APPLICATION FOR AUTHORISATION

DNEL SETTING FOR REPROTOXIC PROPERTIES OF TRIXYLYL PHOSPHATE

SUBSTANCE NAME(S): Trixylyl phosphate (TXP)

IUPAC NAME(S): Reaction product of phosphorous oxytrichloride acid and a mixture of xylenols containing >95% tri (dimethylphenyl and/or ethylphenyl) phosphate

EC NUMBER(S): 246-677-8

CAS NUMBER(S): 25155-23-1

ECHA/RAC/A77-O-0000006922-70-01/F

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DATE: 04 December 2020

NOTE OF THE COMMITTEE FOR RISK ASSESSMENT

APPLICATION FOR AUTHORISATION - DNEL SETTING FOR REPROTOXIC PROPERTIES OF TRIXYLYL PHOSPHATE

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (the REACH Regulation), the Committee for Risk Assessment (RAC) has adopted a note on DNEL setting for reprotoxic properties of trixylyl phosphate for the Application for Authorisation.

I PROCESS FOR ADOPTION OF THE OPINION

The Executive Director of ECHA in the mandate of 06.07.2020[1], requested RAC to prepare a note concluding on the on DNEL setting for reprotoxic properties of trixylyl phosphate for the Application for Authorisation.

Rapporteur, appointed by RAC: **Gerlienke Schuur**

Co-rapporteur, appointed by RAC: **Bert-Ove Lund**

The RAC note was adopted on **04 December 2020**.

The RAC note was adopted by consensus of all members present and having the right to vote.

Preface

Reference values in the form of DNELs for threshold substances and/or dose response relationships for non-threshold (mainly) carcinogens are often published in advance of applications for authorisation, so providing greater consistency and better use of the legally defined periods of opinion-development in the Committee for Risk Assessment (RAC)[2].

The DNEL and dose response relationships so derived serve as non-legally binding 'reference values'. They provide applicants with a clear signal as to how RAC is likely to evaluate these important elements of the risk assessment of applications for authorisation.

In this case, the derivation of the DNELs follows the method as specified in ECHA Guidance on Information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health.[3]

[1] https://echa.europa.eu/documents/10162/13630/rac_mandate_art77_dnel_txp_en.pdf/96c9b641-bb5c-3bcb-8619-702561bf3826

[2] At the 22nd meeting of the Committee for Risk Assessment (RAC) in September 2012, a proposal to set reference DNELs/DMELs and dose response relationships for substances prior to receiving applications for authorisation was introduced. Following a trial exercise, ECHA agreed to continue the practise, recognising its value to the authorisation process.

[3] https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258

Summary

The reference DNELs for all routes of exposure of trixylyl phosphate as agreed by RAC are given in Table 1 below.

Table 1 Overview of derivation of reference DNELs for workers and general population exposed to trixylyl phosphate by the inhalation, oral and dermal route

Point of departure for DNEL derivation by all routes	
Oral (gavage) OECD 422 screening study in rats, 11 animals per sex and dose, administration 7 d/wk, 33 days (males), ca. 48 days (females), substance tested Phosflex TXP (trixylyl phosphate; Anonymous 2, (2004))	
LOAEL (mg/kg bw/day) - based on histopathological effects in testis, ovaries and adrenals	**25**
Inhalation absorption (%)	100
Oral absorption (%)	50
Dermal absorption (%)	10

Derivation of Reference DNELs		
	WORKERS	GENERAL POPULATION
Assessment Factors		
Extrapolation LOAEL to NAEL	3	3
Interspecies, allometric scaling (not for inhalation)	4	4
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Exposure duration		
Quality of whole database	10	10
INHALATION		
Adjustment oral to inhalation	1 / 0.384	1 / 1.15
Correction for exposure regime (day/week)	7 / 5	7 / 7
Absorption percentage rat oral / human inhalation (%)	50 / 100	50 / 100
Breathing rate for workers light activity vs rest	6.7 / 10	--
Corrected LOAEC (mg/m^3)	30.5	10.9
Overall assessment factors	375	750
Reference DNEL INHALATION (mg/m³)	**0.08**	**0.014**
DERMAL		
Correction for exposure regime (day/week)	7 / 5	7 / 7
Absorption percentage rat oral / human dermal (%)	50 / 10	50 / 10
Corrected LOAEL (mg/kg bw/day)	175	125
Overall assessment factors	1500	3000
Reference DNEL DERMAL (mg/kg/day)	**0.12**	**0.04**
ORAL		
Correction for exposure regime (day/week)	—	7 / 7
Absorption percentage rat oral / human oral (%)	—	50 / 50
Corrected LOAEL (mg/kg bw/day)	—	25
Overall assessment factors	—	3000
Reference DNEL ORAL (mg/kg/day)	—	**0.008**

Annex: DNEL setting for the reproductive properties of Trixylyl phosphate

1. Relevant Endpoint(s)

For applicants applying for authorisation under Article 60(2) (adequate control route), in order to conclude whether the adequate control is demonstrated, only endpoints (i.e. properties of concern) for which the substance is included in Annex XIV need to be addressed in the hazard assessment[2]. However, information on other endpoints might be necessary for comparing the risks with the alternatives.

For applicants aiming at authorisation based on Article 60(4) (socio-economic analysis route) Article 62(4)(d) also applies and the socio-economic analysis (SEA) route will as a consequence focus on the risks that are related to the intrinsic properties specified in Annex XIV. The SEA should in turn consider the impacts related to such risks. In practice the applicant is expected to provide this information in their (Chemical Safety Report) CSR for which an update may be advisable. However, for an authorisation to be granted, the applicant should also demonstrate that there are no suitable alternatives. In this latter analysis it may be the case that other endpoints than those for which the substance was listed in 'Annex XIV' become relevant in order to demonstrate that no suitable alternative is available.Trixylyl phosphate is included on Annex XIV due to its reprotoxic properties (Article 57(c) of REACH). The basis for the identification of the substance as SVHC was its classification as toxic for reproduction category 1B (H360F). The DNELs proposed in the present document are therefore based on the reprotoxic properties of this substance affecting fertility and a threshold approach is taken.

Trixylyl phosphate is registered at >1000 tonnes per year. In the REACH registration dossier, the lead registrant submitted reliable studies with the registered substance trixylyl phosphate (only the OECD TG422 study is described below) and with three analogue substances (one study from 1974 in rats exposed for 90 days to tris(methylphenyl)phosphate, a 21 days developmental toxicity study using Reofos 35 (tris(4-isopropylphenyl)phosphate, and a continuous breeding study in mice using tricresyl phosphate) and provided a justification for this read-across approach. All reliable studies performed with the registered substance and with analogue substances are considered in this document. No further publicly available relevant studies could be retrieved.

1.1. Reproductive toxicity / effects on fertility

Results of reproductive studies are summarised below and in Appendix

Table 5.

For trixylyl phosphate only one study regarding repeated dose toxicity and reproductive toxicity has been provided which is a combined repeated dose and reproductive/ developmental toxicity screening test in rats according to OECD TG 422 (previous version from 1999) with oral administration of Phosflex TXP at doses of 25, 200 and 1000 mg/kg bw/day from 2 weeks prior to mating throughout gestation until day 4 post-partum (Anonymous 2, (2004)). RAC notes that the dose ranges differ with a factor 8 (instead of the normally recommended factor 3).

The tested substance Phosflex TXP [reaction product of phosphorous oxytrichloric acid and a mixture of xylenols containing > 95% tri (dimethylphenyl and/or ethylphenyl) phosphates] reflects the registered substance trixylyl phosphate. Food consumption, body weight and body weight gain were slightly reduced in the 1000 mg/kg bw/day group after the first week of treatment. During the later part of gestation, dam food consumption, body weight and body weight gain were lower in the 200 and 1000 mg/kg bw/day groups; this was attributed to the lower rate of successful pregnancies. The number of successful matings (sperm positive) was similar across all groups (11/11 mated in the control, 25 and 200 mg/kg bw/day groups, while 10/11 of the 1000 mg/kg/day group rats were sperm-positive). However, reproductive outcome was adversely impacted in the 200 and 1000 mg/kg groups: in the 25 mg/kg bw/day group all dams delivered litters (11/11) whereas in the 200 and 1000 mg/kg bw/day groups, only 2/11 and 0/11 litter were delivered, respectively. Litter size, survival and offspring body weight were unaffected by treatment at 25 mg/kg bw/day. Because of the pronounced reproductive effects seen in the 200 and 1000 mg/kg bw/day groups, the recovery animals were subsequently mated. The cross-over mating (naive females x high dose recovery males) was performed first and resulted in all 5 naive females becoming pregnant, showing that the high dose males were fertile after 4 weeks of recovery. For the within group matings, 60% of the recovery control dams and 100% of the 1000 mg/kg bw/day recovery dams were sperm-positive. Successful parturition was seen for all sperm-positive dams resulting in 100% pregnancy in the recovery groups (controls and high dose). These findings suggest resolution of the observed functional deficit in reproductive performance. Thus, the functional deficit in reproductive performance was reversed in males after 4 weeks of recovery and after 3 weeks for females. Since reversal was seen in both sexes, it was not possible to determine if the effect on reproductive performance was male- or female-mediated. Based on organ weight data, the adrenals, testes, epididymis, ovaries, heart and liver were identified as target organs. In both sexes, the weight changes of the reproductive organs were combined with histological changes. Lesions consisted of degeneration of the germinal epithelium of the testes with corollary findings of sloughed epithelial cells in the lumen of the epididymis. Findings in the ovaries consisted of distinct mild diffuse hyperplasia of the interstitial cells. The histopathological findings in testes and ovaries were observed all three dose levels. The incidence and severity of the histopathological changes were less pronounced at the lower doses (25 and 200 mg/kg bw/day). Following the recovery period, the incidence and severity of all changes were decreased in the target tissues of 1000 mg/kg/day group, indicating ongoing, successful repair of the damage caused by 4-5 weeks of exposure. It is not known if damage caused by chronic exposure also can be repaired. Tables with the individual findings are presented in the CLH report (RIVM, (2009)[4]). **Since histopathological changes were observed at all dose levels, no NOAEL could be identified from this study and the LOAEL is 25 mg/kg bw/day.**

Read across data

Below follows an analysis of the data available for tricresyl phosphate, an analogue for which there is a reproductive toxicity study, although in mice. Also NTP studies on repeated dose toxicity in mice and rats are considered.
Tris(methylphenyl) phosphate (tricresyl phosphate, EC: 809-930-9; TCP), showed also an impact on fertility (reduced number of live pups per litter) in a continuous breeding experiment in mice with dosing via feed consumption resulting in estimated daily dosages of approximately

[4] https://echa.europa.eu/documents/10162/6ae620c3-58d0-e2fc-f973-1d6e937bbbda

62.5, 124 and 250 mg/kg bw/day (Anonymous 1, 1988; see Appendix Table 5). Crossover mating (exposure of one sex only) found impaired fertility in both sexes with greater effects on females. Reduced sperm motility was observed even at the lowest dose tested of 62.5 mg/kg bw/day (LOAEL). This was not accompanied by histopathological changes in the prostate, seminal vesicles, liver, or kidney of the male F0 mice, or in the ovaries, uterus, vagina, liver, or kidneys of the females. Treatment-related atrophy of the seminiferous tubules was seen in the testes of treated males, ranging from scattered foci of decreased germ cell number, to widespread bilateral loss of germ cells in the high dose mice. While RAC notes that this study is conducted using another species (the mouse), also tricresyl phosphate affected reproduction and the testis. Surprisingly, the cross-over mating still suggested females to be more affected than males.

NTP conducted several studies with tricresyl phosphate using rats and mice and different durations (16 days and 13 weeks gavage, 13 week and 2 year diet) (see Appendix Table 6). In all these studies, histopathology showed ovarian interstitial cell hyperplasia (females) and cytoplasmic vacuolisation of the adrenal cortex, with the lowest LOAEL of 7 mg/kg bw/day (from the 2 year mice study). Testis toxicity was only observed in rats, and it seems that this is duration dependent. In contrast, neurotoxic effects were shown in mice only (NTP, (1994)[5]), indicating differences in the toxicity profile between these species.

With respect to the read-across, there are both similarities and differences between trixylyl phosphate and tricresyl phosphate, making it difficult to use a quantitative read across between these substances, Thus:

- The single trixylyl phosphate study in rats reported histopathological effects on adrenals, testis and ovaries at all dose levels, and the same target organs were seen for tricresyl phosphate in the NTP rat studies..
- The studies with tricresyl phosphate in mice and rats showed that the adrenal and ovary are target organs in both species, while testis is only affected in rats and neurotoxicity only is observed in mice.
- The trixylyl phosphate study used a duration intermediate (33/48 days) compared to the studies performed with tricresyl phosphate, causing uncertainties when trying to compare studies performed on these two substances.
- Anonymous 1, (1988) noted that trimethyl phosphate, tri-*o*-cresyl phosphate (TOCP), dimethyl phosphonate (DMMP) and mixed tricresyl phosphate isomers have been reported to produce structural damage to the testis of rats, supporting the testis toxicity observed for trixylyl phosphate.

All in all, the toxicity profile for tricresyl phosphate differs between rats and mice, and RAC is therefore hesitant to draw conclusions from a comparison of the trixylyl phosphate *rat* screening study with the tricresyl phosphate *mouse* continuous breeding study.

RAC notes the extremely small database for trixylyl phosphate, but that the only available study (TG 422) clearly shows that trixylyl phosphate is a reproductive toxicant. Accordingly, trixylyl phosphate is classified as toxic for reproduction category 1B (H360F).

Point of Departure for DNEL derivation

[5] https://pubmed.ncbi.nlm.nih.gov/12616298/

The approach to DNEL derivation described in the lead registrant's CSR was reviewed but not further described in this document because RAC does not agree with the selection of the Point of Departure, especially with respect to the relevant endpoint fertility. For example, the overall DNEL was in the view of RAC chosen from an inappropriate study (repeated dose toxicity study) on an analogue substance rather than the correct substance. This was accompanied by only some qualitative argumentation for the read across (without including the OECD 422 study with TXP). The registrant assumed 100% absorption for all routes whereas dermal studies were available for the analogue substance showing other absorption percentages.

RAC considers the LOAEL of 25 mg/kg bw/day derived from the OECD 422 screening test with the registered substance trixylyl phosphate (Anonymous 2, (2004)) to be the appropriate Point of Departure (PoD) to derive the DNELs. At the lowest dose, histopathological effects in testes, ovaries and adrenals were still observed but without impact on the reproductive outcome at this dose level.

1.2. Bioavailability

No substance-specific information on absorption via oral, inhalation or dermal route is available. In the registration dossier for trixylyl phosphate information on absorption studies of the analogue substance tricresyl phosphate (EC 809-930-9) are available and discussed below.

Oral

For the analogue tricresyl phosphate, at least 41% of a single gavage dose of 7.8 mg/kg bw C14-labeled substance in rats was excreted in the urine over 7 days following administration (Kurebayashi et al., (1985)). About 12% of a single gavage dose of 89.6 mg/kg in rats was excreted in the urine. Most of the urinary excretion occurred within the 24 hours after administration.

For trixylyl phosphate, RAC assumes an oral absorption of 50%, which is in line with ECHA Guidance R.8. It is recommended that in the absence of route-specific information on the starting route, a default factor of 2 should be included in case of oral-to-inhalation extrapolation. The inclusion of this factor 2 means for example that 50% (instead of 100%) absorption is assumed for oral absorption, and 100% for inhalation.

Inhalation

For trixylyl phosphate RAC assumes an inhalation absorption of 100% following ECHA Guidance R.8. It is recommended that in case of oral-to-inhalation extrapolation, the worst case of 100% for inhalation absorption should be assumed.

Dermal

For an isomeric mixture containing the analogue tricresyl phosphate the dermal penetration has been investigated in vitro using dermatomed abdominal human skin samples and [C14]-labelled test compound. At 24 hours, following an 8 hour exposure to [C14]-tricresyl phosphate 5% v/v formulation, a mean of less than 75% of absorption into the receptor fluid occurred within the first half of the experiment and 89.5% (475 µg equivalent/cm^2) of the applied dose was considered dislodgeable. The percentage directly absorbed which includes the amount

recovered from the receptor fluid, receptor chamber wash, and skin was 6.8% (36.3 µg equivalent/cm^2), and the potentially absorbed dose which also includes the amount recovered from the receptor fluid, receptor chamber wash, skin and tape strips was 10.4% (55.4 µg equivalent/cm^2).

Similar physico-chemical characteristics are shown for trixylyl phosphate, high log Pow of >6.2 and a molecular weight of 410 to 453 as for the analogue tricresyl phosphate with a log Pow of 5.9 and a molecular weight of 368). A dermal absorption of 7% is measured in vitro for the analogue tricresyl phosphate. Concerning toxicological effects, comparable effects (on adrenals, ovaries and testis) were found in corresponding dose ranges, but the comparison is weakened by having two types on studies in two different species (screening study in rats vs a continuous breeding study in mice).

Based on the above, RAC assumes a dermal absorption of 10% for trixylyl phosphate.

2. Derived No Effect Levels (DNELs)

As explained above, RAC considers that the LOAEL of 25 mg/kg bw/day derived from the OECD 422 screening test with Phosflex TXP (Anonymous 2, (2004)) would be the most appropriate basis to derive a DNEL covering reproductive toxicity (fertility).

In addition, RAC proposes absorption percentages of 100%, 50%, and 10% for the inhalation, oral, and dermal routes respectively (see above).

Corrections

Exposure-related corrections include considerations of inhalation rate (higher in workers than in resting rats) and exposure frequency. Thus, in the OECD 422 screening test administration was 7 days per week, and consequently RAC applied adjustment for 5 days per week for workers.

Assessment factors

RAC notes that there is considerable uncertainty in setting a DNEL based on the very limited database, including use of a combined repeated dose and reproductive/developmental toxicity screening test (OECD TG 422).
The finding at the lowest dose (25 mg/kg bw/day) was histopathological damage in male and female reproductive organs (degeneration of germinal epithelium in testes of 2 of the 5 male animals and mild diffuse hyperplasia in interstitial ovary cells in 5 out of 5 females). The incidence and severity of the histopathological changes were dose-dependent and more pronounced at the higher doses (200 and 1000 mg/kg bw/day). Reproductive outcome was adversely impacted in the 200 and 1000 mg/kg groups, and few pups were produced at the 1000 mg/kg/day top dose (14% of control).

Inter- and intraspecies variation

Regarding the assessment factors for inter- and intraspecies variation, the default factors as described in the ECHA Guidance R.8 are used: For intraspecies differences AF 5 for workers and

AF 10 for the general population, and for interspecies differences AF 4 for allometric scaling (when relevant) and AF 2.5 for remaining differences.

LOAEL/NAEL extrapolation

With regard to the assessment factor for LOAEL to NAEL, the default factor of 3 is used.

Quality of the whole database and exposure duration

There is only one single test with trixylyl phosphate using repeated exposure (OECD TG422 screening study). The quality of this study is not under discussion, however the total database of available studies with TXP is very small with only one single study available. As noted above, information from a read across substance is difficult to use, as the toxicity profile for tricresyl phosphate differs between rats and mice. Without other alternative data, the small overall database calls for an AF of 2.

The AF for quality of the database is needed because information on reproductive toxicity available for this Annex X substance is scarce. According to the REACH Regulation, PNDT and EOGRT studies are standard information requirements at this tonnage level. However, due to the clear adverse effects observed in the available OECD TG 422 study, the Registrant has classified as Repr. 1B, which is an adaptation for omitting the definitive reproductive toxicity studies of Annex IX and Annex X of the REACH Regulation. There is also a harmonised classification with Repr. 1B. Furthermore, the OECD TG 422 fails to identify a NOAEL because histopathological findings in male and female rats were observed at the lowest dose level as described in this document. The lack of the definitive PNDT and EOGRT studies as well as the lack of NOAEL identification results in a weak quality of the database.

Regarding assessment factors for exposure duration and quality of the whole database, RAC has considered different arguments.

As noted in the REACH guidance concerning the TG 422:

> "*The purpose of the Reproduction/Developmental Toxicity Screening Test (OECD TGs 421 and 422) is to provide information of the effects on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of conceptus and parturition. It should be regarded as an in vivo screening assay and is not designed as an alternative or a replacement of the reproductive toxicity studies (OECD TGs 414 and 416). These screening tests are not meant to provide complete information on all aspects of reproduction and development such as that obtained from a two-generation reproduction study (OECD TG 416). In particular, the post-natal effects associated with prenatal exposure (such as undetected malformations affecting viability or functional effects) or effects resulting from post-natal exposure or exposure during lactation are not covered in these studies. Furthermore, the exposure duration in these studies does not cover a full spermatogenic cycle and the number of animals per dose group is limited.*
>
> *A positive result in OECD TG 421/422 may be considered sufficient for the calculation of a* $DNEL_{fertility}$ *and/or a* $DNEL_{development}$*; however, an additional assessment factor of 2 to 5, decided on a case-by-case basis, should generally be used to take account of the lower sensitivity of the study, unless there is evidence to support that the lower sensitivity is not*

relevant for the effect mechanism of the substance (e.g. specific teratogenic effects that are the result of a known mechanism of action).”

Unfortunately, the guidance does not explain the basis for the proposed AF of 2-5, and what aspects this should cover. RAC suggests to consider the small size, low power of the study and short duration as explained below and use an AF of 5.

The male and female rats were exposed in the pre-mating period in the actual test (TG 422) for 2 weeks. A standard EOGRTs (TG 443) requires 2 weeks premating exposure which could be adapted for males if testicular toxicity (impairment of spermatogenesis) or effects on sperm integrity and function have been clearly identified in previous studies. ECHA requires in its compliance check decisions 10 weeks premating exposure duration in case of lipophilic substances (which is the case for trixylyl phosphate; log Pow>6.2) and in case the second generation is not triggered. 10 weeks are required to cover the full spermatogenesis and maturation, meaning that the full cycle of development of sperm from spermatogonia into mature sperm is exposed, as well as the folliculogenesis, which lasts around 62 days. Total exposure duration before histopathological evaluation of the parental generation in the OECD TG 422 test is 4 weeks for males and 9 weeks for females. In EOGRTS it is about 18 weeks (incl. 10 weeks pre-mating) for both sexes for lipophilic substances such as trixylyl phosphate (but it can be shorter if not lipophilic). Thus, the available TG 422 study has an exposure period much shorter than what is considered needed to fully assess testis toxicity. Accordingly, the studies with the analogue tricresyl phosphate show a duration dependency on testis effects, indicated that the available TG422 study underestimates the testis toxicity.

Furthermore, in an OECD TG 422 screening test 10 animals per sex and dose are mated but histopathological evaluation of reproductive organs is usually performed on 5 animals per sex and dose. In an EOGRTS histopathological evaluation of reproductive organs is performed on all parental animals (20 animals per sex and dose) in case of treatment related changes. Thus, the TG 422 study uses fewer animals than a EOGRTS, and is therefore likely to be less sensitive. Another reason for a lower sensitivity of TG 422 is that fewer endpoints are assessed than in an EOGRTS.

The TG 422 study is a short term repeated dose toxicity study (there was similar histopathological analysis of the tissue damage as would have been done in e.g. a 28 day study, and similar number of animals for histopathological evaluation), and the exposure duration (33 days) was similar to a 28 day study.
The default approach according to the Reach guidance would be to use an exposure duration AF of 6 extrapolating from a 28 day study to chronic exposure. It might be somewhat conservative (as no functional fertility effects were noted at the LOAEL), but on the other hand it is recognised that a similar testicular toxicity in humans most likely would have bigger functional consequences than in rats that have a known high production capacity of sperms.

Considering the argumentation on AFs above and quality of the database, RAC proposes a combined AF of 10 after considering all the above arguments (shorter duration – full spermatogenesis cycle not covered, few animals, limited end-points).

Conclusion on Assessment factors

RAC proposes a combined AF for exposure duration and quality of the database of 10 after considering all the above aspects (shorter duration, few animals, limited end-points). In addition, default assessment (uncertainty) factors (for inter- and intraspecies variation, and for LOAEL to NOAEL extrapolation) are applied in the absence of substance-specific information, as prescribed in the ECHA Guidance R. 8 on the characterisation of dose [concentration]-response for human health (ECHA, (2012)). Thus, regarding the assessment factor for LOAEL to NAEL extrapolation, the default factor of 3 is used, for intraspecies differences 5 for workers and 10 for the general population, and the AF for interspecies differences are 4 for allometric scaling (when relevant) and 2.5 for remaining differences.

A LOAEL of 25 mg/kg/day was set based on histopathological effects in testes, ovaries and adrenals.

Correction of the LOAEL of 25 mg/kg bw/day to an inhalation LOAEC results in 30.5 mg/m^3 for worker and 10.9 mg/m^3 for the general population. The total AF for inhalation exposure to workers is 375, and two-fold higher for the general population. This results in a DNEL of 0.08 mg/m^3 for worker and 0.014 mg/m^3 for the general population.

Correction of the LOAEL of 25 mg/kg bw/day to a dermal LOAEL results in 175 mg/kg bw/day for worker and 125 mg/kg bw/day for the general population. The total AF for dermal exposure to workers is 1500, and two-fold higher for the general population. This results in a DNEL of 0.12 mg/kg bw/day for worker and 0.04 mg/kg bw/day for the general population.

2.1.1. Inhalation DNELs

Table 2 DNELs for inhalation, systemic, long-term

DNELs INHALATION, systemic, long-term		
Anonymous 2, (2004)		
Oral study (gavage), rat, 11 animals per sex and dose, administration 7 d/wk, 33 days (males), ca. 48 days (females), OECD TG 422, substance tested Phosflex TXP (trixylyl phosphate; Anonymous 2, (2004)		
LOAEL (mg/kg bw/day)	**25**	
CORRECTION	Workers	General population
Correction for exposure regime rat / human (day/wk)	7 / 5	7 / 7
Adjustment route of exposure (rat oral to human inhalation): Worker (0.8 l/min/kg, 8 h): 0.384 m^3/kg bw/8 h General population (0.8 l/min/kg, 24 h): 1.15 m^3/kg bw/24 h	1 / 0.384	1 / 1.15
Route-specific bioavailability: 50% oral rat / 100% inhalation humans	50 / 100	50 / 100
Activity driven differences: At rest 6.7 m^3 , light activity 10 m^3	6.7 / 10	--
Corrected LOAEC for human inhalation (mg/m^3)	**30.5**	**10.9**
ASSESSMENT FACTORS (AFs)	Workers	General population
LOAEC to NAEC (in case no NOAEC)	3	3
Interspecies, allometric scaling	--	--
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Duration: extrapolation sub-acute to chronic	10	10
Quality of database		
Remaining	1	1
Overall AF	**375**	**750**
Reference DNEL, inhalation, systemic, long-term (mg/m^3)	**0.08**	**0.014**

2.1.2. Dermal DNELs

Table 3 DNEL for dermal, long-term

DNELs DERMAL, long-term		
Anonymous 2, (2004)		
Oral study (gavage), rat, 11 animals per sex and dose, administration 7 days/wk, 33 days (males), ca. 48 days (females), OECD TG 422, substance tested Phosflex TXP (trixylyl phosphate; Anonymous 2, (2004)		
LOAEL (mg/kg bw/d)	**25**	
CORRECTION	Workers	General population
Correction for exposure regime rat / human (day/wk)	7 / 5	7 / 7
Route-specific bioavailability: 50% oral rat / 10% dermal humans	50 / 10	50 / 10
Calculation corrected LOAEL for human dermal (mg/kg bw/day)	**175**	**125**
ASSESSMENT FACTORS	Workers	General population
LOAEL to NAEL	3	3
Interspecies, allometric scaling	4	4
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Duration: extrapolation sub-acute to chronic	10	10
Quality of database		
Remaining	1	1
Overall AF	**1500**	**3000**
Reference DNEL, dermal, long-term (mg/kg bw/day)	**0.12**	**0.04**

2.1.3. Oral DNELs

Table 4 DNEL for oral, long-term

DNELs ORAL, long-term		
Anonymous 2, (2004)		
Oral study (gavage), rat, 11 animals per sex and dose, administration 7 days/wk, 33 days (males), ca. 48 days (females), OECD TG 422, substance tested Phosflex TXP (trixylyl phosphate; Anonymous 2, (2004)		
LOAEL (mg/kg bw/day)	**25**	
CORRECTION	Workers	General population
Correction for exposure regime rat / human (day/wk)	—	7 / 7
Route-specific bioavailability: 50% oral rat / 50% oral humans	—	50 / 50
Calculation corrected LOAEL for human oral (mg/kg bw/day)	—	**25**
ASSESSMENT FACTORS	Workers	General population
LOAEL to NAEL (in case no NOAEL)	—	3
Interspecies, allometric scaling	—	4
Interspecies, remaining differences	—	2.5
Intraspecies	—	10
Duration: extrapolation sub-acute to chronic	—	10
Quality of database	—	
Remaining	—	1
Overall AF	—	**3000**
Reference DNEL, oral, long-term (mg/kg bw/day)	—	**0.008**

3. References

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Appendix

Table 5 Reproductive toxicity studies – summary

Study description	Main Observations	NOAEL or LOAEL	Reference	Reliab.
OECD TG 422 (GLP) • Rat (Sprague-Dawley) • 11 animals per sex and dose • Oral (gavage, in corn oil) • 0, 25, 200, 1000 mg/kg bw/day • vehicle: corn oil (2 mL/kg) • 7 days/wk, starting 2 wks prior to mating, for 33 days (males) or ca. 48 days (females), termination PND 4 • Recovery group (high dose; 5 animals per sex and dose) **Phosflex TXP** (EC: 246-677-8)	≥ 25 mg/kg bw/day: • no impaired fertility (all dams produced litters) • degeneration of the germinal epithelium of the testes with corollary findings of sloughed epithelial cells in the lumen of the epididymis • ovaries with distinct mild diffuse hyperplasia of the interstitial cells ≥ 200 mg/kg bw/day: • impaired fertility (only 2/11 females delivered) 1000 mg/kg bw/day: • no female with litter (0/10) • no impact on fertility when exposed males and females were mated following 4 weeks without exposure	LOAEL: 25 mg/kg bw/day	Anonymous 1, (2004) (study report)	1, key study
Read-across: **Tricresyl phosphate** **(EC: 809-930-9)** **98-day continuous breeding study** (non-GLP) • Mouse (CD-1) • 20 animals per sex and group • Oral (diet) • 0, 0.05, 0.1, 0.2% diet • ca. 0, 62.5, 124, 250 mg/kg bw/day	≥ 62.5 mg/kg bw/day: • F0 males: atrophy of the seminiferous tubules (ranging from scattered foci of decreased germ cell number, to widespread bilateral loss of germ cells in the high-dose) • F1 males: sperm motility sign. ↓ (F0 males only HD investigated) • F1: hypertrophy of the adrenal zona fasciculata cells, and brown degeneration of the cells in the adrenal juxtamedullary zone (F0 males only HD investigated) ≥ 124 mg/kg bw/day: • F1: impaired fertility (number of live F2 pups per litter sign. ↓) 250 mg/kg bw/day: • F0 and F1: impaired fertility (number of live F1 and F2 pups per litter sign. ↓	LOAEL: 62.5 mg/kg bw/day	Anonymous 1, (1988) (publication)	2, key study

	• F0: Hypertrophy of the adrenal zona fasciculata cells, and brown degeneration of the cells in the adrenal juxtamedullary zone (no information on lower doses) • crossover mating (exposure of one sex only) found impaired fertility (reduced number of live pups per litter) in both sexes with greater effect on females: ○ none exposed: 8.9 live pups per litter ○ only males exposed: 5.2 live pups per litter ○ only females exposed: 0.33 live pups per litter			

Appendix Table 6 NTP Repeated dose toxicity studies with Tricresyl phosphate – summary

Study description	Main Observations	NOAEL or LOAEL	Reference	Reliab.
Substance used: Mixed isomer preparation of 79% tricresyl phosphate esters (consisting of 21% tri-m-cresyl phosphate, 4% tri-p-cresyl phosphate, less than 1% tri-o-cresyl phosphate, and other unidentified tricresyl phosphate esters)				
16 days gavage • Rat (F344/N) • 10 animals per sex and dose • Oral (gavage, in corn oil) • 0, 360, 730, 1450, 2900, 5800 mg/kg bw/day • five days per week for a total of 13 or 14 doses in a 16-day period	≥ 1450 mg/kg bw/day: • Lower final mean body weights • Mortality (1/10 females) ≥ 2900 mg/kg bw/day: • Mortality (5/10 males, 8/10 females) • Necrosis of mandibular lymph node, spleen and thymus • Diffuse aspermatogenis in male rats.	NOAEL: 360 mg/kg bw/day males 730 mg/kg bw/day for females	NTP, (1994)	1
16 days gavage • Mouse (B6C3F1) • 10 animals per sex and dose • Oral (gavage, in corn oil) • 0, 360, 730, 1450, 2900, 5800 mg/kg bw/day • five days per week for a total of 13 or 14 doses in a 16-day period	≥ 360 mg/kg bw/day: • Higher final mean body weights (females; all doses) • Hindlimb grip strengths lower than controls (males) ≥ 730 mg/kg bw/day: • Hindlimb grip strengths lower than controls (females) ≥ 1450 mg/kg bw/day: • Lower final mean body weights (males) • Mortality (5/10 males, 10/10 females)	LOAEL: 360 mg/kg bw/day	NTP, (1994)	1

	≥ 2900 mg/kg bw/day: • Mortality (all animals) • Necrosis of mandibular lymph node, spleen and thymus ≥ 5800 mg/kg bw/day: • Mortality (4/10 males, 1/10 females)			
13 week gavage • Rat (F344/N) • 10 animals per sex and dose • Oral (gavage, in corn oil) • 0, 50, 100, 200, 400, 800 mg/kg bw/day	≥ 50 mg/kg bw/day: • Cytoplasmic vacuolization of adrenal cortex (with increased severity) • Females: ovarian interstitial cell hypertrophy ≥ 200 mg/kg bw/day: • final mean body weights significantly lower (males) ≥ 400 mg/kg bw/d: • Atrophy of seminiferous tubules	NOAEL: 50 mg/kg bw/day males 730 mg/kg bw/day for females	NTP, (1994)	1
13 week gavage • Mouse (B6C3F1) • 10 animals per sex and dose • Oral (gavage, in corn oil) • 0, 50, 100, 200, 400, 800 mg/kg bw/day	≥ 50 mg/kg bw/day: • Cytoplasmic vacuolization of adrenal cortex (with increased severity) • Females: ovarian interstitial cell hypertrophy ≥ 100 mg/kg bw/day: • Multifocal degeneration of the spinal cord (males and female) • Multifocal degeneration of the sciatic nerve (females) ≥ 200 mg/kg bw/day: • Body weights significantly lower (males) • Multifocal degeneration of the sciatic nerve (males) • Lowered hindlimb grip strengths ≥ 400 mg/kg bw/day: • Body weights significantly lower (females)	LOAEL: 50 mg/kg bw/day	NTP, (1994)	1
13 week diet • Rat (F344/N) • 10 animals per sex and dose • Oral (diet) • 0, 900, 1700, 3300, 6600, 13000	≥ 55/65 mg/kg bw/day: • Cytoplasmic vacuolization of adrenal cortex • hyperplasia of ovarian interstitial cells and inflammation of ovarian interstitium (females) ≥ 220 mg/kg bw/day:	LOAEL: 55 mg/kg bw/day males; 65 mg/kg bw/day for	NTP, (1994)	1

ppm • 0, 55, 120, 220, 430, 750 mg/kg bw/d (males) and 0, 65, 120, 230, 430, or 770 mg/kg bw/day (females)	• Body weights significantly lower (females) ≥ 430 mg/kg bw/d: • Body weights significantly lower (males) • Renal papillary edema and necrosis (females) • Basophilic hypertrophy of the pituitary gland pars distalis (males) • Atrophy of seminiferous tubules (males) ≥ 750 mg/kg bw/d: • Renal papillary edema and necrosis (males)	females		
13 week diet • Mouse (B6C3F1) • 10 animals per sex and dose • Oral (diet) • 0, 250, 500, 1000, 2100, 4200 ppm • 0, 45, 110, 180, 380, or 900 mg/kg bw/day (males) and 0, 65, 130, 230, 530, or 1,050 mg/kg bw /day (females)	≥ 45/65 mg/kg bw/day: • Cytoplasmic vacuolization of adrenal cortex ≥ 110 mg/kg bw/d: • Papillary hyperplasia of gallbladder mucosa (males) ≥ 230 mg/kg bw/day: • Papillary hyperplasia of gallbladder mucosa (females) • Axonal degeneration (females) ≥ 380 mg/kg bw/day: • Axonal degeneration (males) ≥ 530 mg/kg bw/day: • Body weights significantly lower (females) ≥ 900 mg/kg bw/day: • Body weights significantly lower (males)	LOAEL: 45 mg/kg bw/day males; 65 mg/kg bw/day for females	NTP, (1994)	1
2 year diet • Rat (F344/N) • 95 animals per sex and dose • Oral (diet) • 0, 75, 150, 300 ppm • 0, 3, 6, or 13 mg/kg bw/day (males) and 0, 4, 7, or 15 mg/kg bw/day (females) • Additional group of 95 male and	4 mg/kg bw/day: • Cytoplasmic vacuolization of adrenal cortex (males, only at 3 months, not at later time points) ≥ 15 mg/kg bw/day: • cytoplasmic vacuolization of adrenal cortex (females) • hyperplasia of ovarian interstitial cells (in females with incidence and severity increasing at the end)	LOAEL: 15 mg/kg bw/day females; NOAEL: 13 mg/kg bw/day males	NTP, (1994)	1

female rats – diet with 600 ppm (twice the highest dose) for 22 weeks, thereafter control diet • Interim kills of 15 animals at 3, 9, and 15 months				
2 year diet • Mouse (B6C3F1) • 95 animals per sex and dose • Oral (diet) • 0, 60, 125, 250 ppm • 0, 7, 13, or 27 mg/kg bw/day (males) and 0, 8, 18, or 37 mg/kg bw/day (females)	≥ 7/8 mg/kg bw/day: • Ceroid pigmentation of adrenal cortex (males and females) ≥ 13 mg/kg bw/day • Incidences of clear cell foci, fatty change, and ceroid pigmentation of the liver (males)	LOAEL: 7 mg/kg bw/day males; 8 mg/kg bw/day for females	NTP, (1994)	1