Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

N-(2-nitrophenyl)phosphoric triamide

EC Number: 477-690-9

CAS Number: 874819-71-3

CLH-O-0000006850-73-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

17 September 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

***N*-(2-nitrophenyl)phosphoric triamide**

EC Number: **477-690-9**

CAS Number: **874819-71-3**

Index Number: **--**

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Version number: 2 **Date: July 2019**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON *N*-(2-NITROPHENYL)PHOSPHORIC TRIAMIDE

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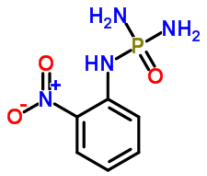
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON *N*-(2-NITROPHENYL)PHOSPHORIC TRIAMIDE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	***N*-(2-nitrophenyl)phosphoric triamide**
Other names (usual name, trade name, abbreviation)	**N-(diaminophosphoryl)-2-nitroaniline** **2-NPT** **Phosphoric triamide, (2-nitrophenyl)-**
ISO common name (if available and appropriate)	**--**
EC number (if available and appropriate)	**477-690-9**
EC name (if available and appropriate)	***N*-(2-nitrophenyl)phosphoric triamide**
CAS number (if available)	**874819-71-3**
Other identity code (if available)	**--**
Molecular formula	**$C_6H_9N_4O_3P$**
Structural formula	
SMILES notation (if available)	NP(N)(=O)NC1=CC=CC=C1[N+]([O-])=O
Molecular weight or molecular weight range	216.134
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	--
Description of the manufacturing process and identity of the source (for UVCB substances only)	--
Degree of purity (%) (if relevant for the entry in Annex VI)	--

1.2 Composition of the substance

Not relevant for the classification of the substance.
Details on the test substance (if available) are given in the study summaries.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 2:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No entry	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	--	*N*-(2-nitrophenyl)phosphoric triamide	477-690-9	874819-71-3	Repr. 1B STOT RE 2 Aquatic Chronic 3	H360FD H373 (kidney) H412	GHS08 Dgr	H360FD H373 (kidney) H412	--	--	
Resulting Annex VI entry if agreed by RAC and COM	--	*N*-(2-nitrophenyl)phosphoric triamide	477-690-9	874819-71-3	Repr. 1B STOT RE 2 Aquatic Chronic 3	H360FD H373 (kidney) H412	GHS08 Dgr	H360FD H373 (kidney) H412	--	--	

Table 3: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	harmonised classification proposed	Yes
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

So far no proposals for harmonised classification for human health and/or the environment were submitted.

RAC general comment
N-(2-nitrophenyl)phosphoric triamide (2-NPT) is used as a urease inhibitor. Urease inhibitors are added to urea containing fertilisers to reduce the release of ammonia due to the hydrolysis of urea.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

Further detail on need of action at Community level

Based on the available data, besides reproductive toxicity also specific target organ toxicity after repeated exposure and environmental hazards have been evaluated.

Regarding specific target organ toxicity after repeated exposure no classification is proposed by the registrants/notifiers. The dossier submitter disagrees with this and proposes to classify as STOT RE 2.

2-NPT is a chemical with wide dispersive outdoor use as an additive in fertilizers and hence intentional exposure of the environment takes place. Therefore, harmonization of classification for the environment is proposed as well, in order to ensure legal clarity, correct handling and thus sufficient protection of the environment.

5 IDENTIFIED USES

According to ECHA dissemination site the substance is registered under REACH in a tonnage band of 10-100 tonnes per year. The substance is used by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

The substance is an additive for urea based fertilizers.

6 DATA SOURCES

The information has been retrieved from REACH registration and from original study reports provided by the registrant(s). Furthermore, Toxplanet[1] (compiling most relevant hazard and toxiocology information sources) has been screened for any further relevant information.

7 PHYSICOCHEMICAL PROPERTIES

Table 4: Summary of physicochemical properties

Property	Value	Reference
Physical state at 20°C and 101,3 kPa	The tested substance is a solid.	REACH registration
Melting/freezing point	2-NPT decomposes at 195.4 °C.	REACH registration
Relative density	The relative density of 2-NPT is 1.558.	REACH registration

[1] https://toxplanet.com/

Property	Value	Reference
Vapour pressure	The vapour pressure of 2-NPT was estimated to be 0.00000005 hPa at 20 °C.	REACH registration
Surface tension	The surface tension of 2-NPT is 70.39 mN/m. Therefore, the test item is not surface active.	REACH registration
Water solubility	The water solubility of the test item is 1394 mg/l at 20 °C.	REACH registration
Partition coefficient n-octanol/water	The log Pow of 2-NPT was estimated to be 0.51.	REACH registration
Flammability	The test item 2-NPT is not highly flammable, not flammable in contact with water and not pyrophoric.	REACH registration
Explosive properties	The test item 2-NPT has no explosive properties.	REACH registration
Self-ignition temperature	The relative self-ignition temperature of 2-NPT was estimated as 292 °C.	REACH registration
Oxidising properties	2-NPT is not an oxidising substance.	REACH registration
Granulometry	The particle size distribution of P 101/04 shows no single peaks, but a continuous distribution roughly between 23 and 104 µm, leaving fractions of approx. 34% above 104 µm and less than 10% below 23 µm. 2.95 % particles were smaller than 11 µm.	REACH registration

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Guideline conform oral toxicokinetic data are not available. The registrants set the oral absorption for *N*-(2-Nitrophenyl)phosphoric triamide (2-NPT) at 100%.

There is only an *in vitro* dermal absorption study available. Based on the outcome of the study the registrants set the dermal absorption rate at 0.26%.

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The available data are not relevant for classification purposes.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not assessed.

10.2 Acute toxicity - dermal route

Not assessed.

10.3 Acute toxicity - inhalation route

Not assessed.

10.4 Skin corrosion/irritation

Not assessed.

10.5 Serious eye damage/eye irritation

Not assessed.

10.6 Respiratory sensitisation

Not assessed.

10.7 Skin sensitisation

Not assessed.

10.8 Germ cell mutagenicity

Not assessed.

10.9 Carcinogenicity

Not assessed.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 5: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD Guideline 421 (Reproduction/	Test substance: *N*-(2-nitrophenyl)phosphoric	Changes in the testes (e.g. testicular atrophy with related tubular atrophy of the seminiferous tubules,	Anonymous

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Developmental Toxicity Screening Test) rat (Crl:CD(SD)) male/female n=80, 40 males and 40 females, n=10 per sex per group Reliability: Klimisch 1	triamide Vehicle: 0.8% aqueous hydroxypropylmethycellulose gel Exposure route: oral gavage 0, 45, 135, 450 mg/kg bw/d (actually ingested) Exposure: males Once daily for 32 days (beginning 2 weeks prior to mating lasting up to the day before sacrifice until a minimum dosing period of 28 days was completed). Exposure: females Once daily, beginning 2 weeks prior to mating and continuing up to, and including, day 3 post-partum or the day before sacrifice (females which have not delivered).	interstitial oedema, loss of germ cell layers in the seminiferous tubules, moderate tubular atrophy with damage (degeneration/necrosis) of the germinal epithelium, loss of spermatogonia, spermatocytes, spermatids and spermatozoa) and epididymis (aspermia with only empty duct(s) or ducts containing cellular debris). Details on histopathological observations in epididymis and testes are provided in table 9. Significant reduction of testis and epididymis weight are depicted in table 8. Macroscopic analysis at the end of the treatment period revealed changes in form of a reduced size of the testes in 1 out of 10 low dosed males, and in all 10 intermediate and high dosed male rats. The findings correlate with the reduced weight of testes noted for the intermediate and high dose group. Adverse effects on **reproductive parameters** at the high dose group were observed, including slightly reduced mean and total number of corpora lutea, implantation sites, reduced mean number of pups at birth, increased number of stillbirth (see table 10). Severely increased number of deceased pups (viability index 29.6%) during the first lactation days, reduced mean and total body weight of the pups at the high dose group (see tables 16 and 17). NOAEL (P): 45 mg/kg bw/d NOAEL (F1, reproduction): 135 mg/kg bw/d	(2012)
OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) rat (Wistar) n=60, male (n=30) and female (n=30) Reliability: Klimisch 1	Test substance: *N*-(2-Nitrophenyl)phosphoric triamide Vehicle: 0.5 % (m/v) solution of Tylose MH 1000 in deionised water Exposure route: gavage 0, 30, 100 and 300 mg/kg bw/d (actual ingested) Duration of exposure: 28 days (once daily), 14 days treatment free recovery period (satellite group)	Main target organs: testes and kidney Reduced testis and epididymis weight (see table 11). Damage of seminiferous tubules in the testes which led to a complete absence of spermatozoa in the epididymis especially in animals of the high dose group and partial in the mid dose group (see tables 13, 14). Dose dependent damage on the kidneys, characterised by flattened and partly degenerated tubular epithelium in the renal cortex and in the renal medulla. NOAEL was set to be 30 mg/kg bw/d based on the normal state of the epididymis, the normal number of spermatozoa and the normal state of kidneys.	Anonymous (2006)

Adverse effects on male reproductive system were detected in an OECD TG 421 study (Reproduction / Developmental Toxicity Screening Test) and in the OECD TG 407 study (Repeated Dose 28-Day Oral Toxicity in Rodents), both studies were carried out under GLP.

In the **OECD TG 421 screening study** rats (Crl:CD(SD)) (n=10/sex/group) were exposed to 0, 45, 135, 450 mg/kg bw/d 2-NPT by gavage for a time period of 32 days (males) or up to and including day 3 post-partum or the day before sacrifice (females).

None of the treated animals died prematurely during the course of the study. No systemic toxicity was noted for the animals treated with 45, 135 and 450 mg/kg bw/d 2-NPT with the exemption of pregnant females. In few individual pregnant females at the highest dose group the following changes were noted: increased salivation, slight to moderate ataxia, pilo-erection and tonic-clonic convulsions. Furthermore, at the highest dose group an extreme yellow discoloured urine in all animals was detected caused by the colour of the test item.

No influence on body weights was noted in males exposed to 45 and 135 mg/kg bw/d 2-NPT compared to control. The body weights of males exposed to 450 mg/kg bw/d 2-NPT were below the control group from day eight onwards (up to 14%) and the difference was statistically significant at day 8, 22, 29 and 33 (see table 6).

Table 6: Body weights of male rats at different time points relative to start date (n=10/group)

		Day 1	**Day 8**	**Day 15**	**Day 22**	**Day 29**	**Day 33**
0 mg/kg bw	Mean SD	344.49 11.43	386.99 20.26	416.15 27.95	432.12 33.59	461.03 36.37	470.67 39.91
45 mg/kg bw	Mean SD	344.11 12.05	384.05 22.53	415.56 29.19	428.70 33.71	462.38 38.30	462.79 44.38
135 mg/kg bw	Mean SD	345.15 11.98	372.64 15.18	401.95 20.53	415.49 24.98	442.12 32.43	449.10 35.04
450 mg/kg bw	Mean SD % difference to control group	344.85 11.93 -	355.87** 16.40 -8	379.78 28.62* -9	383.36** 34.03 -11	402.28** 41.78 -13	405.41** 40.03 - 14

*p<0.05 (Dunnett 2 sided test)
**p<0.01 (Dunnett 2 sided test)

No influence on the body weights was noted in female rats (pre-mating and mating period) exposed to 45, 135 and 450 mg/kg bw/d 2-NPT compared to control.

The body weights of pregnant females exposed to 450 mg/kg bw/d 2-NPT were below the control group (up to 20%) and the difference was statistically significant at day 7, 14, 20 of gestation and on days 1 and 4 of lactation.

Table 7: Body weights of female rats at different time points relative to mating and littering (n=8-10/group)

Dose levels	**Parameter**	**Days relative to mating**				**Days relative to littering**	
		Day 0	**Day 7**	**Day 14**	**Day 20**	**Day 1**	**Day 4**
0 mg/kg bw	Mean SD	251.56 21.32	289.89 17.72	325.32 18.09	405.33 23.07	311.10 18.43	318.17 17.44
45 mg/kg bw	Mean SD	241.09 11.65	280.16 12.63	315.54 11.79	397.40 17.08	297.52 14.76	301.03 10.05
135 mg/kg bw	Mean SD	244.59 14.76	284.11 21.15	325.21 22.54	402.46 31.91	301.18 28.29	305.26 27.72

450 mg/kg bw	Mean SD % difference to Gr. 1	231.20 19.82 -8	263.25** 14.14 -9	287.49** 17.24 -12	335.08** 34.38 -17	251.71** 17.83 -19	256.03** 18.48 -20

** $p < 0.01$ (Dunnett 2 sided test)

Macroscopic analysis at the end of the treatment period demonstrates reduced size of the testes in 1 out of 10 low dosed males, and in all 10 intermediate and high dosed male rats (incidences of animal affected - reduced testes size: 0 mg/kg bw: 0/10, 45 mg/kg bw: 1/10, 135 mg/kg bw: 10/10, 450 mg/kg bw: 10/10). The findings correlate with the reduced weight of testes noted for the intermediate and high dose group, and with histopathological changes observed in the high dosed animals.

In the following table the changes of epididymis and testes weights are summarised. Results indicate a marked decrease in organ weights up to -60% (right testis: 450 mg/kg bw).

Table 8: Changes in absolute epididymis and testes weights compared to the control at terminal sacrifice on test day 33 [%] (males)

Organ	**45 mg/kg bw**	**135 mg/kg bw**	**450 mg/kg bw**
Epididymis, left	-14**	-43**	-46**
Epididymis, right	-11	-42**	-45**
Testis, left	none	-53**	-59**
Testis, right	none	-52**	-60**

** statistically significant: $p \leq 0.01$ (Dunnett´s test or Student´s t-test)

A detailed histopathological examination was performed on the ovaries, testes and epididymis of the adult animals of the control and the high dose group. The examination of the parental animals of the high dose group demonstrates changes in the testes (e. g. testicular atrophy with related tubular atrophy of the seminiferous tubules, interstitial oedema, loss of germ cell layers in the seminiferous tubules, moderate tubular atrophy with damage (degeneration/necrosis) of the germinal epithelium, loss of spermatogonia, spermatocytes, spermatids and spermatozoa) and epididymis (aspermia with only empty duct(s) or ducts containing cellular debris). All male animals at the high dose group were affected (for details see table 9). Ovaries from animals exposed to 450 mg/kg bw/d 2-NPT did not show any adverse effects related to the test item.

Table 9: Histopathological observations in epididymides and testes of male rats (n=10/group)

Observation	**0 mg/kg bw**	**450 mg/kg bw**
Epididymides		
- no abnormalities detected	10	0
- duct(s), aspermia	0	10**
Testes		
- no abnormalities detected	10	0
- atrophy (moderate)	0	10**
- interstitial oedema (moderate)	0	10**
- tubular atrophy (moderate)	0	10**

- seminiferous tubules; germinal epithelium; degeneration/necrosis (marked)	0	10**

** statistically significant: $p \leq 0.01$ (Fisher´s test)

No test item related influence was noted on the pre-coital time of the animals and there was no influence on the female fertility index or on the gestation length of the treated animals in none of the treatment groups (see table 10).Treatment with the high dose of 450 mg /kg bw/d 2-NPT resulted in the following test item related changes: a slightly reduced mean and total number of corpora lutea, implantation sites (both reduced by 25%, statistically not significant at $p \leq 0.01$) and, subsequently, a reduced mean number of pups at birth (value reduced by 33%, statistically not significant at $p \leq 0.01$).

An increased number of stillbirths resulted in a slightly increased post implantation loss of 16% compared to the control group.

Subsequently, a reduced mean number of live born pups and a reduced live birth index of 91% (compared to 99% in the control group) were noted. The total number of live born pups was reduced accordingly.

No differences were noted between treated and control groups in gestation index, the birth index and the pre-implantation loss (details see table 10).

Table 10: Summary of fertility and reproduction parameters

Parameter		**0 mg/kg bw**	**45 mg/kg bw**	**135 mg/kg bw**	**450 mg/kg bw**
Pre-coital time (days), females (n=10/group)[a]	Mean ± SD	4.9 ± 4.7	2.4 ± 1.2	2.1 ± 1.1	6.2 ± 5.9
Fertility Index[b]	%	90	100	80	80
Number of dams evaluated for the following paramters	Nr.	9	10	8	8
Gestation length[b]	Mean ± SD	22.2 ± 0.4	22.2 ± 0.6	22.5 ± 0.5	22.9 ± 0.4
Gestation index[b]	%	100	100	100	100
Corpora lutea[c]	Total Mean ± SD	163 18.1 ± 3.9	183 18.3 ± 3.5	145 18.1 ± 2.9	108 13.5 ± 5.6
Implantation Sites[a]	Total Mean ± SD	150 16.7 ± 1.3	159 15.9 ± 1.2	127 15.9 ± 2.2	101 12.6 ± 5.0
Number of pups at birth (alive and dead)[a]	Total Mean ±SD	139 15.4 ± 1.4	146 14.6 ± 1.6	116 14.5 ± 2.4	93 11.6 ± 5.0
Number of stillbirth	Nr.	2	4	0	10
Number of dams with stillborn pups	Nr.	2	1	0	4
Number of live	Total	137	142	116	83

born pups[a]	Mean ± SD	15.2 ± 1.4	14.2 ±2.0	14.5 ± 2.4	10.4 + 4.6**
Birth index[d]	%	92.8	91.7	91.9	92.4
Live birth index[d]	%	98.6	97.3	100.0	90.6**
Pre-implantation loss[d]	Mean % ± SD	6.0 ± 10.9	10.9 ± 13.4	11.6 ± 10.1	4.8 ± 9.6
Post-implantation loss[d]	Mean % ± SD	8.4 ± 7.8	10.8 ± 9.0	8.9 ± 13.4	16.2 ± 15.2*

[a] $p \leq 0.01$ (Student´s t-test)
[b] $p \leq 0.05$ or $p \leq 0.01$ (Fisher test)
[c] $p \leq 0.01$ (Dunnet test)
[d] $p \leq 0.05$ or $p \leq 0.01$ (Chi^2 test)

No abortion or any malformed foetuses were noted in any of the tested dose groups.

Results demonstrate severely increased numbers of deceased pups during the first four lactation days. In total 55 pups of the high dose group were found dead compared to 17 deceased or cannibalised pups in the control group. Subsequently, the viability index of only 30% was calculated for the high dose group (see table 16). Reduced mean and total body weight of the pups were observed at the high dose group (see table 17).

These results are considered as adverse effects on development and are addressed in more detail in chapter 10.10.4.

In the **OECD TG 407 subacute oral repeated dose toxicity study** Wistar rats (30 males and 30 females) were exposed daily to 0, 30, 100, or 300 mg /kg bw 2-NPT by oral gavage followed by a 14 day recovery period for the control and the highest dose group (satellite group).

The most abundant toxic effect of the test substance was a dose dependent damage to the testes, which resulted in the high dose group in a complete absence of spermatozoa in the epididymis. The exposure to the test substance in the high dose group disturbed the normal development of testes and epididymis completely. These observations are depicted in tables 11, 12, 13 and 14.

The absolute and relative organ weights of the testes and the epididymis were irreversibly, dose dependently and statistically significantly decreased in animals of the mid (epididymis right) and the high dose group (all testparameter) (see table 11).

The body weight gain was not affected by substance administration. Further, organ weight changes includestatistically significant increase in kidney weights (high dose group - males and females, irreversible in males) and increase in spleen weight (high and medium dose group - males, irreversible).

Table 11: Relative testes and epididymis weight (n=5)

Testparameter	**0 mg/kg bw**	**0 mg/kg bw[a]**	**30 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**300 mg/kg bw[a]**
Testis left	0.488 ± 0.0428	0.432 ± 0.1198	0.487 ± 0.0590	0.336 ± 0.1512	0.187 ± 0.0131*	0.204± 0.0406*
Testis right	0.493 ± 0.0495	0.433 ± 0.1043	0.487 ± 0.0547	0.328 ± 0.1546	0.192 ± 0.0148*	0.207± 0.0336*
Epididymis left	0.0845 ± 0.00742	0.0813 ± 0.01694	0.0833 ± 0.02225	0.0646 ± 0.01290	0.0584 ± 0.00633*	0.0545 ± 0.01244
Epididymis right	0.0870 ± 0.01033	0.0757 ± 0.01434	0.0801 ± 0.00640	0.0596 ± 0.00695*	0.0582 ± 0.00801*	0.0575 ± 0.01337

[a] satellite group (14 day recovery period), statistical evaluation within this group
* statistically significant $p \leq 0.05$ (Dunnett 2 sided test)

Macroscopic pathological findings indicate micro-orchidia and reduced epididymis size in all male rats of the high dose group (5 out of 5) and in 3 out of 5 in the middle dose group (both the right and the left organ were affected). The effect was irreversible. A severe micro-orchidia and epididymis with reduced size and an alteration of testes (alteration of the seminiferous tubules) was observed in one animal of the satellite control group, too. However a complete absence of spermatozoa was not observed in this case, indicating that the cause in this case was different from the effects seen in the dosed groups (details see table 12).

Table 12: Incidences of micro-orchidia and epididymides with reduced size (concerning the left and right organ) (n=5)

Dose	**0 mg/kg bw**	**0 mg/kg bw[a]**	**30 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**300 mg/kg bw[a]**
Micro-orchidia	0	1	0	3	5	5
Epididymides with reduced size	0	1	0	3	5	5

[a]satellite group (14 day recovery period)

The main finding of histopathological observations was a dose-dependent damage of the testes, which led in the high dose group to a complete absence of spermatozoa in the epididymis.

The seminiferous tubules damage was evaluated using a grading system (grade 1-4, grade 5-8 mentioned in the literature were not observed in the present study).

A dose-dependency with regard to the severity, degree and numbers of affected animals is observed (see table 13). Although according to the literature (Yuan, V.D and McEntee K., 1987) a damage up to grade 4 is considered reversible, no reversibility could be observed after the 14 day recovery period (satellite group). There was only very slight decrease of damage in the satellite group.

Table 13: Incidences (all, many, middle or few) of observed seminiferous tubulus with different grades of damage[b] (n=5)

	Dose	**0 mg/kg bw**	**0 mg/kg bw[a]**	**30 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**300 mg/kg bw[a]**
Left testis	Grade 1[b]	All (5/5)	All (4/5)	All	Many (1/5)	-	-

				(1/5) Many (3/5) Middle (1/5)	Few (1/5)		
	Grade 2	-	Few (1/0)	Middle (1/5) Few (3/5)	Middle (2/5) Few (1/5)	-	Middle (1/5)
	Grade 3	-	Middle (1/0)	-	Many (2/5) Few (2/5)	Many (4/5) Middle (1/5)	All (1/5) Many (3/5) Middle (1/5)
	Grade 4	-	Many (1/0)	-	Many (2/5) Few (1/5)	Many (1/5) Middle (4/5)	Few (4/5)
Right testis	Grade 1	All (5/5)	All (4/5)	Many (3/5) Middle (1/5) All (1/5) (	Many (1/5) Middle(1/5)	-	-
	Grade 2	-	Few (1/5)	Few (3/5) Middle (1/5)	Many (1/5) Middle(1/5) Few (1/5)	-	Few (1/5)
	Grade 3	-	Middle (1/5)	0	Middle(1/5) Few (3/5)	Many (4/5) Middle (1/5)	All (1/5) Many (4/5)
	Grade 4	-	Many (1/5)	0	Many (2/5) Few (1/5)	Many (1/5) Middle (4/5)	Few (4/5)

[a] satellite group (14 day recovery period)

[b] grade 1: normal stage, tubulus are normal and consist of a normal germinal epithelium with several layers of cells. The full thickness of the germinal epithelium varies slightly, according to the stage of the spermatogenic cycle; grade 2: tubules contain one or more vacuoles in the germinal epithelium without any reduction in its thickness; grade 3: tubules include those with a reduction in the thickness of the germinal epithelium. Most of the epithelium, however, still consists of some germinal cells in addition to the spermatogonia and Sertoli´s cells. Multinucleated giant cells derived from spermatids or spermatocytes are first seen in this stage of degeneration; grade 4: tubules have the majority of their germinal epithelium lined by spermatogonia and Sertoli´s cells.

The testes damage had an adverse effect on the epididymis (see table 14). There was a complete lack of spermatozoa in the epididymis of the animals of the high dose group. There were only spermatocytes, multinucleated giant cells, fragments of spermatozoa and/or cell detritus observed and no reversibility was observed after 14 day recovery period. In the low dose group the epididymis of the animals contained a normal number of spermatozoa.

Table 14: Summary of incidences of findings in the epididymis (n=10)

Findings in the lumina	**Incidence**	**0 mg/kg bw**	**0 mg/kg bw[a]**	**30 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**300 mg/kg bw[a]**
Spermatozoa observed	Normal Many Few None	10 0 0 0	8 0 2 0	10 0 0 0	0 4 0 6	0 0 0 10	0 0 0 10
Fragments of spermatozoa observed	Single	0	0	0	2	0	0
Spermatocytes observed	Many Single	0 0	0 2	0 0	2 8	0 8	0 10
Multinucleated giant cells observed	Single Rarely	0 0	2 0	0 0	6 0	5 2	5 2
Cell detritus observed	Many Single	0 0	0 2	0 0	2 8	0 10	0 10

[a] satellite group (14 day recovery period)

Since a slight damage of the seminiferous tubules in the testes was observed also in the animals of the low dose group, a NOEL (No Observed Effect Level) for testes effects cannot be established.

No other relevant information (e.g. human information) has been identified.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Two TG and GLP compliant studies (OECD TG 421 and 407) demonstrate that 2-NPT exposure has an adverse effect on the male reproduction organ.

In addition to statistically significantly reduced organ weights (testes and epididymis), also dose – dependent macroscopic observations (micro-orchidia, reduced epididymis and testes size) clearly indicate that 2-NPT administration results in testes damage.

Those findings are substantiated by histopathological examinations, which indicate a dose-dependent, irreversible damage of the testes characterised by reduced spermatozoa up to complete absence of spermatozoa in the highest dose group and changes in the testes (e. g. testicular atrophy, interstitial oedema, loss of germ cell layers in the seminiferous tubules, moderate tubular atrophy with damage (degeneration/necrosis) of the germinal epithelium, loss of spermatogonia, spermatocytes, spermatids and spermatozoa) and epididymis (aspermia with only empty duct(s) or ducts containing cellular debris).

Slight damage of the seminiferous tubules in the testes were observed in animals of the 30 mg/kg bw/day group in the OECD TG 407 study and thus a NOEL could not be established.

In the OECD TG 421 study other reproductive parameters were altered in the highest dose group, such as slightly reduced mean and total number of corpora lutea, implantation sites, reduced mean number of pups at birth, increased number of stillbirth.

10.10.3 Comparison with the CLP criteria

According to CLP Regulation Nr. 1272/2008 for the purpose of classification for reproductive toxicity, substances are allocated to one of two categories:

"Category 1: "Known or presumed human reproductive toxicant (category 1 A: "Known human reproductive toxicant", category 1B: " Presumed human reproductive toxicant"). Category 2: "Suspected human reproductive toxicant"

The classification in category 1A is largely based on evidence from humans. There are no data indicating reproductive effects of 2-NPT in humans, therefore classification in category 1A is not appropriate.

The classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate. "

Clear indication for adverse effects on male reproductive organs (testes/epididymis damage) and on reproduction parameters was observed in two reliable rat studies (OECD TG 407 and OECD TG 421).

According to above classification criteria there is "clear evidence" for adverse effects on fertility, characterised by changes of the testis (testicular atrophy with related tubular atrophy of the seminiferous tubules, interstitial oedema, loss of germ cell layers in the seminiferous tubules, moderate tubular atrophy with damage (degeneration/necrosis) of the germinal epithelium, loss of spermatogonia, spermatocytes, spermatids and spermatozoa) and epididymis (aspermia with only empty duct(s) or ducts containing cellular debris).

In the OECD TG 407 study seminiferous tubules damage has been already observed at the lowest dose group (30 mg/kg bw/d), whereas altered kidney parameters (kidney weight, histopathological observations) were present at the highest dose group (300 mg/kg bw). Therefore, the observed toxic effects on the reproductive organ of males are not considered as secondary to other toxic effects.

The severity of effects and the number of affected animals are dose dependent. It is demonstrated (28 day study with a recovery period of 14 days) that most of the adverse effects are not reversible.

There is no mechanistic information that raises doubt that the effects are not relevant for humans. Therefore a classification in category 2 is not considered appropriate.

In the 28 day study (OECD TG 407) effects on the semniferous tubulus have been already observed at a dose level of 30 mg/kg bw thus a NOEL for testes cannot be established. In the developmental toxicity screening test (OECD TG 421) adverse effects have been observed already at a dose level of 135 mg/kg bw, thus a NOAEL (P) of 45 mg/kg bw is deduced.

The reliable studies unambiguously demonstrate adverse effects on the male reproductive system and therefore a classification into Repr. 1B (H360F) is considered most appropriate.

10.10.4 Adverse effects on development

Table 15: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD Guideline 421 (Reproduction/ Developmental Toxicity Screening	Test substance: *N*-(2-nitrophenyl)phosphoric triamide Vehicle: 0.8% aqueous hydroxypropylmethylcellulose gel Exposure route: oral gavage	Adverse effects on reproductive parameters at the high dose group were observed, including slightly reduced mean and total number of corpora lutea, implantation sites, reduced mean number of pups at birth, increased number of stillbirth (see chapter	Anonymous (2012)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Test) rat (Crl:CD(SD)) male/female n=80, 40 males and 40 females, n=10 per sex per group Reliability: Klimisch 1	0, 45, 135, 450 mg/kg bw/d (actual ingested) Exposure: males Once daily for 32 days (beginning 2 weeks prior to mating lasting up to the day before sacrifice until a minimum dosing period of 28 days was completed). Exposure: females Once daily, beginning 2 weeks prior to mating and continuing up to, and including, day 3 post-partum or the day before sacrifice. (once daily)	10.10.1, table 10). Severely increased number of deceased pups (viability index 29.6%) during the first lactation days, reduced mean and total body weight of the pups at the high dose group (see table 16 and 17) are considered as adverse effects on development. NOAEL (P): 45 mg/kg bw/d NOAEL (F1, reproduction): 135 mg/kg bw/d	

At 450 mg/kg bw no systemic toxicity was observed, except in few individual pregnant females, who had symptoms such as slightly increased salivation (n=1) and moderate (n=1) to slight (n=2) ataxia, pilo-erection (n=2) and tonic-clonic (n=1) convulsions. In total, five out of ten animals had one or two of the aforementioned symptoms.

At the end of treatment the body weight was reduced in the highest dose group in males (by 14%, statistically significant at $p \leq 0.01$) and in females (by 16% statistically significant at $p \leq 0.01$). For females the body weight was not affected during the pre-mating and mating periods, but during pregnancy and lactation period the body weight was considerably below the body weight of the control group (by up to 20% on gestation days 7, 14, 20 and on lactation days 1 and 4) (see table 16).

Table 16: Body weight (g) of females relative to mating and relative to littering (n=8-10)

	Day(s) relative to Mating				**Day(s) relative to Littering**	
	0	**7**	**14**	**20**	**1**	**4**
Control mean	251.56	289.89	325.32	405.33	311.10	318.17
450 mg/kg bw mean	231.20	263.25**	287.49**	335.08**	251.71**	256.03**
difference (%)	-8%	- 9%	-12	-17	-19	-20

** statistically significant $p \leq 0.01$ (Dunnet´s test)

The data indicate that litter weight has no influence on the body weight of dams since it was statistically reduced at day 20 - relative to mating - and also on day 1 - relative to littering.

It has been previously demonstrated that feed restriction induced reductions in maternal gestational body weight (up to 50 %) only caused reduction in fetal body weight and had no influence on the viability of F1 pups. Even body weight loss (up to 15%) had no influence on fetal viability in rats, but reduced fetal body weights significantly enough to induce minor changes in skeletal development. No external, visceral or skeletal malformations were associated with 15% body weight loss (Fleeman et al., 2005).

As reported in chapter 10.11 (specific target organ toxicity – repeated exposure) 2-NPT has also an impact on kidney at a dose level of 300 mg/kg bw in a 28 day toxicity study. No relationship between kidney damage (observed in the OECD TG 407) and developmental toxicity parameter (OECD TG 421) can be established. At a dose level of 450 mg/kg bw very pronounced effects on the viability of the foetuses during the first four lactation days have been observed not indicative that those pronounced effects are due to kidney damage. Furthermore, in the OECD TG 421 study no test item- related macroscopic changes were observed in any of the organs or tissues of the females treated with 45, 135 or 450 mg/kg bw/day. At 450 mg/kg bw a statistically significant decrease in the mean number of live pups was observed at birth (450 mg/kg bw: 10.4 vs Control 15.2)) and also after the first 4 days of lactation (450 mg/kg bw: 3.5 vs Control 13.3) (see table 10).

55 pups of the high dose group were found dead or were cannibalised on lactation days 1 to 4 compared to 17 deceased or cannibalised pups in the control group (see table 17).

There was no correlation identified between dams with the aforementioned clinical symptoms (increased salivation, ataxia, piloerection, tonic-clonic convulsions) and reduced number of live pups at birth and after 4 days of lactation.

The number of deceased pups and the viability index during the first four lactation days is depicted in table 17.

Table 17: Viability of F1 pups during the first 4 lactation days[a]

Parameter	Control	45 mg/kg bw/day	135 mg/kg bw/day	450 mg/kg bw/day
Number of deceased pups during the first 4 lactation days	17(4)[b]	2	2	55
Viability index (%)	88.3 (97.0)[b]	98.4	98.5	29.6**

[a] number of pups at birth (alive&dead) (mean ± SD: C: 15.4 ± 1.4, 45 mg/kg bw: 14.6 ± 1.6, 135 mg/kg bw/day: 14.5 ± 2.4, 450 mg/kg bw: 11.6 ± 5.0)

[b] the values in the parenthesis represent data of the control group after exlusion of 1 dam (see text below)

** statistically significant at $p \leq 0.01$ (Chi^2 test)

The death of 14 pups out of 17 in one dam of the control group is regarded to be incidental. There were no test item related behavioural abnormalities, nor any macroscopic organ changes for the parental female, no external visible abnormalities were observed for the deceased pups.

The viability index of only 30% compared to 88% (or 97%) is statistically significant compared to the control group ($p \leq 0.01$).

In the low and mid dose group no test item related influence was noted on the mean litter values and the total litter weights of the pups.

Exposure of the parental animals to 450 mg/kg bw/d 2-NPT resulted in a reduced mean and total body weight (-22% and -61%) of male and female pups on lactation day 4 (see table below).

Table 18: Changes in mean and total body weights on lactation day 1 and 4 at a dose level of 450 mg/kg

	Male pups		**Female pups**		**Male and female pups combined**	
Parameter	**Day 1**	**Day 4**	**Day 1**	**Day 4**	**Day 1**	**Day 4**
Body weight [g] (mean litter weight)	6.1 (6.4)[a]	6.7 (9.0)	6.1 (6.0)	6.9** (9.0)	6.1 (6.2)	6.9 (8.8)
Total body weight [g] (total litter weight)	32.7 (49.7)	24.4 (63.6)	25.7 (43.8)	23.3** (65.2)	58.4** (93.5)	47.4** (121.6)

[a] values in parenthesis are values from the control group
** statistically significant at $p \leq 0.01$ (Dunnett test or Student´s t-test)

The total body weight of the pups at the high dose group was already reduced on lactation day 1 compared to the control due to the low number of live born pups (male pups by 34%, male/female pups combined by 38%).

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In the OECD TG 421 developmental screening study clear indication for adverse effect on development of F1 pups was observed at the highest dose level of 450 mg/kg bw/day 2-NPT , which includes reduced viability index, reduced mean and total body weight of the pups.

At 450 mg/kg bw no systemic toxicity was observed, except in few individual pregnant females, who had symptoms such as slightly increased salivation and moderate to slight ataxia, pilo-erection and tonic-clonic convulsions. In total, five out of ten animals had one or two of the aforementioned symptoms. There was no correlation between number of live born pups and those symptoms.

The body weight was decreased in the highest dose group in males (up to 14%) and in pregnant females (up to 20%). It has been previously demonstrated that feed restriction induced reductions in maternal gestational body weight (up to 50 %) only caused reduction in fetal body weight and had no influence on other development toxicity parameters. The most abundant toxicological observation in parental animals is the severe damage of testes, which might lead to the adverse developmental effects in F1 pups, but a causal relationship has not been established.

The effects on F1 generation (reduced viability index during the first lactation days, reduced body weight of pups on lactation day four) -are considered as adverse effects on the development and thus are considered for classification.

The effects on the F1 generation are considered as treatment related.

10.10.6 Comparison with the CLP criteria

According to the CLP Regulation Nr. 1272/2008, for the purpose of classification for reproductive toxicity, substances are allocated to one of two categories:

Category 1: “Known or presumed human reproductive toxicant” (category 1A: “ Known human reproductive toxicant”, category 1B: “Presumed human reproductive toxicant”).

Category 2: “Suspected human reproductive toxicant”

The classification in category 1A is largely based on evidence from humans. There are no data indicating develomental effects of 2-NPT in humans, therefore classification in category 1A is not considered appropriate.

The classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate.

There is no mechanistic information that raises doubt, that the observed developmental effects are not relevant for humans. Therefore a classification in category 2 is not considered appropriate.

Clear indication for adverse effects on the development of F1 pups was observed in the screening study, which includes a statistical significant reduced viability index and statistically reduced mean and total body weight of the pups in the highest dose group.

According to above classification criteria there was "clear evidence" for developmental toxicity, which cannot be identified as secondary non-specific consequence of other toxic effects. The study has no serious deficiencies, thus a classification into Repr. 1B (H360D) is most appropriate. There are no indications that dermal or inhalatory route can be excluded from the hazard statement.

10.10.7 Adverse effects on or via lactation

The adverse effects obtained in OECD TG 421 study (see chapter 10.10.1 and 10.10.4) are considered predominantly as adverse effects on fertility and development and not as adverse effects on or via lactation.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Clear indication for adverse effects on fertility (testes damage) and on the development of F1 pups was observed in the two rat toxicity studies.

According to above classification CLP criteria there was "clear evidence" for reproductive toxicity. A classification into Repr. 1B, H360DF is proposed.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The dossier submitter (DS) proposed classification for effects on sexual function and fertility in category 1B based on a dose-dependent increase in effects on the testes and epididymis including effects on organ weight, size and histopathology in two studies (conducted in accordance with OECD TG 407 and TG 421). At the highest dose these effects were not reversible within 14-days in the OECD TG 407 study. Further, it was stated that there was no mechanistic information that raises doubt that the effects are not relevant for humans.

Developmental effects

The DS proposed classification for effects on development in category 1B based on clear developmental effects of the F1 pups, including reduced viability index as well as reduced

mean and total litter weight. The developmental effects were observed in the presence of some clinical toxicity and decreased body weights in up to 20% of the dams. However, as feed restriction studies have shown, a reduction in maternal gestational body weight up to 50% only caused reductions in foetal body weight and had no influence on other developmental toxicity parameters. The observed developmental effects were not considered to be secondary non-specific consequences of other toxic effects and no mechanistic information was available that raises doubt on their relevance to humans.

Effects on or via lactation

The DS considered that the adverse effects obtained in the OECD TG 421 study are considered predominantly as adverse effects on fertility and development and not as adverse effects on or via lactation.

Comments received during consultation

The proposed classification with Repr. 1B, H360FD was supported by a Member State Competent Authority (MSCA).

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

There are two relevant studies for assessing the effects on sexual function and fertility which are described below. The results of both studies are split into effects on males, females and fertility.

In an oral 28-day repeated dose toxicity study conducted according to OECD TG 407 and GLP, groups of 5 rats per sex were exposed to dose levels of 2-NPT of 0, 30, 100 and 300 mg/kg bw/day by gavage. The study included a 14-day recovery group for the high dose and the controls (Anonymous, 2006).

In an oral screening study conducted according to OECD TG 421 and GLP, groups of 10 rats per sex were exposed to 2-NPT at dose levels of 0, 45, 145 and 450 mg/kg bw/day from 14 days before mating until day 3 post-partum for females and from 14 days before mating until day 32 in males (Anonymous, 2012).

Effects on males

In the repeated dose 28-day study, effects were observed on relative testes and epididymis weight (reduced) at the high dose, a reduction in their size (mid and high dose) and an increase in histopathological changes of both organs (see the tables below) (all dose levels). The effects showed a clear dose-effect relationship. The effects at the highest dose were not reversible and included the complete absence of spermatozoa in the epididymis.

***Table**: Incidences (all, many, middle or few) of observed seminiferous tubules with different grades of damage[b] (n=5)*

Dose (mg/kg bw/day)		**0**	**0[a]**	**30**	**100**	**300**	**300[a]**
Left testis	Grade 1[b]	All (5/5)	All (4/5)	All (1/5) Many (3/5) Middle (1/5)	Many (1/5) Few (1/5)	-	-
	Grade 2	-	Few (1/0)	Middle (1/5) Few (3/5)	Middle (2/5) Few (1/5)	-	Middle (1/5)

	Grade 3	-	Middle (1/0)	-	Many (2/5) Few (2/5)	Many (4/5) Middle (1/5)	All (1/5) Many (3/5) Middle (1/5)
	Grade 4	-	Many (1/0)	-	Many (2/5) Few (1/5)	Many (1/5) Middle (4/5)	Few (4/5)
Right testis	Grade 1	All (5/5)	All (4/5)	Many (3/5) Middle (1/5) All (1/5)	Many (1/5) Middle(1/5)	-	-
	Grade 2	-	Few (1/5)	Few (3/5) Middle (1/5)	Many (1/5) Middle(1/5) Few (1/5)	-	Few (1/5)
	Grade 3	-	Middle (1/5)	0	Middle(1/5) Few (3/5)	Many (4/5) Middle (1/5)	All (1/5) Many (4/5)
	Grade 4	-	Many (1/5)	0	Many (2/5) Few (1/5)	Many (1/5) Middle (4/5)	Few (4/5)

[a] satellite group (14-day recovery period)
[b] grade 1: normal stage, tubules are normal and consist of a normal germinal epithelium with several layers of cells. The full thickness of the germinal epithelium varies slightly, according to the stage of the spermatogenic cycle; grade 2: tubules contain one or more vacuoles in the germinal epithelium without any reduction in its thickness; grade 3: tubules include those with a reduction in the thickness of the germinal epithelium. Most of the epithelium, however, still consists of some germinal cells in addition to the spermatogonia and Sertoli´s cells. Multinucleated giant cells derived from spermatids or spermatocytes are first seen in this stage of degeneration;
grade 4: tubules have the majority of their germinal epithelium lined by spermatogonia and Sertoli´s cells.

Table: *Summary of incidences of findings in the epididymis (n=10)*

Findings in the lumina	**Incidence**	**0 mg/kg bw/day**	**0 mg/kg bw/day [a]**	**30 mg/kg bw/day**	**100 mg/kg bw/day**	**300 mg/kg bw/day**	**300 mg/kg bw/day[a]**
Spermatozoa observed	Normal Many Few None	10 0 0 0	8 0 2 0	10 0 0 0	0 4 0 6	0 0 0 10	0 0 0 10
Fragments of spermatozoa observed	Single	0	0	0	2	0	0
Spermatocytes observed	Many Single	0 0	0 2	0 0	2 8	0 8	0 10
Multinucleated giant cells observed	Single Rarely	0 0	2 0	0 0	6 0	5 2	5 2
Cell detritus observed	Many Single	0 0	0 2	0 0	2 8	0 10	0 10

[a] satellite group (14-day recovery period)

General toxicity in this study was mainly on the kidney and warranted classification with STOT RE 2 as described below. Body weight gain was not affected at any of the dose levels. Clear effects on the kidney were observed in males at 300 mg/kg bw/day and included increased absolute and relative kidney weights and microscopic changes of the cortex and the medulla (see tables under STOT RE section below). In addition, a 10-fold increase in the glucose level of the urine was observed as well as an increase of erythrocytes in the urine sediment. At 100 mg/kg bw/day only slight effects were observed.

Similar effects on the testis and epididymis were observed in an oral screening study conducted according to OECDTG 421 and GLP. Groups of 10 rats per sex were exposed to 2-NPT at dose levels of 0, 45, 135 and 450 mg/kg bw/day via gavage. Testes size was reduced in all mid and high dose males. Absolute epididymis weights were reduced at all dose levels and testes weights at the mid and high dose level. Histopathology examination of the reproductive organs was limited to the high dose animals and controls and showed clear adverse effects on the epididymis, including aspermia, and on the testes, including marked degeneration/necrosis of the germinal epithelium (see table below).

***Table**: Histopathological observations in epididymides and testes of male rats (n=10/group)*

Observation	0 mg/kg bw/day	450 mg/kg bw/day
Epididymides - no abnormalities detected - duct(s), aspermia	 10 0	 0 10**
Testes - no abnormalities detected - atrophy (moderate) - interstitial oedema (moderate) - tubular atrophy (moderate)	 10 0 0 0	 0 10** 10** 10**

**p ≤ 0.01 (Fisher's test)

General toxicity parameters included in a screening study according to OECD TG 421 are very limited. Male body weights were statistically significantly reduced at 450 mg/kg bw/day from day 8 of exposure, resulting in a 14% reduction on day 33 (see table below). No lethality or clinical toxicity was observed.

***Table**: Body weights of male rats at different time points relative to start date (n=10/group)*

Dose (mg/kg bw/day)		Day 1	Day 8	Day 15	Day 22	Day 29	Day 33
0	Mean SD	344.49 11.43	386.99 20.26	416.15 27.95	432.12 33.59	461.03 36.37	470.67 39.91
45	Mean SD	344.11 12.05	384.05 22.53	415.56 29.19	428.70 33.71	462.38 38.30	462.79 44.38
135	Mean SD	345.15 11.98	372.64 15.18	401.95 20.53	415.49 24.98	442.12 32.43	449.10 35.04
450	Mean SD % difference to control group	344.85 11.93 -	355.87** 16.40 -8	379.78 28.62* -9	383.36** 34.03 -11	402.28** 41.78 -13	405.41** 40.03 - 14

*p ≤ 0.05 (Dunnett 2-sided test)
**p ≤ 0.01 (Dunnett 2-sided test)

Effects on Females

No effects on female reproductive organs were observed in the available screening studies.

Fertility

A decrease (25%) in the mean and total number of corpora lutea and implantation sites was observed in the screening study at 450 mg/kg bw/day (see table below). This effect was not statistically significant at p ≤ 0.01. It is noted that the statistical significance at the more usual p-level of 0.05 is not provided in the CLH report.

***Table**: Summary of fertility and reproduction parameters*

Parameter		0 mg/kg bw	45 mg/kg bw	135 mg/kg bw	450 mg/kg bw
Pre-coital time (days), females (n=10/group)[a]	Mean ± SD	4.9 ± 4.7	2.4 ± 1.2	2.1 ± 1.1	6.2 ± 5.9
Fertility Index[b]	%	90	100	80	80
Number of dams evaluated for the following parameters	Nr.	9	10	8	8
Gestation length[b]	Mean ± SD	22.2 ± 0.4	22.2 ± 0.6	22.5 ± 0.5	22.9 ± 0.4
Gestation index[b]	%	100	100	100	100
Corpora lutea[c]	Total Mean ± SD	163 18.1 ± 3.9	183 18.3 ± 3.5	145 18.1 ± 2.9	108 13.5 ± 5.6
Implantation sites[a]	Total	150	159	127	101

	Mean ± SD	16.7 ± 1.3	15.9 ± 1.2	15.9 ± 2.2	12.6 ± 5.0
Number of pups at birth (alive and dead)[a]	Total Mean ±SD	139 15.4 ± 1.4	146 14.6 ± 1.6	116 14.5 ± 2.4	93 11.6 ± 5.0
Number of stillbirths	Nr.	2	4	0	10
Number of dams with stillborn pups	Nr.	2	1	0	4
Number of live born pups[a]	Total Mean ± SD	137 15.2 ± 1.4	142 14.2 ±2.0	116 14.5 ± 2.4	83 10.4 + 4.6**
Birth index[d]	%	92.8	91.7	91.9	92.4
Live birth index[d]	%	98.6	97.3	100.0	90.6**
Pre-implantation loss[d]	Mean % ± SD	6.0 ± 10.9	10.9 ± 13.4	11.6 ± 10.1	4.8 ± 9.6
Post-implantation loss[d]	Mean % ± SD	8.4 ± 7.8	10.8 ± 9.0	8.9 ± 13.4	16.2 ± 15.2*

[a] $p \leq 0.01$ (Student's t-test)
[b] $p \leq 0.05$ or $p \leq 0.01$ (Fisher's test)
[c] $p \leq 0.01$ (Dunnet's test)
[d] $p \leq 0.05$ or $p \leq 0.01$ (Chi^2 test)

General toxicity in the screening study included clinical toxicity in some pregnant females and reduced body weights (see table below) being statistically significant from day 7 after mating at 450 mg/kg bw/day. The effects on body weight are a combination of the number of implantations (mainly post implantation loss)/reduction in number of pups and maternal effects.

***Table**: Body weights of female rats at different time points relative to mating and littering (n=8-10/group)*

Dose levels (mg/kg bw/day)	**Parameter**	**Days relative to mating**				**Days relative to littering**	
		Day 0	**Day 7**	**Day 14**	**Day 20**	**Day 1**	**Day 4**
0	Mean SD	251.56 21.32	289.89 17.72	325.32 18.09	405.33 23.07	311.10 18.43	318.17 17.44
45	Mean SD	241.09 11.65	280.16 12.63	315.54 11.79	397.40 17.08	297.52 14.76	301.03 10.05
135	Mean SD	244.59 14.76	284.11 21.15	325.21 22.54	402.46 31.91	301.18 28.29	305.26 27.72
450	Mean SD % difference to Gr. 1	231.20 19.82 -8	263.25** 14.14 -9	287.49** 17.24 -12	335.08** 34.38 -17	251.71** 17.83 -19	256.03** 18.48 -20

**p ≤ 0.01 (Dunnett 2-sided test)

Conclusion

There is a clear effect of 2-NTP on the male reproductive organs in two studies at dose levels of 30 mg/kg bw/day and above. This effect did not result in a decrease in implantation sites compared to the corpora lutea. However, in this screening study mating occurred on day 14 of exposure whereas histopathology and other parameters on male reproductive organs were performed on day 33. RAC considers it likely that the observed effects on male sexual function on day 33 will result in reduced fertility. The effects on the testes and epididymis were dose dependent and included changes in organ weights, macroscopic and microscopic changes (reduced spermatozoa, absence of spermatozoa in the high dose group, testicular atrophy, loss of germ cell layers in the seminiferous tubules, tubular atrophy with damage (degeneration/necrosis) of the germinal epithelium, loss of spermatogonia, spermatocytes, spermatids and spermatozoa and epididymis aspermia with only empty duct or ducts containing cellular debris). The effects were most pronounced at 450 mg/kg bw/day, a level at which also general toxicity such as changes in body weight and effects on kidneys were

observed. However, at lower doses with minimal to no general toxicity effects on testes and epididymis were also noted (slight damage to the seminiferous tubules of the testes at 30 mg/kg bw/day). Therefore, the observed effects on the testes and the epididymis are not considered secondary to other toxicity. No information is available on the mechanism of the effects or the human relevance. The effects on male reproductive organs are considered relevant to humans. Therefore, classification with Repr. 1B, H360 for fertility is warranted.

The reduction in corpora lutea in the screening study at 450 mg/kg bw/day may be considered an indication of an effect on female fertility. However, the effect was not significant which is probably due to the limited number of animals, and it was observed in the presence of maternal toxicity (clinical toxicity and reduced body weight up to -20% on post-natal day 4 and kidney effects). On this basis it is concluded that the available data does not warrant classification for female reproduction. However, in view of the limitations of a reproduction screening study, the information is insufficient for drawing firm conclusions regarding effects on female reproduction.

Developmental effects

In an oral screening study conducted according to OECD TG 421 and GLP, groups of 10 rats per sex were exposed to 2-NPT at dose levels of 0, 45, 145 and 450 mg/kg bw/day from 14 days before mating until day 3 post-partum for females and from 14 days before mating until day 32 in males.

A decrease (25%) in the mean and total number of corpora lutea and implantation sites was observed in the screening study at 450 mg/kg bw/day (see table "Summary of fertility and reproduction parameters", above). This effect was not statistically significant at $p \leq 0.01$. These effects are considered as effects on fertility and are discussed in the relevant section. The mean number of live born pups per dam was statistically significantly reduced at the highest dose (see table "Summary of fertility and reproduction parameters", above). However, as the number of corpora lutea and implantation sites was also reduced and the birth index was not affected, this is not considered an effect on development. Further, there was a statistically significant decrease in the live birth index and an increase in the post-implantation loss at the high dose. Post-natally, there was a strong reduction of the viability index of day 0 to day 4 to 29.6% (see table "Viability of F1 pups during the first 4 lactation days", below) at the high dose, which was accompanied by a reduction in pup growth (see table "Changes in mean and total body weights on lactation day 1 and 4 at a dose level of 450 mg/kg bw/day", below). No abortion or any malformed foetuses were noted in any of the tested dose groups.

***Table**: Viability of F1 pups during the first 4 lactation days*[a]

Parameter	Control	45 mg/kg bw/day	135 mg/kg bw/day	450 mg/kg bw/day
Number of deceased pups during the first 4 lactation days	17(4)[b]	2	2	55
Viability index (%)	88.3 (97.0)[b]	98.4	98.5	29.6**

[a] number of pups at birth (alive & dead) (mean ± SD: C: 15.4 ± 1.4, 45 mg/kg bw/day: 14.6 ± 1.6, 135 mg/kg bw/day: 14.5 ± 2.4, 450 mg/kg bw/day: 11.6 ± 5.0)
[b] the values in the parenthesis represent data of the control group after exclusion of 1 dam (see text below)
**$p \leq 0.01$ (Chi^2 test)

***Table**: Changes in mean and total body weights on lactation day 1 and 4 at a dose level of 450 mg/kg bw/day*

	Male pups		**Female pups**		**Male and female pups combined**	
Parameter	**Day 1**	**Day 4**	**Day 1**	**Day 4**	**Day 1**	**Day 4**
Body weight [g] - mean litter weight	6.1 (6.4)[a]	6.7 (9.0)	6.1 (6.0)	6.9** (9.0)	6.1 (6.2)	6.9 (8.8)
Total body weight [g] - total litter weight	32.7 (49.7)	24.4 (63.6)	25.7 (43.8)	23.3** (65.2)	58.4** (93.5)	47.4** (121.6)

[a] values in parenthesis are values from the control group
**p ≤ 0.01 (Dunnett test or Student's t-test)

General toxicity in the screening study included clinical toxicity in some pregnant females and reduced body weights being significant from day 7 after mating at 450 mg/kg bw/day. The decrease compared to controls ranged from 8% at mating to 20% on day 4 after birth (see table above). A part of the decrease in maternal body weight could be secondary to the decrease in the number foetuses per dam at this dose level. Further, as some effects on the kidney weights (see table "Kidney weights, relative (%) (n=10/group)", below), kidney pathology (see table "Incidence of findings in the kidneys") and effects showing malfunction of the kidneys were observed in the 28-day study at 300 mg/kg bw/day in females, it is considered likely that at least comparable kidney effects occurred in the dams in the screening study at 450 mg/kg bw/day with an exposure period of at least 37 days.

Overall, clear evidence of pre-natal and post-natal developmental effects were observed at 450 mg/kg bw/day in the presence of maternal toxicity. RAC agrees with the DS that a study with feed restriction indicates that a small weight reduction of 20% is unlikely to induce the observed serious developmental effects (Freeman *et al.*, 2005). However, in addition to the effects on body weight also effects on the kidney and its function were observed (increase in urine glucose and erythrocytes in the urine sediment). It is unknown whether and how such kidney effects affect the pre- and post-natal development of the pups. Therefore, RAC considers that it cannot be concluded that the observed developmental effects are not a secondary non-specific consequence of the observed and anticipated maternal toxicity.

RAC agrees that classification in Category 2 for effects on development is warranted, based on clear developmental effects with likely maternal kidney effects for which it is unclear whether it can induce the observed developmental effects. Furthermore, no developmental study is available and the only information available is from a reproduction screening study. RAC notes that this endpoint may require reassessment when developmental toxicity studies become available. RAC agrees that there are no indications that the dermal or inhalation route can be excluded from the hazard statement.

Effects on or via lactation

The effects considered relevant for the assessment of effects on or via lactation are limited to a statistically significant increase in deceased pups during the first 4 lactation days (see table "Viability of F1 pups during the first 4 lactation days") and the reduced pup body weight gain during this period for the dose of 450 mg/kg bw/day in the screening study (see table "Changes in mean and total body weights on lactation day 1 and 4 at a dose level of 450 mg/kg bw/day"). These effects could be due to the *in utero* exposure of the pups having been expressed only post-natally or due to the effect on lactation or via exposure to 2-NTP via the milk. The available information does not allow a distinction to be made between these possibilities. Therefore, RAC advises not to classify for effects on or via lactation due to inconclusive data.

Overall conclusion

RAC agrees with the DS to classify for effects on sexual function and fertility as Repr. 1B, based on the clear adverse effects on male reproductive organs in two studies which are not considered secondary to the general toxicity and relevant for humans. However, RAC does not support the proposed classification in category 1B for effects on development, but proposes category 2, while noting that no developmental toxicity studies are available to RAC. RAC agrees with no classification for effects on or via lactation due to inconclusive data. Together this results in a **classification for reproductive toxicity as Repr.1B, H360Fd**.

10.11 Specific target organ toxicity-repeated exposure

Table 19: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) rat (Wistar) n=60, male (n=30) and female (n=30) Reliatibility: Klimisch 1	Test substance: *N*-(2-nitrophenyl)phosphoric triamide Vehicle: 0.5 % (m/v) solution of Tylose MH 1000 in deionised water Exposure route: gavage 0, 30, 100 and 300 mg/kg bw/d (actual ingested) Duration of exposure: 28 days (once daily), 14 days treatment free recovery period	Main target organs: testes and kidney Damage of seminiferous tubules in the testes which led to a complete absence of spermatozoa in the epididymis. Dose dependent damage on the kidneys, characterised by flattended and partly degenerated tubular epithelium in the renal cortex and in the renal medulla. Increased organ weight of the spleen indicates that the total organism (presumable via immune system) is involved in the reaction to the test item. NOAEL was set to be 30 mg/kg bw/d based on the normal state of the epididymis, the normal number of spermatozoa and the normal state of kidneys.	Anonymous (2006)

In a subacute oral repeated dose toxicity study according to OECD TG 407 (GLP compliant) Wistar rats (30 males and 30 females) were exposed daily to 0, 30, 100, or 300 mg/kg bw 2-NPT by oral gavage followed by a 14 day recovery period.

No animals died during the course of investigations. Apart from discoloration of the urine (yellow or orange), caused by the colour of the test item, no other alteration of general state of well-being was observed. The body weight gain and the food consumption were not influenced by the administration of the test item.

The most abundant toxic effect of the test substance was dose dependent damage to the testes, which resulted in the high dose group in a complete absence of spermatozoa in the epididymis. The exposure to the test substance in the high dose group disturbed the normal development of testes and epididymis completely. Slight damage to the seminiferous tubules in the testes was observed also in the animals of the low dose group, thus a NOEL (No Observed Effect Level) for testes effects could not be established.

According to the results of the study the testes/epididymis (for more details see chapter 10.10.1) and the kidneys can be assumed to be the target organs of the test item. The testes toxicity effects are considered as relevant for the reproductive endpoint and thus in this section primarily adverse effects on kidneys are considered.

The study authors summarize that the absolute and relative organ weights of the kidneys were statistically significantly increased in all animals of the high dose group. This effect was reversible in female however not in male animals. The increase was up to 15% (absolute organ weight, right kidney, 300 mg/kg bw/day, males) (see table 20).

Also the spleen weight was irreversible, statistically significant increased in the males (not in females) in the high and middle dose group.

Table 20: Kidney weights, relative (%) (n=10/group)

Dose		**0 mg/kg bw**	**0 mg/kg bw[a]**	**30 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**300 mg/kg bw[a]**
Males							
Kidney left	Mean ± SD	0.399 ± 0.0315	0.403 ± 0.0261	0.434 ± 0.0339	0.441 ± 0.0369	0.442 ± 0.0238	0.429 ± 0.0398
Kidney right	Mean ± SD	0.394 ± 0.0282	0.403 ± 0.0354	0.441 ± 0.0294	0.440 ± 0.0458	0.469 ± 0.0225*	0.417 ± 0.0369
Females							
Kidney left	Mean ± SD	0.413 ± 0.0082	0.404 ± 0.0202	0.403 ± 0.0127	0.389 ± 0.0248	0.443 ± 0.0178*	0.387 ± 0.0216
Kidney right	Mean ± SD	0.427 ± 0.0156	0.417 ± 0.0459	0.412 ± 0.0128	0.396 ± 0.0359	0.442 ± 0.0147	0.394 ± 0.0227

[a] satellite group (14 day recovery period)
* statistically significant $p \leq 0.05$ (Dunnett test)

Macroscopic examinations demonstrate brightened kidneys in the highest dose group in males (5/5) and females (1/5) and yellowish discoloured renal pelvis in the highest dose group in males (5/5). This effect occurred only slightly in the male animals of the middle dose group and in the male animals of the satellite group. In the mid dose group multiple dark foci (approx. 1.1 mm) have been detected in kidneys (both) of one male animal. In the control group no macroscopic findings in the kidney were observed (see details table 21).

Table 21: Incidence of the observation for kidney brightened and renal pelvis yellowish discoloured (n=5)

		0 mg/kg bw	0 mg/kg bw[a]	30 mg/kg bw	100 mg/kg bw	300 mg/kg bw	300 mg/kg bw[a]
Males							
Kidney brightened		0	0	0	0	5	1+1[b]
Renal pelvis yellowish discoloured		0	0	0	1	5	0
Females							
Kidney brightened		0	0	0	0	1	1[b]
Renal pelvis yellowish discoloured		0	0	0	0	1	0

[a] satellite group (14 day recovery period)
[b] kidney marbeled

Histopathological examination indicates a dose dependent change in kidney parameters (see table 22), characterised by flattened and partly degenerated tubular epithelium in the renal cortex and in the renal medulla and vascular dilatation of the capillary network in the renal cortex in male and female animals. The males seem to be slightly more sensitive. The degree of severity (low, moderate, severe) has been recorded for the histopathological findings. All recorded findings were ranked to be of low severity.

The observed damage was only partly reversible after the 14 day recovery period.

Partial degeneration of the tubular epithelium in the renal cortex occurred also in the kidneys of two control animals. The study authors conclude that a slight degeneration of tubular epithelium in the renal cortex in single cases seems to be a normal process in the kidneys.

Table 22: Incidence of findings in the kidneys

Findings	**Incidence of findings from 10 kidneys, each in**											
	male animals of dose group [mg/kg bw]						**Female animals of dose group [mg/kg bw]**					
	0	0[a]	30	100	300	300[a]	0	0[a]	30	100	300	300[a]
flattened tubular epithelium in the renal cortex	0	0	0	0	1	0	0	0	0	2	0	0
flattened tubular epithelium in the renal medulla	0	0	0	1	10	2	0	0	0	2	6	3
partial degeneration of the tubular epithelium in the renal medulla	0	0	0	0	4	0	0	0	0	0	1	0
vascular dilatation of the capillary network in the renal cortex	0	0	0	0	2	0	0	1	0	2	1	4
partial degeneration of the tubular epithelium in the renal cortex (partial only one focus)	0	2	1	4	2	4	0	0	0	0	2	0
necrotic cells in the tubules	0	0	0	1	0	0	0	0	0	0	0	0
necrotising inflammation in the area of the papilla renalis**	0	0	1	0	0	0	0	0	0	0	0	0
severe lymphocytic infiltration in the renal pelvis	0	0	0	0	0	0	0	1	0	0	0	0

[a] satellite group (14 day recovery period), statistical evaluation within this group
** necrotic tubules in the centre, capillary multiplication and connective tissue covered in the area of renal pelvis by epithelium of the urinary tract

Urinalysis demonstrates a dose dependent increase in glucose levels, which reached statistical significance in the high dose group in males and females. The content of glucose in males was 15.92 mg/dl at the highest dose vs 1.54 mg/dl in the control group, and in females 12.28 mg/dl vs 1.68 mg/dl, respectively. Glucose content in the blood was not affected by treatment with the substance.

Furthermore erythrocytes were found in the urine sediment of the treated animals of the middle and the high dose group without complete reversibility.

A dose dependent increase in test item concentration in urine was seen in all treated animals and corresponded to the discoloration of the urine.

The study authors conclude that the test item is rapidely excreted via the kidneys in case of intake of not damaging doses.

Furthermore the study author states that the increased organ weight of the spleen shows that the total organism (presumable via the immune system) is involved in the reaction to the test item administration.

The absolute and relative organ weights of the spleen were irreversible, statistically significantly increassd only in the male animals of the middle and high dose group. These effects was not observed in females.

These increased organ wights correspond to the dose dependently slightly increased leucocyte count, which was statistically significant in the male animals of the high dose group.

The activities of the alkaline phospatase and of the aspartate aminotransferase of the serum were reversible decreased in the animals of the high dose group.

A NOEL could not be established, based on the discoloration of the urine (caused by the colour of the test item) and the slight damage of the seminiferous tubules in the testes observed also in the animals of the low dose group. Since there was normal state of kidneys and no abnormality of the epididymis in the animals of the low dose group the NOAEL is set at 30 mg/kg bw.

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

In the OECD TG 407 study severe adverse effects on male reproductive organs (see chapter 10.10.1) and negative effects on the kidney, such as organ weight changes, histopathological changes and changes in the urineanalysis parameters were detected. The study was conducted under GLP and has a reliability of 1.

Table 23: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	**Effective dose (mg/kg/d)**	**Length of exposure**	**Extrapolated effective dose when extrapolated to 90-day exposure**	**Classification supported by the study**
Anonymous (2006)	300 mg/kg bw/day	28 days	100 mg/kg bw/day	Yes

10.11.2 Comparison with the CLP criteria

Administration of 2-NPT leads to a dose dependent severe damage to the testis (details see Chapter 10.10.1). Also the kidney is a target organ of 2-NPT ´s toxicity.

At the highest dose group (300 mg/kg bw) the following histopathological findings in the kidney are recorded: flattened tubular epithelium in the renal cortex (m: 1/10, f: 2/10) and in the renal medulla (m:10/10, f: 6/10), partial degeneration of the tubular epithelium in the renal medulla (m: 4/10, f: 1/10), vascular dilatation of the capillary network in the renal cortex (m: 2/10, f:1/10), partial degeneration of the tubular epithelium in the renal cortex (partial only one focus) (m: 2/10, f: 2/10). The severity of the findings has been ranked as low. The results indicate that males are more sensitive than females.

Macroscopic examination reveals brightened kidneys as well as yellowish discoloured renal pelvis in the highest dose group particularly in males. Absolute and relative organ weights of the kidneys were statistically significantly increased in all animals of the high dose group. The effect was not reversible in males.

The results of the urineanalysis demonstrate a dose-dependent, but reversible, increase in glucose content in all dose groups. The increase reached statistical significance in the high dose group (males and females, up to 10-fold increase). No effects on blood glucose content were observed, indicative of considerable kidney damage. Further indication of injury is the observation of erythrocytes in the urine in treated animals in the middle and high dose group, which was not reversible within the recovery period.

According to CLP Regulation Annex I: 3.9.1.1. significant health effects that impair the function, which are both reversible and irreversible, immediate or delayed should be considered for STOT RE classification. Results demonstrate that the function of the kidney is severely impaired, since there is a statistically significant change in the glucose content at the highest dose group and there have been erythrocytes detected in the urine in treated animals, the latter effect was not reversible. Although the study authors ranked the histopathological findings (e.g. partial degeneration of the tubular epithelium in the renal medulla and cortex) as not severe, the sum of the observed findings leads to significant alteration of kidney function, indicating injured kidneys.

The results clearly indicate that the function and morphology of the kidney (macroscopic and histopathological observations, increased organ weight) have toxicologically significantly changed and thus Annex I, 3.9.1.3. of CLP Regulation is fulfilled.

Based on the study results a classification for specific organ toxicity (repeated exposure) is proposed according to definitions laid down in CLP Regulation (Annex I, 3.9.1.1 to Annex I, 3.9.1.3, and further specified in Annex I, 3.9.2.7.3.).

Since the observed adverse effects in the 28 day toxicity study are above the guidance values for STOT RE 1 classification (≤ 30 mg/kg bw/day), no classification into this hazard category is proposed.

The guidance values for STOT RE 2 classification (oral 28 day toxicity studies (rat)) are according to CLP Regulation between 30 and 300 mg/kg bw/day. Thus, based on the severity of the detected effect at 300 mg/kg bw/day classification with STOT RE 2 (H373, kidney) is proposed.

10.11.3 Conclusion on classification and labelling for STOT RE

Based on the results of the 28 day toxicity study a classification for STOT RE 2; H373 (kidney) is proposed. There are no indications that dermal or inhalatory route can be excluded from the hazard statement.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed classification as STOT RE 2 for effects on the kidneys and their function observed at 300 mg/kg bw/day in an oral 28-day study conducted according to OECD TG 407 with the kidneys as the target organ.

Comments received during consultation

The proposed classification with STOT RE 2, H373 (kidney) was supported by a MSCA.

Assessment and comparison with the classification criteria

The available repeated dose toxicity information is limited to an oral 28-day study conducted according to OECD TG 407 and GLP, using 4 groups of 5 rats per sex at dose levels of 0, 30, 100 and 300 mg/kg bw/day by gavage and including a recovery group for the high dose and the controls (Anonymous, 2006). No effects were observed on mortality, clinical effects, body weight gain and food consumption. The main effects were limited to the kidneys and the testes. The effects to the testes were taken into account for the classification for adverse effects on sexual function and fertility. The kidney effects at 300 mg/kg bw/day included increased absolute and relative kidney weights in males (see table "Kidney weights, relative", below), discolouration of the kidneys, renal pelvis and urine and microscopic changes of the cortex and the medulla (see table "Incidence of findings in the kidneys", below). The effects were more severe in male rats. In addition, a 10-fold increase in the glucose level of the urine but not in the blood was observed at the high dose in males and females as well as an increase of erythrocytes in the urine sediment at the mid and high dose in rats. Some of the observed effects were reversible within 14 days. Overall, RAC considers the observed effect at 300

mg/kg bw/day to be clear evidence of a functional effect on the kidneys. Some of these effects were also observed at 100 mg/kg bw/day although with a lower incidence and/or severity but not at 30 mg/kg bw/day.

***Table**: Kidney weights, relative (%) (n=10/group)*

Dose (mg/kg bw/day)		**0[a]**	**0**	**30**	**100**	**300**	**300[a]**
Males							
Kidney left	Mean ± SD	0.399 ± 0.0315	0.403 ± 0.0261	0.434 ± 0.0339	0.441 ± 0.0369	0.442 ± 0.0238	0.429 ± 0.0398
Kidney right	Mean ± SD	0.394 ± 0.0282	0.403 ± 0.0354	0.441 ± 0.0294	0.440 ± 0.0458	0.469 ± 0.0225*	0.417 ± 0.0369
Females							
Kidney left	Mean ± SD	0.413 ± 0.0082	0.404 ± 0.0202	0.403 ± 0.0127	0.389 ± 0.0248	0.443 ± 0.0178*	0.387 ± 0.0216
Kidney right	Mean ± SD	0.427 ± 0.0156	0.417 ± 0.0459	0.412 ± 0.0128	0.396 ± 0.0359	0.442 ± 0.0147	0.394 ± 0.0227

[a] satellite group (14 days recovery period)
*p ≤ 0.05 (Dunnett test)

***Table**: Incidence of findings in the kidneys*

Findings	**Incidence of findings from 10 kidneys, each in**											
	male animals of dose group (mg/kg bw/day)						**Female animals of dose group (mg/kg bw/day)**					
	0	0[a]	30	100	300	300[a]	0	0[a]	30	100	300	300[a]
Flattened tubular epithelium in the renal cortex	0	0	0	0	1	0	0	0	0	2	0	0
Flattened tubular epithelium in the renal medulla	0	0	0	1	10	2	0	0	0	2	6	3
Partial degeneration of the tubular epithelium in the renal medulla	0	0	0	0	4	0	0	0	0	0	1	0
Vascular dilatation of the capillary network in the renal cortex	0	0	0	0	2	0	0	1	0	2	1	4
Partial degeneration of the tubular epithelium in the renal cortex (partial only one focus)	0	2	1	4	2	4	0	0	0	0	2	0
Necrotic cells in the tubules	0	0	0	1	0	0	0	0	0	0	0	0
Necrotising inflammation in the area of the papilla renalis**	0	0	1	0	0	0	0	0	0	0	0	0
Severe lymphocytic infiltration in the renal pelvis	0	0	0	0	0	0	0	1	0	0	0	0

[a] satellite group (14-day recovery period), statistical evaluation within this group
** necrotic tubules in the centre, capillary multiplication and connective tissue covered in the area of renal pelvis by epithelium of the urinary tract

In addition, an irreversible increase in spleen weight was observed in males at 100 and 300 mg/kg w/day and an increase in leukocyte counts in the high dose males. The activities of the alkaline phosphatase and of the aspartate aminotransferase of the serum were also reversibly decreased in the animals of the high dose group.

RAC agrees with the proposal of the DS to classify 2-NTP with **STOT RE 2, H373** with the target organ **kidneys**, based on the observed effects on the kidney and the related indication of kidney malfunction at 100 and 300 mg/kg bw/day in a 28-day study as the effects were observed within the guidance values for STOT RE 2 for a 28-day study (30 – 300 mg/kg bw/day). RAC also agrees that there is no information to exclude specific target organ toxicity via other routes.

10.12 Specific target organ toxicity-single exposure

Not assessed.

10.13 Aspiration hazard

Not assessed.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 24: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Part C: C4.-B OECD Screening test (similar to OECD TG 301E) GLP performed Test item: *N*-(2-nitrophenyl) phosphoric triamide 28d exposure Tested on DOC removal	No marked DOC removal was measured. Pass level of 70% for ready biodegradation was not reached. The test item is seen to be not readily biodegradable. Study revealed no toxicity towards microorganisms.	Valid, Klimisch Reliability: 1	Anonymous, 2006c
OECD TG 111 (1981) GLP performed Test item: *N*-(2-Nitrophenyl) phosphoric triamide 30d exposure pH 4,7 and 9 (DT_{50} were calculated, first order kinetics)	pH 4: DT_{50} 28.5h (25°C) pH 7: DT_{50} 148.4d (25°C) pH 9: DT_{50} 48.2h (25°C)	Valid, Klimisch Reliability: 2	Anonymous, 2008

11.1.1 Ready biodegradability

A ready biodegradation study is available using 2-NPT following the modified OECD screening test method Part C: C.4-B (similar to OECD Test Guideline 301E). The study was conducted under GLP conditions.

Material and methods

The used inoculum was taken from the effluent of a waste water treatment plant, a predominant domestic sewage. The flasks were incubated for a period of 28 days in the dark at a temperature of 20.0 – 24°C. The number and composition of the used flasks was the same as described in the guidance, only the order was different (see table below). Sodium benzoate was used as positive reference substance. In the study a test concentration of 110 mg/L for the test item, which is 13-fold lower than the water solubility (1392 mg/L) and 60 mg/L for the reference substance was used.

Test run flasks

No.	Name	Composition	Number of flasks
1+2	Inocolum control	Mineral medium+control	2
3+4	Test item	Mineral medium+inoculum+test item	2
5	Procedure control	Mineral medium+inoculum+reference substance	1
6	Toxicity control	Mineral medium+inoculum+reference substance	1
7	Abiotic control	Mineral medium+test item+sterilisation agent	1
8	Adsorption control	Mineral medium+inoculum+test item+sterilisation agent	1

Results

The pH was measured at the beginning in all flasks with 7.4±0.1. DOC concentration was measured at day 0,1,3,6,9,13,17,20,24 and 28 and the percentage of the DOC removal was calculated. For the test item no marked removal of DOC was observed at any time (see Fig.1). In the procedure control the reference substance was degraded for 6% at day 1, 68% at day 3, 91% at day 6 and almost completely to 93% at day 9. The pass level for ready biodegradability (70 %) was reached at day 3. No inhibitory effect of the test item was observed. The abiotic and adsorption control did not give any indication for a loss of DOC. Toxicity and adsoption were not the drivers for the non-biodegradability of the test item. The validity criteria a.) the percentage degradation of the reference substance has reached the pass level of 70% on day 6 and b.) for the test item solutions the difference of extremes of replicate values at the end of the test, were met according to the guideline.

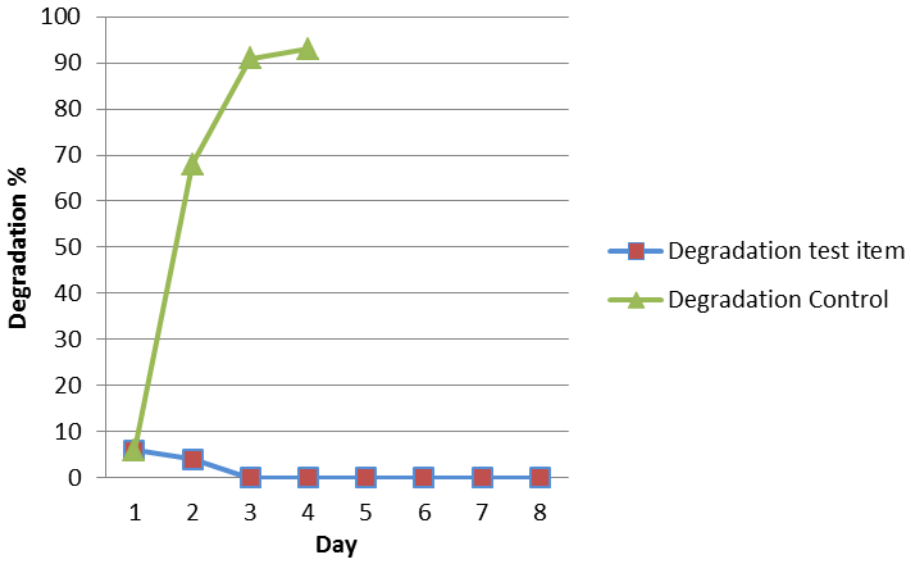


Figure 1: Degradation (DOC % removal) of the test item and the reference substance (taken from Anonymous, 2006c)

It can be concluded, that the test item 2-NPT is not readily biodegradable.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

A hydrolysis study was performed using 2-NPT following the OECD test guideline 111 (adopted 1981). The study was conducted under GLP conditions.

Material and methods

2-NPT was tested in the pre-test and the definite test at pH 4, 7 and 9 at 25°C in buffered mediums in the dark. In the pre-test the used test concentration was 0.432 mg/L in triplicate of the tested pH values. Specimen were taken in the pre-test at day 0 and 3. The definite test was also performed with a concentration of 0.432 mg/L, well below of the water solubility. Specimen were taken at pH4 at 0,4h, 1d, 2d, 3d and 4d, at pH 7 at 0,3d, 8d, 15d, 22d and 30d and at pH 9 at 0,4h, 1d, 2d, 3d, 4d and 5days at duplicate. In the pH 4 buffer potassium citrate, NaOH and reagent water, in the pH 7 buffer potassium phosphate monobasic together with NaOH with reagent water and in the pH 9 buffer boric acid, KCl, NaOH and reagent water was used. To detect the test item in the aqueous solution a valid LC-MS/MS methodology was applied.

Results

In the pre-test after 3 days approximately 20% of the initial concentration was found at pH 4 buffer and 30% at pH 9 buffer. At pH 7 buffer the concentration of the test item remained constant after 3 days. In the definite test the recovery rates for pH 4 ranged from 101% at time 0 to 9% at time 4d, for pH 7 from 96% at time 0 to 84% at day 30 and at pH 9 from 91% at time 0 to 16% at day 5. The occurrence of any degradation products was not reported in the study. First order kinetics (DT_{50} and DT_{90} if possible) were calculated for the test item for all tested pH values (see table below).

Kinetics of 2-NPT

pH value	DT_{50} 1st order	DT_{90} 1st order
4	28.5h	94.6h
7	148.4d	Calculation not possible
9	48.2h	~ 150h

Conclusion:

The substance 2-NPT undergoes hydrolysis at pH 4 (DT_{50} of 28.5h) and at pH 9 (DT_{50} of 48.2h), whereas at pH 7 (the most relevant pH value) a very slow hydrolytic reaction occurred (DT_{50} of 148.4d). In the CLP guidance (ECHA, 2017)[2] it is stated, that "*data on hydrolysis e.g. OECD 111 might be considered for classification purposes only when the longest half-life t1/2 determined within the pH range 4-9 is shorter than 16 days*". Since the longest half life time with 148.4 days (measured at the most relevant pH value of 7)

[2] Guidance on the application of the CLP criteria (ECHA, 2017)

is above the limit value of 16 days, this is another indication that the test item is not rapidly degradable in the environment. However it is further stated in the CLP guidance "*Only when it can be satisfactorily demonstrated that the hydrolysis products formed do not fulfil the criteria for classification as hazardous for the aquatic environment, data from hydrolysis studies could be considered*". For 2-NPT no information on degradation products is available, therefore hydrolysis is not taken into account for classification purposes.

11.1.4 Other convincing scientific evidence

No additional data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

In an aerobic soil degradation study according to OECD TG 307 (adopted april 2002) the degradation of the test item 2-NPT was tested in three different soils for an incubation time of 29 days. The recoveries of the test item were between 84% and 95%. Samples were taken at time zero, 1, 3, 7, 10, 14, 15, 22 and 29 days. DT_{50} values related to dissipation (1^{st} order kinetic) were calculated for each soil type and ranged from 3.9 - 8.6 days and DT_{90} values from 13.0 - 28.6 days. Three reference substances were used in the test: 2-Nitroaniline (CAS 88-74-4), 4-Amino-3-nitrophenol (CAS 610-81-1) and 1,2-Phenylendiamine (CAS 95-54-5). One major metabolite (2-Nitroaniline) was detected with a maximum of ~50% at day 15 in relation to the parent compound and remained on a constant level around 30% until day 29. 4-Amino-3-nitrophenol was not detected in any of the soils. No other metabolite was observed. The determination of 1,2-Phenylendiamine in the soil matrix failed. The quantity of non-extractable residues (NER) was not reported in the study. A harmonised classification was found in the C&L inventory for 2-Nitroaniline (H412, Aquatic Chronic 3).

On this basis 2-NPT does not have an ultimate degradation half-life less than 16 days, as stated in the CLP guidance (ECHA, 2017).

Overall conclusion on degradation data

The presented degradation information does not provide sufficient information to prove that 2-NPT is ultimately degraded within 28days (equivalent to a half life <16days). In a ready biodegradability test the test substance did not show any remarked removal of DOC. In the hydrolysis test no degradation products were determined. Hence 2-NPT is considered not rapidly degradable according to the definition of the CLP Regulation.

11.1.4.4 Photochemical degradation

No data available.

11.2 Environmental transformation of metals or inorganic metals compounds

The substance is not a metal.

11.2.1 Summary of data/information on environmental transformation

The substance is not a metal.

11.3 Environmental fate and other relevant information

The substance is not a metal.

11.4 Bioaccumulation

Table 25: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
EU method A.8	Mean log P_{ow} of 0.51 for 2-NPT	Klimisch Reliability: 1	Anonmymous, 2006d

11.4.1 Estimated bioaccumulation

No data available.

11.4.2 Measured partition coefficient and bioaccumulation test data

A study according to EU method A.8: Partition coefficient was performed to determine the log K_{ow} of the test item 2-NPT. According to the guideline the HPLC method was used. A correlation graph of log k versus log P for seven reference compounds was plotted. All tested reference substances are recommended in the guideline. Based on the correlation graph a corresponding log Kow of 0.51 was calculated for the test item 2-NPT. The reporting of the method and the results were considered as sufficient and the validity criteria mentioned in the guideline were met. data available.

Conclusion:

According to the CLP regulation Annex I, 4.1.2.8 with a log Kow of 0.51 2-NPT does not have a potential for bioaccumulation.

11.5 Acute aquatic hazard

Table 26: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
OECD 203 (1992): 96h Fish Acute Toxicity Test Static conditions Test performed under GLP conditions Observed Endpoint: mortality Used Test concentrations < water solubility Purity ≥ 99% Test temperature: 21.7 – 22.5°C Dissolved oxygen: 8.24 – 9.04 mg/L pH: 7.06 – 7.66	*Danio rerio (Brachydanio rerio)*	*N*-(2-nitrophenyl)-phosporic triamide	LC_{50}: 100 mg/L (based on nominal concentration)	Klimisch Reliability: 1	Anonymous, 2005a
OECD 202 (2004): 48h Acute Immobilisation Test Static conditions Test concentration: 100 mg/L Test performed under GLP conditions Observed Endpoint: mobility Used Test concentrations < water solubility Purity ≥ 99% Test temperature: 20.1 – 20.5°C Dissolved oxygen: 8.45 – 8.69 mg/L pH: 7.91 – 8.04	*Daphnia magna* Straus	*N*-(2-nitrophenyl)-phosporic triamide	EC50: 100 mg/L (based on measured concentration)	Klimisch Reliability: 1	Anonymous, 2005b

OECD 201 (1984): 72h Algae growth inhibition test Static conditions Nominal test concentrations: 3.13, 6.25, 12.5, 15, 50 and 100 mg/L Test performed under GLP conditions Observed Endpoint: cell density, growth rate Used Test concentrations < water solubility Purity ≥ 99% Test temperature: 23.5 – 24.1°C pH: 7.96 – 9.17	*Desmodesmus subspicatus* (algae)	*N*-(2-nitrophenyl)-phosporic triamide	E_rC_{50}: 51.4mg/L (growth rate, based on nominal concentrations) No valid NOEC or LOEC could be determined.	Klimisch Reliability: 2	Anonymous, 2005c

[1] Indicate if the results are based on the measured or on the nominal concentration

[2] bold value indicate the most sensitive acute endpoint

11.5.1 Acute (short-term) toxicity to fish

In all three acute studies the concentrations of the test item were well below the water solubility of 1394 mg/L.

In a 96h short-term toxicity test according to OECD 203 (1992) following GLP zebrafish (*Danio rerio*) were exposed under static conditions to a nominal concentration of 100 mg/L of the test substance *N*-(2-nitrophenyl) triamide.

Material and methods

The fish were of a length of 2.4 – 3.0 cm, the age was not reported in the original study. Before the study started the fish were acclimatised for seven days in the dilution water which was used in the study. In a range finding test up to a concentration of 100 mg/L no mortalities of fish were observed, therefore the definite study was performed with the highest concentration of 100 mg/L. The test solution was prepared according to ISO 6341 which is recommended in the guideline. A stock solution was prepared by weighing 1000 mg of the test item and by adding 1000 ml of dilution water. To achieve a nominal concentration of 100 mg/L, the stock solution was filled with dilution water up to volume of 10 L in the test tanks. The control vessel contained also a volume of 10 L only with water. 10 fish each in the control and treatment group were used with a maximum loading of 1.0g fish/L in the tanks. The test concentration was analytically measured at the beginning and at the end of the test via a valid HPLC method.

Results

No mortalities were observed over the complete observation period at 3, 6, 24, 48, 72 and 96h, neither in the control nor in the treatment group. No abnormalities or behavioural effects of the fish occurred in the testing

phase. The testing parameters dissolved oxygen [mg/L], pH and the temperature [°C] were measured at the beginning of the test (0h), after 24, 48, 72 and 96h. The measured concentrations at 0h ranged from 99.5 – 102.3% and after 96h from 97.9 – 102.5% of the nominal concentration. The validity criteria a.) mortality in the control should not exceed 10% at the end of the test, b.) dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test and c.) concentration of the substance being tested should be at least 80% of the nominal throughout the test and the parameters for the recommended fish species mentioned in the guideline were met. The NOEC of the test item was set at ≥100 mg/L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

In a 48h short term toxicity test, according to OECD 202 (2004) following GLP, invertebrates (*Daphnia magna* Straus) were exposed under static conditions to a nominal concentration of 48 mg/L of the test substance *N*-(2-nitrophenyl) triamide.

Material and methods

The used daphnids were <24h old and were held in a M4 medium before used in the test. In a range finding test up to a concentration of 100 mg/L no immobilities of the daphnids were observed, therefore the definite test was performed with the highest concentration of 100 mg/L. In the test system 20 daphnids were used divided into 4 groups á 5 daphnids/vessel. The test was performed under static conditions. The test water was prepared according to ISO 6341 which is recommended in the guideline. At the beginning and at the end of the test the pH, dissolved oxygen and the temperature was measured. The test item was analytically measured at the start and end of the test via a validated HPLC method. In order to test the sensitivity of the test method the reference item potassium dichromate was tested at five different concentrations.

Results

No effects (immobilisation) were observed in the control and in the treatment group after 24 and 48h at the test concentration of 100 mg/L neither. The measured concentration of the test item in the test solution were 99.4 – 99.7% at 0h and 93.8 – 94.1% 48h, respectively. The validity criteria a.) in the control, including the control containing the solubilising agent, not more that 10% of the daphnids should have been immobilised and b.) the dissolved oxygen concentration at the end of the test should be 3 mg/l in control and test vessels mentioned in the guideline were met. The NOEC of the test item was set at ≥100 mg/L.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

A static algae growth inhibition test according to OECD 201 (1984) and GLP with the species *Desmodesmus subspicatus* is available. The surveyed endpoints were cell density and growth rate.

Material and methods

As test organism the freshwater green algae *Desmodesmus subspicatus* (former genus Scenedesmus) was used in this study. Before the initiation of the study a stock solution was prepared by dissolving the test item in the test medium. The test medium was prepared according to the guideline. No solvent was used. The nominal exposure range of the test substance *N*-(2-nitrophenyl) triamide was 3.13, 6.25, 12.5, 25, 50 and 100 mg/l (geometric series with a factor of 2). The study was run under constant illumination, a temperature range of 23.5 – 24.1°C and pH of 7.96 – 9.11. The pH was measured at the beginning and at the end of the study. The initial cell concentration was set at $1x10^4$ Desmodesmus cells. For each concentration 3 replicate vessels were used and for the control group six vessel replicates. Each vessel contained 100 ml testing solution. The algae were exposed to each concentration up to 72h. The cell concentration after 24, 48 and 72h was determined by a cell counter. From these data the cell density (E_bC_{50}) and the growth rate (E_rC_{50}) were examined. The statistical Dunett test ($p>0.05$, one sided) was used to identify statistical significance between the differences of the control and the test concentrations. Probit analysis was used to determine NOEC, LOEC and the effect concentrations for the endpoints biomass and growth rate.

For the determination of the test substance concentrations a working calibration function with a range of 1.98 mg/L – 23.76 mg/L was used. The LOQ and LOD based on the working calibration function were 1.215 mg/L and 0.503 mg/L, respectively . For the validation of the method a reference calibration function with a range of 0.0198 – 0.2970 mg/L was used. This function revealed a LOQ and LOD of 0.010 mg/L and 0.004 mg/L, respectively. The study authors set as a quality criterion a relative standard deviation of 0.5% for the single calibration points. For the reference calibration function the relative standard deviation was > 0.5% and for the working calibration function it was < 0.5%. Therefore, the working calibration function was used for the determination of the test item down to a concentration of 0.4 mg/L. Values below 0.4 mg/L were not further quantified (see table 25). For the nominal test concentration 3.13 mg/L the chromatogram was attached to the study. Hence, it was possible to recalculate via a linear regression the measured concentration at time point 0 and 72h. The results for this concentration with 2.994 mg/L at 0h and 0.367 mg/L at 72h, respectively are comparable to the results presented in the study (see table 25).

Results

Analysis by a valid HPLC-UV at 0 hours showed 93% to 98.3% of nominal concentrations. Recovery rates after 72 hours were between 7.8 and 81.3% of nominal for the concentrations from 12.5 mg/L to 100 mg/L, whereas in the lowest concentrations (3.13 mg/L and 6.25 mg/L) no test item was found after 72 hours (see Table 25).

Table 25: Analytical results

Test concentration of 2-NPT (mg/l; nominal)	Measured concentrations of 2-NPT			
	0h		72h	
	mg/L	% of nominal	mg/L	% of nominal
control	<0.4	-	<0.4	-
3.13	2.936	93.8	<0.4	-
6.25	5.988	95.8	<0.8	-
12.5	11.917	95.3	0.991	7.9
25.0	23.579	94.3	15.505	62.0
50.0	47.418	94.8	39.899	79.8
100.0	93.808	93.8	81.262	81.3

The NOEC and LOEC for biomass and growth rate were determined at nominal test concentrations of 6.25 mg/L and 12.5 mg/L respectively. Statistically significant differences (p>0.05) were determined for concentrations exceeding 6.25 mg/L considering the testing period of 72h for the determined endpoints biomass and growth rate. The calculated E_bC_{50} (0-72h) for biomass was calculated with 28.3 mg/L and the E_rC_{50} (0-72h) for growth rate was 51.4 mg/L (based on nominal concentrations). There was a high uncertainty of the analytical results for the two lowest test concentrations 3.13 and 6.25 mg/L after 72h observed. The measured values for both nominal concentrations were below the LOD of 0.503 mg/L of the working calibration function and not further quantified, even if it would have been theoretically possible, as the LOQ of the reference calibration function was 0.010 mg/L and the corresponding LOD was 0.004 mg/L. Therefore, it was not possible to determine an exact NOEC. Nevertheless, it is clear from the analytical results, that the measured concentrations after 72h will be far below the triggervalue of <20% of nominal concentration and therefore, the results have to be based on measured concentrations. Hence, the nominal NOEC of 6.25 mg/L is considered invalid. Based on mean measured concentrations the NOEC could be estimated around 1.2 mg/L, but as stated above, this value is calculated from measurements for 72h with a high degree of uncertainty. Therefore, the NOEC is not used for classification purposes. The ErC_{50} of 51.4

mg/L (based on nominal concentration) is considered valid since the recovery rates at this concentration are within the ±20% of the nominal at the start and the end of the test.

Table 26: Nominal, measured and mean measured concentrations of the test item 2-NPT

Nominal (mg/L)	0h (mg/L)	72h (mg/L)	Geom Mean (mg/L)	Inhib (%) cell growth rate
0	<0.4	<0.4	<0.4	0
3.13	2.936	<0.4	(0.859)*	0.1
6.25	5.988	<0.8	(1.227)*	0.5
12.5	11.917	0.991	3.437	4.7
25	23.579	15.505	19.120	9.9
50	47.418	39.899	43.496	37.6
100	93.808	81.262	87.310	92.4

*values in parantheses were calculated using for the 72h-concentration values the LOD/2 of the working calibration function (0.503/2 = 0.2515 mg/L).

The validity criterium "The cell concentration in the control cultures should have increased by a factor of at least 16 within three days" mentioned in the guidance were met.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.6 Long-term aquatic hazard

No data available.

11.6.1 Chronic toxicity to fish

No data available.

11.6.2 Chronic toxicity to aquatic invertebrates

No data available.

11.6.3 Chronic toxicity to algae or other aquatic plants

No data available.

11.6.4 Chronic toxicity to other aquatic organisms

No data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data on 2-NPT are available for fish, invertebrates and algae. Acute endpoints for fish and daphnids are above 100 mg/L. The lowest acute value is the 72-h ErC_{50} of 51.4 mg/L (nominal) for algae. Based on this data set *N*-(2-nitrophenyl)phosphoric triamide doesn't need to be classified for acute hazards.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Valid chronic toxicity data is not available. Therefore the surrogate approach is used for classification into the chronic categories. Based on the most sensitive species (algae) in the short term toxicity studies with an ErC_{50} of 51.4 mg/L in combination with "non rapidly degradability" the substance" 2-NPT has to be classified with Aquatic Chronic 3 according to the CLP regulation Annex I, table 4.1.0 (iii).

CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Classification: Aquatic Chronic 3, H 412;

Labelling: Aquatic Chronic 3, H412; P273, P501

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed not to classify 2-NPT for aquatic acute hazards and to classify it as Aquatic Chronic 3, H412 for chronic hazards.

The measured water solubility of 2-NPT is 1394 mg/L at 20°C and pH 6.2. Data indicate that the vapour pressure for 2-NPT is low at 5.0 x 10^{-6} Pa at 20°C. The measured partition coefficient of 2-NPT is 0.51 at 25°C.

Acute aquatic toxicity data on 2-NPT is available for fish, invertebrates and algae. Acute endpoints for fish and daphnids are above 100 mg/L. The lowest acute value is the 72h E_rC_{50} of 51.4 mg/L (nominal) for algae. As this value is above 1 mg/L, 2-NPT is not classified for acute hazards, according to the DS.

2-NPT is not rapidly degradable in the environment and has a low potential for bioaccumulation in aquatic organisms (Log K_{OW} = 0.51, namely below the CLP threshold value of 4). The surrogate approach was used for chronic classification. Based on the most sensitive species (algae) in the acute toxicity studies with an E_rC_{50} of 51.4 mg/L in combination with the substance being considered by the DS as non-rapidly degradable, an Aquatic Chronic 3 classification is proposed.

Degradation

Stability

A hydrolysis study was performed following OECD TG 111 and conducted under GLP conditions. 2-NTP was tested in the pre-test and the definite test at pH 4, 7 and 9 at 25°C in buffered mediums in the dark. 2-NPT underwent hydrolysis at pH 4 (DT_{50} of 28.5h) and pH 9 (DT_{50} of 48.2h). Whereas at pH 7 (the most relevant pH), the substance is considered stable to hydrolysis with a DT_{50} value of 148.4 days at a temperature of 25°C. No information on the degradation products is available.

Biodegradation screening test

Ready biodegradation was tested following the modified OECD screening test method Part C: C.4-B (similar to the OECD TG 301E) following GLP principles. The study was run using 110 mg/L 2-NPT for 28 days in the dark at a temperature of 20 - 24°C and pH 7.4 ± 0.1. Dissolved organic carbon (DOC) concentrations were measured, and the percentage of DOC removal was calculated. For 2-NPT no marked removal of DOC was observed by the end of

the study on day 28 and no inhibitory effect was observed. The control pass level for ready biodegradability (70%) was reached at day 3. The abiotic and adsorption control did not give any indication for a loss of DOC. Toxicity and adsorption were not the drivers for the non-biodegradability of the test item. The DS stated that the validity criteria for the study were met according to the guidance and concluded that 2-NPT is not readily biodegradable.

Biodegradation soil simulation test

In an aerobic soil degradation study according to OECD TG 307, the degradation of 2-NPT was tested in three different soils for an incubation time of 29 days. DT_{50} values related to dissipation rates (1st order kinetic) for each soil type ranged from 3.9 – 8.6 days and DT_{90} values from 13.0 – 28.6 days.

DS conclusion on rapid degradation

The presented degradation information did not provide sufficient information to demonstrate that 2-NTP is ultimately degraded above 70% within 28 days (equivalent to a half-life < 16 days). In a ready biodegradation test, no marked removal of DOC was recorded. In the hydrolysis test, despite hydrolysis being demonstrated, there was no information on the degradation products. Hence, 2-NPT was considered by the DS as not rapidly degradable, according to the criteria of the CLP regulation.

Bioaccumulation

Experimental BCF data on fish is not available for 2-NPT. Based on experimental data, 2-NPT has a measured log K_{OW} of 0.51 (pH 6.3) at 25°C (Shake flask). The DS concluded that 2-NPT does not have the potential for bioaccumulation.

Aquatic toxicity

The aquatic toxicity data and study reliabilities as proposed by the DS are summarised in the following table (key data is highlighted in bold).

Test Guideline	Test Organism	Exposure		End point	Result (mg/L)	Remark	Reference
		Design	Duration				
Short-term toxicity to fish							
OECD TG 203 (1992) GLP Purity ≥ 99%	*Danio rerio (Brachydanio rerio)*	Static	96 hours	LC_{50}	> 100	Mortality Based on nominal concentrations Ri = 1	Anonymous, 2005a
Short-term toxicity to aquatic invertebrates							
OECD TG 202 (2004) GLP Purity ≥ 99%	*Daphnia magna*	Static	48 hours	EC_{50}	> 100	Mobility Based on mean concentrations Ri = 1	Anonymous, 2005b
Toxicity to algae and aquatic plants							
OECD TG 201 (1984) GLP Purity ≥ 99%	*Desmodesmus subspicatus (formerly Selenastrum capricornutum)*	Static	72 hours	**E_rC_{50}**	**51.4** No valid NOEC could be determined	Growth rate based on nominal concentrations Ri = 2	Anonymous, 2005c

Acute aquatic toxicity

Valid acute aquatic toxicity data are available for fish, invertebrates and algae with algae being the most sensitive trophic level.

In a 96-hour short-term toxicity test, according to OECD TG 203 following GLP, zebrafish (*Danio rerio*) were exposed under static conditions to a nominal concentration of 100 mg/L of the test substance. The measured concentrations of the test substance were 99.5 – 102.3% at 0-hour and 97.9 – 102.5% at 96-hour of the nominal concentrations. No mortalities were observed over the complete observation period, neither in the control nor in the treatment group. No abnormalities or behavioural effects of the fish occurred in the testing phase. The reported 96-hour LC_{50} value was greater than 100 mg/L (nominal).

In a 48-hour short term toxicity test, according to OECD TG 202 following GLP, invertebrates (*Daphnia magna*) were exposed under static conditions to a nominal concentration of 48 mg/L of the test substance. The measured concentrations of the test substance were 99.4 – 99.7% at 0-hour and 93.8 – 94.1% at 48-hour of the nominal concentrations. No immobilisation was observed in the control nor in the treatment group.

A static algal growth inhibition study was conducted following OECD TG 201 and according to GLP. Nominal concentrations at 0 hours ranged from 93.8 to 95.8%. Recovery rates after 72 hours were between 7.9 and 81.3% of nominal concentrations from 12.5 mg/L to 100 mg/L, whereas in the two lowest concentrations (3.13 mg/L and 6.25 mg/L) no test item was found after 72 hours. The calculated 72h E_bC_{50} was 28.3 mg/L and the E_rC_{50} was 51.4 mg/L (based on nominal concentrations). The E_rC_{50} of 51.4 mg/L is considered valid since the recovery rates at this concentration are within the ± 20% of the nominal at the start and end of the test.

Chronic aquatic toxicity

Long-term toxicity data on 2-NPT is not available for fish and aquatic invertebrates, although chronic toxicity data is available for algae.

A 72-hour growth inhibition test with algae was carried out in a static test system at concentrations of 0, 3.13, 6.25, 12.5, 25, 50, and 100 mg/L (OECD TG 201, GLP). Nominal concentrations at 0 hours ranged from 93.8 to 95.8%. Recovery rates after 72 hours were between 7.9 and 81.3% of nominal concentrations from 12.5 mg/L to 100 mg/L, whereas in the two lowest concentrations (3.13 mg/L and 6.25 mg/L), no test item was found after 72 hours. Statistically significant effects compared to the control occurred at 12.5 mg/L for biomass and growth rate. The nominal NOEC and LOEC for biomass and growth rate were determined at test concentrations of 6.25 mg/L and 12.5 mg/L, respectively. The DS reported a high uncertainty in the analytical results for the two lowest test concentrations of 3.13 and 6.25 mg/L, after 72 hours. The measured values for both nominal concentrations were below of 0.0503 mg/L of the working calibration function and not further quantified. The limit of quantification of the reference calibration was 0.010 mg/L and the corresponding LOD was 0.004 mg/L. In light of this, the DS reported that it is impossible to determine an exact NOEC and concluded that the nominal NOEC of 6.25 mg/L is invalid. As a result, the NOEC is not used for classification purposes.

Based on the available information for aquatic toxicity, the DS concluded that 2-NPT is not acutely toxic to the environment based on the lowest toxicity value E_rC_{50} of 51.4 mg/L. No classification for acute hazards is warranted because this value is above 1 mg/L. The DS considered that valid chronic toxicity data were not available and used the surrogate

approach for chronic classification. Based on the most sensitive species (algae) in the acute aquatic toxicity studies with an E_rC_{50} of 51.4 mg/L and in combination with the substance being considered by the DS as 'non-rapidly degradable', an Aquatic Chronic 3 classification was proposed.

Comments received during consultation

Four MSCAs provided public comments. Two agreed with the proposed classification with comments. One concurred with the proposed classification and commented that if the measured concentrations were used instead of nominal in the E_rC_{50} calculation, the classification conclusion would have been the same. Another MSCA suggested using the 12.5 mg/L test concentration as the 72h NOEC, however agreed with the proposed classification of Aquatic Chronic 3. They also indicated minor editorial mistakes in the CLH report.

One MSCA asked the DS to present the 72-hour E_rC_{50} and E_rC_{10} based on geometric mean measured dose-response curve. They noted that a geometric mean concentration of 1.227 mg/L was reported in the CLH report at the test concentration of 6.25 mg/L. Also, the REACH registration dossier (ECHA, 2019) included a nominal E_rC_{10} of 22 mg/L. As the algal growth inhibition study forms the basis of the classification proposal, the MSCA pointed out that the validity of the study controls should be confirmed. As requested, the DS reported the geometric mean based E_rC_{50} and E_rC_{10} values, 49.93 mg/L and 9.99 mg/L, respectively. The DS considered the nominal E_rC_{50} value of 51.4 mg/L valid because the recovery rates were within the ± 20% of the nominal at the end of the test. They mention that both E_rC_{50} values, based on nominal and geometric means concentrations, were almost equal and did not alter the classification proposal. The E_rC_{10} value of 9.99 mg/L was considered unreliable by the DS because of the uncertainty associated with the analytical results at the nominal concentration of 6.25 mg/L, which was below the limit of detection of 0.503 mg/L. The DS also expressed that the validity criteria of the algal study were met.

Another MSCA pointed out that, following the CLP Guidance, where concentrations at the end of the test are below the analytical detection, such concentrations shall be considered to be half that detection limit (LOD = 0.503 mg/L). As a result, the MSCA proposed a NOEC value of 0.252 mg/L. A classification of Aquatic Chronic 2 was proposed as more appropriate by this MSCA, based on the NOEC value of 0.252 mg/L and the fact that the substance is not rapidly degradable. The DS pointed out that the LOD/2 cannot be used to derive the NOEC. The LOD/2 refers to the 72-hour value wherever concentrations at the end of the test are below the detection limit.

Assessment and comparison with the classification criteria

Degradation

2-NPT is not susceptible to hydrolysis at relevant environmental pHs; hydrolysis half-life (at 25°C) of 148.4d at pH 7, with shorter half-lives as conditions become more acidic (pH 4 at 28.5h or basic (pH 9 at circa 48h). In a ready biodegradability study, removal of DOC was not observed within 28 days, so 2-NPT is considered not readily biodegradable. In an aerobic soil degradation study, dissipation DT_{50} values ranged from 3.9 – 8.6 days at 20°C. One significant metabolite (2-nitroaniline) was detected at a maximum level of 50% to 30% on day 29. 2-Nitroaniline has a harmonized classification of Aquatic Chronic 3. Consequently, this does not support that 2-NPT would fulfil the criteria for ultimate degradation within 28 days.

RAC agrees with the DS proposal to consider 2-NPT as not rapidly degradable for the purpose of classification and labelling.

Aquatic Bioaccumulation

Measured BCF values are not available for the substance. However, a reliable, experimentally derived partition coefficient n-octanol/water study is available. The low bioaccumulation potential of 2-NPT is supported by the experimental Log K_{OW} of 0.51, which is below the CLP trigger value of Log $K_{OW} \geq 4$. Therefore, RAC agrees with the DS proposal to consider 2-NPT as a substance with a low potential to bioaccumulate.

Aquatic toxicity

Aquatic acute toxicity data on 2-NPT are available for fish, invertebrate and algae. 2-NPT is of low toxicity to fish and invertebrates with valid LC_{50}/EC_{50} values above 100 mg/L. The lowest toxicity value is an E_rC_{50} of 51.4 mg/L (nominal concentration) for algae (Anonymous, 2005c). As noted by two MSCAs during the consultation, the initial exposure concentrations in the algae were not maintained throughout the testing period. At the lowest concentrations, 3.13 and 6.25 mg/L, no test item was found, and the recovery rates for test concentrations 12.5 and 25.0 mg/L ranged from 7.8 to 81.3% of the nominal. In the two highest dose levels, 50 and 100 mg/L, the measured concentrations were close 80% (79.8% and 81.3%, respectively) of the nominal concentrations. For static tests, where the concentrations do not remain within 80 – 120% of nominal, the effects concentration should be expressed relative to the geometric mean of the measured concentration at the start and end of the test (CLP guidance and OECD TG 23). Considering the test concentrations were not entirely maintained during the test as a whole as prescribed in the guidelines, RAC prefers to express the effect concentrations as the geometric mean of the measured concentration. When calculating the geometric mean test concentrations using the methodology described in OECD TG 23 (based on growth rate) and statistical analysis (log-logistic in Graph pad), RAC derives a 72-hour E_rC_{50} of 49.8 mg/L. This value is roughly the same as the 72-hour E_rC_{50} based on nominal concentrations 51.4 mg/L, derived by the DS. As the lowest from all acute aquatic toxicity tests acute toxicity (E_rC_{50} of 49.8 mg/L) is above 1 mg/L, 2-NPT does not fulfil the criteria for acute toxicity, based on Table 4.1.0 (a) and does not warrant classification as Aquatic Acute 1.

Aquatic chronic toxicity data on 2-NPT is available for one trophic level, algae. The CLH reports $NOEC_{biomass}$ and $LOEC_{growth}$ values at nominal concentrations of 6.25 mg/L and 12.5 mg/L, respectively. The DS considered that a valid NOEC cannot be determined because the NOEC at the nominal concentration of 6.25 mg/L was below the detection limit of 0.503 mg/L. Analysis of the concentration of 2-NPT showed no test item for the two lowest test concentrations of 3.13 and 6.25 after 72 hours. It was argued that the calculated NOEC value is from a 72h measurement with a high degree of uncertainty and therefore not used for classification purposes.

RAC would like to point out that where a measured concentration at the end of the exposure period is absent or where a substance is not detected, the validity of the chronic test result should be reconfirmed. The DS stated that the validity criteria were met in the study, albeit they did not provide data to verify this. As a result, RAC decides to check the validity of the algal test. The average number of cells per mL increased from 1100 to 597900 cells/mL, which is a factor of 54.4. This value exceeds the validity criterion for cell growth of the controls by a factor of at least 16 within three days. The mean coefficient of variation (CV) for section by section specific growth rate in the controls resulted in 17.7%. This value does

not exceed the 35% limit. The CV of the average specific growth rate during the whole test period in the replicate controls resulted in 1.4%. This value does not exceed the 7% limit. RAC considers that the validity criteria of the test were met.

RAC reviewed the technical study report for 2-NPT in algae, Anonymous (2005c) reported the following results:

Test Guideline	Test Organism	Results (mg/L)		Remark
		Biomass 72-hour	Growth rate 72-hour	
OECD TG 201 (1984) GLP Purity ≥ 99%	*Desmodesmus subspicatus* *(formerly Selenastrum capricornutum)*	E_bC_{50} = 28.28 E_bC_{10} = 11.72 LOEC = 12.50 NOEC = 6.25	ErC_{50} = 51.37 ErC_{10} = 22.02 LOEC = 12.50 NOEC = 6.25	Based on nominal concentrations

Effect levels based on the nominal concentration, where analytical methods cannot quantify test concentrations, might result in an underestimation of the toxicity. Therefore, RAC understands the DS's concerns with regard to the validity of using the NOEC value of 6.25 mg/L (nominal concentration) for classification purposes. According to the CLP guidance (I.4.1.a), where concentrations at the end of the test are below the analytical detection limit, such concentrations shall be considered to be half of that detection limit. In these cases, it is good practice to use half of the limit of detection to calculate a mean exposure concentration and final concentration. Taking this into account and the fact the study is valid, RAC considers that a calculated E_rC_{10} based on geometric mean measured concentration, suitable for classification purposes.

When calculating the geometric test concentrations using the methodology described in OECD TG 23 (based on growth rate) and statistical analysis (log-logistic in Graph pad), RAC derived a 72-hour E_rC_{10} of 29.0 mg/L. RAC considers the 72-hour E_rC_{10} value of 29.0 mg/L valid and it can be used for classification purposes.

Aquatic chronic toxicity data on 2-NPT is available for one trophic level, algae. In absence of long-term toxicity data for fish and aquatic invertebrates, the surrogate method is applied as recommended in CLP Regulation Annex I, 4.1.2.3. and Figure 4.4.1. The substance is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation potential.

- Classification based on adequate chronic toxicity data: Algal testing resulted in a 72-hour E_rC_{10} of 29.0 mg/L. The E_rC_{10} is above 1 mg/L and the substance is not rapidly degradable. 2-NPT does not fulfil the criteria for chronic classification, based on Table 4.1.0 (b)(i).
- Classification based on surrogate data for other trophic levels. Two acute limit tests for fish and aquatic invertebrates resulted in $L(E)C_{50}$ values > 100 mg/L and the substance is not rapidly degradable. 2-NPT does not fulfil the criteria for chronic classification, based on Table 4.1.0 (b)(iii).
- Overall conclusion: no Chronic classification

Conclusion on Classification

RAC concludes that 2-NPT **does not warrant classification for either aquatic acute or chronic toxicity.**

Supplemental information - In depth analyses by RAC

Table*: Determination of geometric mean measured 2-NPT concentrations*

2-NPT nominal concentrations	Mean measured 2-NPT (mg/L)		Mean measured 2-NPT as nominal (%)		Geometric mean measured 2-NPT concentrations (mg/L)
	0 hour	72 hours	0 hour	72 hours	
Control	< 0.40	< 0.40	-	--	
3.13	2.94	0.002*	93.8	--	0.0767
6.25	5.99	0.002*	95.8	--	0.1095
12.5	11.92	0.99	95.3	7.9	3.44
25.0	23.58	15.51	64.3	62.0	19.12
50.0	47.42	39.90	94.8	79.8	43.50
100.0	93.81	81.26	93.8	81.3	87.31

*Reference calibration function (LOD=0.004 mg/L) resulting in LOD/2 = 0.004 mg/L/2 = 0.002 mg/L

Table: *% inhibition of cell growth rate*

Geometric mean measured 2-NPT concentrations (mg/L)	% inhibition
Control	
0.0767	0.1
0.1095	0.5
3.44	4.7
19.12	9.9
43.50	37.6
87.31	92.4

Calculation of E_rC_{50} = 49.8 mg/L and calculation of E_rC_{10} = 29.0 mg/L

RAC evaluation of additional information

After the RAC consultation, additional information was received by the DS regarding the analytical uncertainties of the algae study. They stated that the LOD/2 cannot be used, independently of which LOD is applied for the 72h calculation of the two lowest concentrations:

- Working calibration function (LOD = 0.503 mg/L) will lead to an overestimation and
- Reference calibration function (LOD = 0.004 mg/L) will lead to an underestimation.

Due to these uncertainties, the DS still considered that no verified NOEC/EC_{10} can be derived. They therefore proposed classification based solely on acute toxicity, which leads to a classification of Aquatic Chronic 3.

Based on the arguments, RAC considers that it is preferred to use the LOD from the reference calibration curve, as this covers a worst-case approach. The EC_{50} and EC_{10} were recalculated replacing the exposures by the geometric mean of the mean measured concentration for t=0 and the LOD/2 of 0.002 mg/L.

LOD	EC_{50} (mg/L) (95% CI)	EC_{10} (mg/L) (95% CI)
Working calibration function (0.503 mg/L)	49.7* (44.5 - 55.6)	28.7* (20.2 – xxx)
Reference calibration function (0.004 mg/L)	**49.8 (44.5 - 55.8) R^2 of 0.99**	**29.0 (20.31 - xxx) R^2 of 0.99**

*Initial EC_{50} and EC_{10} values derived by RAC

The LOD choice in the calculations had little effect on the EC_{10} (28.7 mg/L vs 29.0 mg/L) and the error introduced by the uncertainties is minimal. That the precise concentration of the two lowest exposures has little effect on the outcome is explained by the limited inhibition of 0.1 and 0.5% observed at these concentrations (see table "% inhibition of cell growth rate", above). This can also be seen in the figure below, as these points are located in the flat top part of the fitted curve, their horizontal position has little effect on the curve's actual shape. Although the confidence interval for the EC_{10} has no upper limit, the lower limit matters most for classification purposes and it is close to the geometric mean measures exposure of 19.12 mg/L where an inhibition of 9.9% is observed (see table "% inhibition of cell growth rate", above).

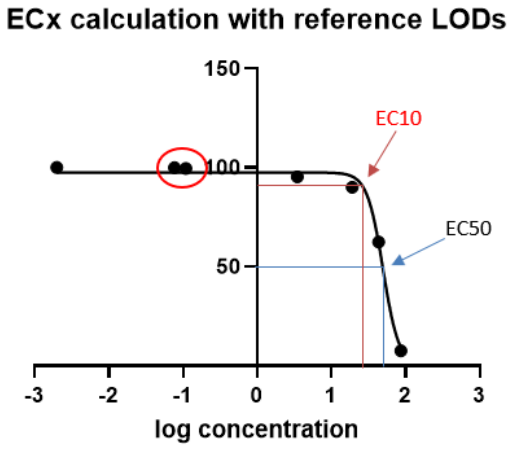


Figure: fitted curve for the ECx calculation with the use of reference LODs

RAC finds that the calculated EC_{10} is within the range of the geometric mean exposure concentrations 3.44, 19.12, 43.5 and 87.31 mg/L that are also regarded as reliable by the DS. For these concentrations, the effect on inhibition was 4.7, 9.9, 37.6 and 92.4% respectively, which indicates that the 10% effect level is unlikely to be lower than 1 mg/L. RAC also notes that the EC_{10} is calculated based on the same fitted curve as the EC_{50}, considering that the values for the EC_{10} and EC_{50} are relatively close, the EC_{10} and EC_{50} should both be accepted or disregarded. Therefore, it is also considered inappropriate to disregard the EC_{10} and apply the surrogate method with the EC_{50} derived from the same fitted curve.

Altogether, there is considered to be sufficient evidence that the EC_{10} will be higher than 1 mg/L. In an overview, this conclusion is based on the following points:

- The two lowest exposure concentrations have only a limited effect on the EC_{10}
- The EC_{10} calculated is within the range of concentrations considered to be reliable (interpolation within the reliable values)

- A 9.9% effect is observed at 19 mg/L (mean measured)
- EC_{10} and EC_{50} should not be assessed separately

Based on the above, RAC considers that a calculated E_rC_{10} based on geometric mean measured concentration using the LOD/2 (reference calibration function), suitable for classification purposes and concludes that 2-NPT does not warrant classification for aquatic chronic hazards.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

No data available.

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not assessed.

12.1.2 Comparison with the CLP criteria

Not assessed.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not assessed.

13 ADDITIONAL LABELLING

Not necessary.

14 REFERENCES

Anonymous (2012). Reproduction/developmental toxicity screening study of *N*-(2-nitrophenyl)phosphoric triamide in rats by oral administration according to OECD TG 421. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Anonymous (2008). Final Report Hydrolysis of *N*-(2-nitrophenyl)phosphoric triamide according to OECD 111. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Anonymous (2006a). Final Report 28-Day Oral (Gavage) Toxicity Study with *N*-(2-nitrophenyl) phosphoric triamide accoring to OECD TG 407. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Anonymous (2006b). Final Report Aerobic Soil Degradation of *N*-(2-nitrophenyl)phosphoricacid triamide according to OECD 307. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Anonymous (2006c). Final Report Determination of the ready biodegradability of *N*-(2-nitrophenyl)phosphoricacid triamide according to OECD-screening test. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Anonymous (2006d), Final Report Determination of the Partition Coefficient of *N*-(2-nitrophenyl)phosphoricacid triamide according to EU method A.8 Partition coefficient. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Anonymous (2005a). Final Report Acute toxicity of P101/04 to the zebra fish Brachydanio rerio according to OECD 203. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Anonymous (2005b). Final Report Acute toxicity of P101/04 to Daphnia magna according to OECD 202. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Anonymous (2005c). Final Report Algae growth inhibition test with P101/04 on Desmodesmus subspicatus according to OECD 201. Sponsor: SKW Stickstoffwerke Piesteritz GmbH.

Fleeman T.L., Cappon G.D., Chapin R.E., Hurtt M.E. (2005) Effects of feed restriction during organogenesis on embryo-fetal development. Birth defects research (Part B) 74:442-449

Yuan, Y.D. and McEntee K.: Testicular Degeneration, Rat. In Monographs on pathology of laboratory animals. Genital System. T.C. Jones, U., Mohr, R.D. Hunt (Eds.); Springer-Verlag Berlin, Heidelberg 1987: p. 212-218