): 15 room air changes/hour
- Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- Oral via gavage (stomach tube)
- Exposure:
  - Frequency of treatment: once daily
  - Number of animals per sex per dose: 25
  - Control animals: 25 male and 25 female Wistar rats were dosed daily with the vehicle only (Polyethylene glycol 400).
  - Rationale for animal assignment: random
  - The duration of treatment covered a 2-week premating and mating period in both sexes, about three weeks postmating in males, and the entire gestation period as well as approximately 4 days of the lactation period in females with litters, and about 3 weeks of postmating period in non-pregnant females.
- Positive control: N/A
- No historical control data provided
- Vehicle: polyethylene glycol 400; chosen based on the results of previous studies
- Concentration in vehicle: 0 or 5 g / 100 mL
- Amount of vehicle (if gavage): 5 mL/kg bw/d vehicle was chosen based on previous studies (OECD 422 85R0600/11X396)
- Dose: 250 mg/kg body weight/day
- Preparation of dosing solutions:
  - The test substance preparations in Polyethylene glycol 400 were prepared at the beginning of the administration period and daily thereafter.
  - Initially, the test substance was melted at approximately 50 degrees Celsius, aliquotted into suitable portions and stored at room temperature in the dark until preparation of the formulation.
  - For the test substance preparations, Polyethylene glycol 400 was added to each pre-weighed aliquot in a calibrated beaker and the formulations were heated up (at approximately 55°C) and stirred continuously with a magnetic stirrer (for approximately 15 minutes) to achieve homogeneity at a visually acceptable level. Subsequently, dose preparations were allowed to cool down to a maximum of 40 degrees Celsius before dosing. During administration, the preparations were kept homogeneous with a magnetic stirrer.

- Analytical verification of doses or concentration: Yes.
  - The homogeneity and concentration control analyses were carried out at the Analytical Chemistry Laboratory of the Experimental Toxicology and Ecology of BASF SE, Ludwigshafen, Germany.
  - Analytical verifications of the stability of the test substance in Polyethylene glycol 400 over a period of 6 hours at room temperature were carried out prior to the start of the study in a separate GLP study (WIL Research Europe B.V., Hambakenwetering 7, 5231 DD ‘s-Hertogenbosch, The Netherlands, Project 499381, BASF Project number 85R0600/11X396).
  - Samples of the test substance preparations were sent to the analytical laboratory twice (at the beginning and towards the end of the administration period) for verification of the concentrations. The samples taken for the concentration control analyses at the beginning of the administration period were also used to verify the homogeneity of the samples of the high-concentration (250 mg/kg bw/d). Three samples (one from the top, middle and bottom) were taken from the preparation vessel with the magnetic stirrer running.
  - Of each sample, one additional reserve sample was retained. Details of the sampling schedule were recorded with the raw data.
  - The analytical investigations of the test substance preparations were performed according to control procedure 11/0600_02.
- Analyses confirmed the overall accuracy of the prepared concentrations, the homogeneous distribution and the stability of the test substance in Polyethylene glycol 400 over a period of 6 hours at room temperature.

Description of test design:

- Mating procedure:
  - Each of the male and female animals was mated overnight (from about 16.00 h until 7.00-9.00 of the following morning) at a 1 : 1 ratio for a maximum of 2 weeks. Throughout the mating period, each female animal was paired with a predetermined male animal from the same dose group. Deviations from the specified times were possible on weekends and public holidays and were reported in the raw data.
  - A vaginal smear was prepared after each mating and examined for the presence of sperm. If sperm was detected, pairing of the animals was discontinued.
  - The day on which sperm were detected was denoted "gestation day (GD) 0" and the following day "gestation day (GD) 1".
- Reproductive indices:

- The pairing partners, the number of mating days until vaginal sperm was detected in the female animals, and the gestational status of the females were recorded for F0 breeding pairs.
- For the males, the follwoing mating and fertility indices were calculated for F1:
  - Male mating index
  - Male fertility index
- For the females, the following mating, fertility and gestation indices were calculated for F1:
  - Female mating index
  - Female fertility index
  - Gestation index
  - Live birth index
  - Postimplantation loss

- Observations and tests for P:
  - Cage side observations for moribund or dead animals: at least twice a day on working days and once a day on saturdays, sundays or on public holidays. (If animals were in a moribund state, they were sacrificed and necropsied. The examinations of these animals were carried out according to the methods established at the BASF SE Laboratory for Pathology, Experimental Toxicology and Ecology, Ludwigshafen, Germany.)
  - Cage side observation for any sign of morbidity, pertinent behavioral changes and signs of overt toxicity: once a day, morbidity, pertinent behavioral changes and signs of overt toxicity. Individual data of daily observations can be found in the raw data.
  - Observation of the parturition and lactation behavior of the dams: evaluated in the mornings in combination with the daily clinical inspection of the dams. On weekdays, the parturition behavior of the dams was additionally inspected in the afternoons. Only particular findings (e.g. inability to deliver) were documented on an individual dam basis. The day of parturition was considered the 24-hour period from about 15.00 h of one day until about 15.00 h of the following day.
  - Body weight:
    - In general, the body weight of the male and female parental animals was determined once a week in the morning until sacrifice. Body weight changes of the animals were calculated from these results.
    - During the mating period the parental females were weighed on the day of positive evidence of sperm (GD 0) and on GD 7, 14 and 20.
    - Females with litter were weighed on the day of parturition (PND 0) and on PND 4.
    - Females without positive evidence of sperm, without litter or waiting for necropsy, were also weighed weekly. These body weight data were solely used for the

calculations of the dose volume; therefore these values are not reported in the Summary but in the Individual Tables.

- Food consumption: Weekly
  - exceptions:
    - Food consumption was not determined after the 2nd premating week (male parental animals) and during the mating period (male and female F0 animals).
    - Food consumption of the F0 females with evidence of sperm was determined on gestation days (GD) 0-7, 7-14 and 14-20.
    - Food consumption of the F0 females, which gave birth to a litter, was determined on PND 1-4.
    - Food consumption was not determined in females without positive evidence of sperm during gestation period and females without litter during lactation and post-mating period.
- Oestrous cyclicity: N/A
- Sperm parameters: N/A

- Post mortem examinations for P0:
  - All parental animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology, special attention being given to the reproductive organs.
  - The following animals died intercurrently (No. 132, 133, 135 and 150) or were sacrificed moribund (No. 29) and were necropsied and assessed by gross pathology as soon as possible after their death.
  - Organ weights determined from all animals sacrificed on schedule:
    - Epididymides
    - Testes
  - Organ/tissue fixation in 4% neutral-buffered formaldehyde solution or in modified Davidson’s solution:
    - All gross lesions
    - Cervix
    - Coagulating glands
    - Epididymides (modified Davidson’s solution)
    - Ovaries (modified Davidson’s solution)
    - Oviducts
    - Prostate gland
    - Seminal vesicles
    - Testes (modified Davidson’s solution)

- Vagina
- Uterus
- Specific procedures:
  - The uteri of all cohabited female P0 parental animals were examined for the presence and number of implantation sites. The uteri of apparently nonpregnant animals or empty uterus horns were placed in 1% ammonium sulfide solutions for about 5 minutes in order to be able to identify early resorptions or implantations (SALEWSKI's method (1)). Then the uteri were rinsed carefully in physiologic salt solution (0.9 % NaCl).
  - The ovaries, testes and epididymides of animals that died or were sacrificed intercurrently were fixed in 4% neutral-buffered formaldehyde solution.

- Histopathology: only the stomach (forestomach and glandular stomach) was examined

- Observations for F1:
  - Initial observation on the day of birth for
    - Total number of pups
    - Sex: determined by the distance between the anus and the base of the genital tubercle; normally, the anogenital distance is considerably greater in male than in female pups.
    - Number of liveborn and stillborn pups (“stillborn” defined as dead before initial examination)
    - Macroscopically evident changes
  - Mortality / viability:
    - Check for moribund or dead pups twice a day on workdays (once in the morning and once in the afternoon) and once a day on Saturadays, Wundays or public holidays
    - The number and percentage of dead pups on the day of birth (PND 0) and of pups dying between PND 1-4 (lactation period) were determined. Pups, which died accidentally or were sacrificed due to maternal death, were not included in these calculations. The number of live pups/litter was calculated on the day after birth, and on lactation day 4. The viability index was calculated.
  - Sex ratio:
    - Final confirmation of sex at necropsy
    - The sex ratio calculated at day 0 and day 4 after birth
  - Clinical observations: The live pups were examined daily for clinical symptoms (including gross-morphological findings) during the clinical inspection of the dams and documented for each pup.
  - Body weight:

- The pups were weighed on the day after birth (PND 1) and on PND 4. The individual weights were always determined in the morning. Body weight change was calculated from these results.
- Pups that weigh less than 75% of the mean weight of the respective control pups on day after birth (PND 1) were determined and called “runts”.

- Post mortem observation F1:
  - Necropsy pups:
    - All pups with scheduled sacrifice on PND 4 were sacrificed under isoflurane anesthesia with CO2. All pups were examined externally and eviscerated; their organs were assessed macroscopically.
    - All stillborn pups and all pups that died before PND 4 were examined externally, eviscerated and their organs were assessed macroscopically.
    - All pups without notable findings or abnormalities were discarded after their macroscopic evaluation. Animals with notable findings or abnormalities were evaluated on a case-by-case basis, depending on the type of finding noted.

Results and discussion

- First parental generation (P0)
  - General toxicity:
    - Salivation:
      - Nearly all male and female animals of all test groups (0 and 250 mg/kg bw/d) showed salivation after treatment during the whole study period. This transient salivation for a few minutes (up to 10 minutes) immediately after treatment was likely to be induced by the unpleasant taste of the vehicle (Polyethylene glycol 400) or by local irritation of the upper digestive tract.
      - This observation is not considered to be a sign of systemic toxicity.
    - Three females (Nos. 132, 133 and 135) of test group 1 (250 mg/kg bw/d) died during the parturition process on GD 23. Two of them showed adverse clinical findings preceding death:
      - Female No. 132 showed apathy (GD 22-23), piloerection and a reddish, brown vaginal discharge (GD 23, respectively).
      - Female No. 133 showed apathy on GD 22.
    - For one female of test group 1 (No. 140) dystocia was recorded on GD 22.
    - One test substance-treated dam had only stillborn pups (female no. 143 with 13 pups).

- Furthermore, one control animal (no. 103) and one test-substance treated animal (no. 137) had a complete litter loss on PND 0 (including stillborn pups and pups that died during the first observation day).
- One sperm-negative female of test group 0 and 1, each, (No. 110 – 0 mg/kg bw/d; No. 136 – 250 mg/kg bw/d) did not deliver F1 pups. In one female of test group 0 (No. 121 – 0 mg/kg bw/d) no sperm was detected in the vaginal smears (no GD 0), but the animal delivered pups.
- Mortality:
    - Four P0 females of test group 1 (250 mg/kg bw/d) died during the gestation period. Females Nos. 132, 133 and 135 were unable to deliver and were found dead on GD 23, respectively (see Table 17). Furthermore, female No. 150 was found dead on GD 10 without showing any clinical findings which could explain the premature death.
    - One P0 male (No. 29 – 250 mg/kg bw/d) was sacrificed moribund due to a gavage error on day 16 of the post-mating phase.

Table 17: Summary delivery report of female P0 rats

		control	250 mg/kg bw/d
No. of females at start	N	25	25
No. of females mated	N	25 f-	25
	%	100.0	100.0
Pregnant	N	24 f-	24
	%	96.0	96.0
Dead	N	0	4
Without delivery	N	1	5
- Pregnant	N	0	4
- Not pregnant	N	1	1
Delivering	N	24 f-	20
	%	100.0	83.3
With liveborn pups	N	24 f-	19 *
Gestation Index	%	100.0	79.2
Gestation days	Mean	22.0 n	22.4 **
	S.d.	0.3	0.6
	N	23	20
Litters with stillborn pups	N	3 f+	6
	%	12.5	30.0
Litters with all pups stillborn	N	0 f+	1
	%	0.0	5.0

Statistic Profile = Fisher's exact test (one-sided-), Student's t-test (two-sided), Fisher's exact test (one-sided+), * p<=0.05, ** p <=0.01
f=FISHER-EXACT; n=STUDENT

- Body weight:
  - Mean body weights of the substance-treated P0 males and females (250 mg/kg bw/d) were generally comparable to the respective concurrent control group during the entire study period (see Table 18).
  - The body weight gain of the substance-treated P0 males was statistically significantly reduced during pre-mating days 0-7 (35% below control) and post-mating days 14-20 (68% below control). Furthermore, mean body weight gain of the test substance-treated P0 females was statistically significantly reduced during GD 0-14 (up to 20% below the concurrent control); see Table 19 and Table 20 . As these apparent sporadic reductions in body weight change had no effect on the body weight in the treated group, they were considered to be spontaneous and unrelated to treatment.

Table 18: Summary of of body weights in P0 animals[g]

		control	250 mg/kg bw/d
Male			
Day 13 (pre-mating)	MEAN	354.3 n	352.0
	ST.DEV	14.6	14.9
	N	25	25
Post-mating (day 20)	MEAN	383.9 n	377.2
	ST.DEV	25.9	24.9
	N	25	24
Female			
Day 13 (pre-mating)	MEAN	214 .0 n	212.7
	ST.DEV	7.2	6.8
	N	25	25
Gestation day 0	MEAN	213.2 n	216.0
	ST.DEV	7.6	7.0
	N	23	24
Gestation day 20	MEAN	322.7 n	319.7
	ST.DEV	16.5	13.7
	N	23	23
Lactation day 4	MEAN	256.7 n	256.7
	ST.DEV	12.6	12.6
	N	23	18

Statistic Profile = Student's t-test (two-sided), * p<=0.05, ** p <=0.01

n=STUDENT

Table 19: Summary of body weight gain – premating in male and female P0 animals [g]

		control	250 mg/kg bw/d
Male			
days 0 - 7	MEAN	14.2 n	9.2 *
	ST.DEV	6.2	7.6
	N	25	25
days 7 - 13	MEAN	5.0 n	6.0
	ST.DEV	4.1	4.3
	N	25	25
days 0 - 13	MEAN	19.1 n	15.2
	ST.DEV	8.2	9.2
	N	25	25
Female			
days 0 - 7	MEAN	4.5 n	4.1
	ST.DEV	5.7	7.0
	N	25	25
days 7 - 13	MEAN	6.8 n	6.7
	ST.DEV	3.9	5.5
	N	25	25
days 0 - 13	MEAN	11.3 n	10.8
	ST.DEV	4.7	5.8
	N	25	25

Statistic Profile = Student's t-test (two-sided), * p<=0.05, ** p <=0.01

d = day; n=STUDENT

Table 20: Summary of body weights gain - post-mating in male and female P0 animals[g]

		control	250 mg/kg bw/d
Male - Post-mating			
days 0 - 7	MEAN	5.3 n	4.3 *
	ST.DEV	6.2	4.7
	N	25	25
Days 7 - 14	MEAN	3.8 n	5.7
	ST.DEV	9.8	7.3
	N	25	25
days 14 - 20	MEAN	5.9 n	1.9 *
	ST.DEV	5.5	6.0
	N	25	24
days 0 - 20	MEAN	15.0 n	11.8
	ST.DEV	11.2	8.4
	N	25	24
Female - Gestation			
days 0 - 7	MEAN	25.3 n	20.3 **
	ST.DEV	5.1	4.4
	N	23	24
days 7 - 14	MEAN	29.0 n	26.5 *
	ST.DEV	4.3	3.1
	N	23	23
days 14 - 20	MEAN	55.3 n	57.2
	ST.DEV	11.3	9.0
	N	23	23
days 0 - 20	MEAN	109.5 n	103.6
	ST.DEV	15.2	10.3
	N	23	23
Female - Lactation			
days 0 - 4	MEAN	9.3 n	7.6
	ST.DEV	13.7	8.9
	N	23	18

Statistic Profile = Student's t-test (two-sided), * p<=0.05, ** p <=0.01

d = day; n=STUDENT

- Food consumption and compound intake:
  - Food consumption of the substance-treated F0 males and females (250 mg/kg bw/d) was generally comparable to the respective concurrent control group during the entire treatment period. This includes the statistically

significantly increased food consumption value in the F0 males during premating days 7-13, which is considered to be spontaneous in nature.

- Organ weight findings including organ / body weight ratios: no effects observed
- Histopathological findings:
  - Treatment-related findings were observed in the limiting ridge of the forestomach of males and females,.
  - The hyperkeratosis in the forstomach at the limiting ridge was observed either alone or accompanied by hyperplasia of the squamous epithelium. Within the keratin masses, the hyperkeratosis frequently included vesicles containing serum, erythrocytes and/or cellular debris, which most probably grossly mimicked ulcerations or erosions.
  - All other findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment.

- Reproductive function / performance: treatment related effects
  - Male reproductive data:
    - For all but two P0 parental males copulation was confirmed by the presence of sperm or sperm plug in the vaginal canal. Copulation was not confirmed for control male No. 10 paired with control female No. 110 and for test substance-treated male No. 36 paired with test substance-treated female No. 136. For control male No. 21 no sperm or sperm plug was detected, thus no GD 0 was defined for control female No. 121, but this female delivered pups. The male mating index was 96% both in the control and test group 1 (250 mg/kg bw/d).
    - Fertility was proven for most of the P0 parental males within the scheduled mating interval for F1 litter. One control male (No. 10) and one test substance-treated male (No. 36) did not generate F1 pups. Thus, the male fertility index was 96% in all test groups. This reflects the normal range of biological variation inherent in the strain of rats used for this study.
    - All respective values are within the range of the historical control data of the test facility.
  - Female reproduction and delivery data:
    - The female mating index calculated after the mating period for F1 litter was 96% in both test groups (0 and 250 mg/kg bw/d). The mean duration until sperm was detected (GD 0) varied between 1.9 and 2.4 days.

- All female rats delivered pups or had implants in utero with the following exceptions:
    - Control female No. 110 (mated with male No. 10, no confirmed copulation) did not become pregnant.
    - Test substance-treated female No. 136 (mated with male No. 36, no confirmed copulation) did not become pregnant.
- The female fertility index was 100% both in the control and in test group 1. These values reflect the normal range of biological variation inherent in the strain of rats used for this study.
- Implantation was not affected by the treatment since the mean number of implantation sites was comparable between the test substance-treated group and the control, taking normal biological variation into account (11.6 and 11.5 implants/dam in test groups 0 and 1, respectively). Furthermore, there were no indications for test substance-induced intrauterine embryo-/fetolethality since the postimplantation loss did not show any statistically significant differences between the groups, and the mean number of F1 pups delivered per dam remained unaffected (10.8 and 10.9 pups/dam in test groups 0 and 1).
- The mean duration of gestation was statistically significantly prolonged in test group 1 (22.4** [$p \leq 0.01$] days vs. 22.0 days in control). For one female (No. 140) of test group 1 (250 mg/kg bw/d) dystocia was recorded on GD 22, while three females (Nos. 132, 133 and 135) of this group were unable to deliver and died during the parturition process on GD 23. These effects at the 250 mg/kg bw/d dose level are considered to be treatment-related.
- Thus, a significantly lower number of pregnant test substance-treated females (19* [$p \leq 0.05$]) had liveborn pups, in comparison to 24 pregnant females in the control. This resulted in a lower gestation index in the treatment group (79.2% in test group 1 vs. 100% in the control).
- The number and rate of liveborn pups was affected by the test substance, as indicated by a reduced live birth index of 84.5% in test group 1, in comparison to 98.1% in the control. Moreover, the number of stillborn pups was increased in test group 1 (34 stillborn vs. 5 in control). One test substance-treated dam (No. 143) had only stillborn pups in its litter and one further animal in this group had complete litter loss on PND 0 (including stillborn pups and pups that died during PND 0) (see Table 21).

Table 21: Summary litter report

		control	250 mg/kg bw/d
Litters with liveborn pups	N	24	19
Pups delivered	N	259	219
Pups found dead	N	7	3
	%	2.7	1.4
Pups stillborn	N	5	34
	%	1.9	15.5
Pups live born	N	254	185
	%	98.1	84.5
Pups cannibalized	N	1	8
	%	0.4	3.7
Pups sacrificed scheduled	N	246	174
	%	95.0	79.5
Litters not surviving Day 4	N	1	2
	%	4.2	10.0
Viability Index	MEAN %	95.3 x-	88.0 **
	ST.DEV	20.5	23.9
	N	24	19
% Live male pups Day 0	MEAN %	48.0 x	45.7
	ST.DEV	10.2	22.5
	N	24	19
% Live female pups Day 0	MEAN %	52.0 x	54.3
	ST.DEV	10.2	22.5
	N	24	19
% Live male pups Day 4	MEAN %	48.0 x	43.5
	ST.DEV	10.4	19.6
	N	23	18
% Live female pups Day 4	MEAN %	52.0 x	56.5
	ST.DEV	10.4	19.6
	N	23	18

Statistic Profile = Wilcoxon test (one-sided-), Wilcoxon test (one-sided+), Wilcoxon test (two-sided), Fisher's exact test (one-sided-), Fisher's exact test (one-sided+), * p<=0.05, ** p <=0.01, X = Group excluded from statistics, x=WILCOX

- F1 generation
  - General toxicity
    - Clinical signs: no effects observed
    - Mortalilty / viability:

- Pup number and status at delivery: The mean number of delivered F1 pups per dam was comparable between the test groups. However, the rate of liveborn F1 pups was decreased (live birth indices: 98.1% and 84.5% in test groups 0 and 1), and the rate of stillborn F1 pups was increased in the test substance-treated group (5 in control vs. 34 in test group 1). These changes are considered to be treatment-related.
- The viability index indicating pup mortality during lactation (PND 0-4) differed between the test group 1 (88.0%** [$p \leq 0.01$]) in and the control (95.3%) . A slightly higher number of decedents (cannibalized/dead pups) in test group 1 (250 mg/kg bw/d) compared to the control (8 vs. 1) is considered to be treatment-related (see Table 21) .

- Sex ratio: The sex distribution and sex ratios of live F1 pups on the day of birth and on PND 4 did not show significant differences between the control and the test group 1; slight differences were regarded to be spontaneous in nature.
- Body weight and weight changes: non-treatment-related effects (see Table 22)
  - Mean body weights of the test substance-treated F1 male and female pups (250 mg/kg bw/d) were statistically significantly below the concurrent control values on PND 1 (-9%) and PND 4 (-7%).
  - Mean pup body weight change of the test substance-treated group was comparable to the concurrent control group.
  - Three male and eight female runts were noted in test group 1 (250 mg/kg bw/d).

Table 22: Summary pup report on body weights [g]

		control	250 mg/kg bw/d
day 1 Runt	Males	0	3
	Females	0	8
day 1 Males	MEAN	6.8 n	6.3 **
	ST.DEV	0.5	0.9
	N	23	17
	Deviation vs Control		-8.6
day 1 Females	MEAN	6.5 n	6.0 **
	ST.DEV	0.5	0.8
	N	23	18
	Deviation vs Control		-8.5
day 1 Males + Females	MEAN	6.7 n	6.1 **
	ST.DEV	0.4	0.8
	N	23	18
	Deviation vs Control		-8.4
day 4 Males	MEAN	10.4 n	9.6 *
	ST.DEV	1.1	1.2
	N	23	17
	Deviation vs Control		-7.5
day 4 Females	MEAN	10.0 n	9.3 *
	ST.DEV	1.0	1.2
	N	23	18
	Deviation vs Control		-6.8
day 4 Males + Females	MEAN	10.2 n	9.5 *
	ST.DEV	1.0	1.2
	N	23	18
	Deviation vs Control		-6.9

Statistic Profile = Student's t-test (two-sided), * p<=0.05, ** p <=0.01, X = Group excluded from statistics, n=STUDENT

- Other effects:
  - A few F1 pups showed spontaneous findings at gross necropsy, such as reddish discolored testis and a small lower jaw (control – dam No. 118, female pup No. 8). These findings occurred without any relation to dosing and/or can be found in the historical control data at comparable or even higher incidences.

3.11 Specific target organ toxicity – single exposure

Evaluation not performed for this substance.

3.12 Specific target organ toxicity – repeated exposure

Evaluation not performed for this substance.

3.13 Aspiration hazard

Evaluation not performed for this substance.

4 ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance.