Outcome of assessment of 3-Nitrooxypropanol “3-NOP” - Appendix 1 List of toxicological studies

Tolerance and residue studies

Study	Year	OECD	Animals	Doses tested
Pilot tolerance study, 90 days	2018	N/A	16 (4 x4 groups) dairy cows	0, 1.6, 8, 16g 3-NOP/cow/day = 100, 500 and 1000mg/kg feed DM
Pivotal tolerance study, 56 days	109	N/A	80 (20 x 4 groups) dairy cows	0, 80, 100, 200 mg 3-NOP/kg DM
Milk analysis for NOPA from University of Reading efficacy study	2019	N/A	5 dairy cows	Milk samples from 5 cows receiving 3-NOP at approx 60 mg/kg, during 3 days in week 1, 6 and 15.

ADME

Study	Year	OECD	Animals	Doses tes
Stability of 3-NOP under Different Conditions	2015	N/A	N/A	202 µmol/
Stability of 3-NOP under Different Conditions II – Plasma Protein Binding and Chemical Oxidation	2017	N/A	Wistar rat plasma	34 µmol/L

Study	Year	OECD	Animals	Doses tes
Plasma Protein Binding of 14C-NOPA	2019	N/A	Wistar rat plasma	31.3 µmol/ and 6.26 µmol/ at 37c for to 24 hour
Stability of 3-NOP under Different Conditions III – In-vitro Incubations Leading to the Major Metabolite NOPA	2017	N/A	Rat (Wistar and Sprague Dawley), Dog (Beagle) and Human Liver Function	34 to 36 µmol/L
Metabolite Profiles and Kinetics of 3-NOP after In-vitro Incubation	2014	N/A	Cow rumen fluid	2.2 and 23 mg/L at 38 degree for 24 hou
Metabolite Profiles of 3-NOP after In-vitro Incubation	2016	N/A	Sheep, Goat and Cow Rumen Fluid	1 mg/L at degrees fo 16 hours
ADME tissue distribution and plasma kinetics	2013	417	Wistar rats	505 mg/kg bw
ADME in the Rat Following Single and Multiple Oral Administration	2018	N/A	4M/4F Wistar rats	2 exps eac with 50 an 500 mg/kg bw (exps i total). 50 given as a single dos and as a 5 5 daily dos 500 just as single dos

Study	Year	OECD	Animals	Doses tes
ADE with volatiles	2015	417	Wistar rats	506 mg/kg bw
Metabolites in plasma, liver and GIT	2014	417	Wistar rats	505 mg/kg bw
Nitrate/ nitrite in plasma	2014	417	Wistar rats	100 and 5 mg/kg bw
3-NOP in lactating goats	2015	503	2 goats	7 daily dos of 4.34 an 3.28 mg/k bw being equiv to 1 102 mg / k DM (feed)
ADME in Dairy Cattle Following Multiple Oral Administration	2018	N/A	4 dairy cows	Every 12 hours for 7 days at do level of 3.6 mg / kg bw d (1.8 g / animal / d) being equi to 150-160 mg / kg DM (feed)
ADME in Dairy Cattle Following Multiple Oral Administration (part 2)	2021	N/A	10 dairy cows	Every 12 hours for 5 days at do level of 3.6 mg / kg bw d (2.1 g/animal/d being approxima equivalent 150 mg/kg dry feed

Study	Year	OECD	Animals	Doses tes
NOPA and nitrate analysis of plasma	2016	N/A	4 Beef cattle and 4 controls	29 days of mg/kg bw / animal) being equi to 284 mg/ (feed)
NOPA and nitrate analysis of plasma	2016	N/A	28 beef cattle per dosing group	0,100,200 mg/kg feed for 238 da

Toxicity

Study	Year	OECD	Animals	
In-vitro Ames Microsuspension Test	2010	471	N/A	
In-vitro Salmonella typhimurium and Escherichia coli reverse mutation assay	2014	471	N/A	
In-vitro Salmonella typhimurium and Escherichia coli reverse mutation assay II	2015	471	N/A	
Screening in-vitro Micronucleus Test in Chinese Hamster V79 Cells	2010	487	N/A	
In-Vitro V79 Micronucleus Assay	2020	487	N/A	
In-vitro Micronucleus assay in cultured peripheral human lymphocytes	2014	487	N/A	
In-vitro mammalian cell gene mutation test (Mouse lymphoma assay)	2015	476	N/A	

Study	Year	OECD	Animals	
Cell transformation (SHE) assay	2013	N/A (followed OECD draft proposal)	N/A	
In-Vitro TK6 Micronucleus Assay	2021	487	N/A	
Salmonella typhimurium and Escherichia coli reverse mutation assay (NOPA)	2020	471	N/A	
Micronucleus Test in Human Lymphocytes In vitro (NOPA)	2020	487	N/A	
Acute Oral Toxicity Test	2014	423	Wistar rats	
Assessment of acute inhalation toxicity	2017	436	Wistar rats	
Micronucleus test in bone marrow cells of the mouse (screening)	2011	474	NMRI Male mice (intraperitoneal)	
Micronucleus test in bone marrow cells of the rat	2014	474	Wistar rats	
10-day dose range finding study	2012	N/A	Wistar rats (n= 3 per group per sex)	
Combined 28-day repeated dose toxicity study and reproduction / developmental toxicity screening test	2013	422, 407	Wistar rats	
90-day oral gavage toxicity study	2015	408	Wistar rats	

Study	Year	OECD	Animals	
Dose range finding study and the maximum Tolerated Dose (MTD study)	2014	N/A	Beagle dogs, n = 2 (1 xM, 1x F) DRF, n = 2 per sex per dose MTD	
14-day oral gavage toxicity study	2016	N/A	Beagle dogs 2 x M and 2 x F per dose	
3-months oral gavage toxicity study	2016	409	Beagle dogs	
1 year oral gavage toxicity study	2016	452	Wistar rats	
2-year carcinogenicity study	2019	451	Wistar rats	
6-day DRF in mice	2018	451 and 417	CbyB6F1 hybrid mouse	
28-day study in mice	2019	451	CbyB6F1 hybrid mouse	
NOPA In-Vivo 14-Day Dose Range Finder Assay in Rats	2021	N/A	Fischer rats	
NOPA In-Vivo Mutation Assay at the cII Locus and In-Vivo Micronucleus Assay in Male and Female Big Blue® Transgenic F344 Rats	2021	488, 474	Fischer rats	

Reprotoxicity

Study	Year	OECD	Animals	Doses

28-day oral gavage mechanistic study	2014	Based on 407	Wistar rats	0, 100 kg bw
Prenatal developmental toxicity study	2015	414	Wistar rats	0, 100 mg/kg
Prenatal developmental toxicity study	2016	414	NZW Rabbits	0, 50, bw
Two-generation reproduction study	2016	416	Wistar rats	0, 25, and Fe satellit female mg/kg
6-10-day preliminary mechanistic study	2017	N/A	Wistar rats (n=9 across the two dosing levels)	800 ar bw
Dose range finding (mechanistic)	2018	N/A	Wistar rats (n=1 per dosing group)	3-NOP bw (Or NOPA 75,250 (IV) HPA (r 250, 4((IV) HPA: 7 mg/kg
Influence of metabolites on testicular toxicity in male rats, 10-day study	2018	N/A	Wistar rats (n=5 per dosing group)	3-NOP bw (Or NOPA (IV) HPA: 3 (day 3 mg/kg HPA: 3 (SC)
Single dose transcriptomics study	2017	N/A	Wistar rats (n=8 per dosing group)	0, 100 bw

Benchmark-Dose-Modelling	2019	N/A	N/A	N/A
In-vitro Steroidogenesis	2015	N/A	Human adrenal cells	0, 0.00 0.01, 0 (3-NOI HPA)
Ex-vivo model testicular toxicity evaluation (3-NOP, NOPA, HPA, inorganic nitrate)	2015	N/A	Sprague Dawley rat	0, 0.00 mM (a
Ex-vivo model testicular toxicity evaluation of NOPA	2016	N/A	Sprague Dawley rat	0, 0.02 (NOPA
In-vitro / ex-vivo species comparison study using NOPA	2019	N/A	Testicular tissue from Wistar rats, Beagle dog, and Cynomolgus monkey (n=34 tissue samples for each species)	0,1,20 µM (N