Committee for Risk Assessment

RAC

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

Adopted

17 September 2020

17 September 2020

CLH-O-0000006850-73-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: ***N*-(2-nitrophenyl)phosphoric triamide**

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

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

The proposal was submitted by **Austria** and received by RAC on **9 July 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **26 August 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **25 October 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Betty Hakkert**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **17 September 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 current Annex VI entry										
Dossier submitters proposal	TBD	*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			
RAC opinion	TBD	*N*-(2-nitrophenyl)phosphoric triamide	477-690-9	874819-71-3	Repr. 1B STOT RE 2	H360Fd H373 (kidney)	GHS08 Dgr	H360Fd H373 (kidney)			
Resulting Annex VI entry if agreed by COM	TBD	*N*-(2-nitrophenyl)phosphoric triamide	477-690-9	874819-71-3	Repr. 1B STOT RE 2	H360Fd H373 (kidney)	GHS08 Dgr	H360Fd H373 (kidney)			

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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

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]
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 $p \leq 0.01$. 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 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 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 ≤ 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**.

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.

ENVIRONMENTAL HAZARD EVALUATION

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.**

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).