Helsinki, 12 April 2013
RAC/24/2013/08 rev. 2
Agreed in written procedure

24TH MEETING OF THE COMMITTEE FOR RISK ASSESSMENT

5 - 8 MARCH 2013

HELSINKI, FINLAND

Concerns:	**Authorisation, establishing reference DNELs for DEHP**
Agenda Point:	**7 a) i. DNEL setting (DEHP)**
Action requested:	**For agreement**

Background

At the 22nd meeting of the Committee for Risk Assessment (RAC) in September 2012, the ECHA Secretariat presented a proposal to set DNELs and dose-response curves for substances prior to receiving applications for authorisation (AfAs). This was approved by RAC as a trial exercise.

The DNELs and dose response curves so derived will serve as non-legally binding 'reference' values. They would provide applicants with a clear signal as to how RAC is likely to evaluate these important elements of the risk assessment of AfA.

This initiative is intended to improve the efficiency of the AfA process as a whole by discussing and when possible publishing reference values in advance of applications, so providing greater consistency and better use of the legally defined period of opinion-development in the RAC. The trial will be evaluated in terms of efficiency after the first applications have been discussed in Committee.

Requested action

Following the Committee's agreement on the document, it will be published on the ECHA website at:

http://echa.europa.eu/web/guest/applying-for-authorisation/additional-information.

Progress

The trial was started with two substances, RAC agreeing to establish 'reference' DNELs for DEHP (diethylhexyl phthalate) and TCEP (tris(2-chloroethyl)phosphate). However, it was later decided that DBP (dibutyl phthalate) had greater urgency and this substance was therefore selected as a second candidate instead of TCEP.

The Secretariat prepared this document for the first substance (DEHP) which was reviewed at RAC 23 and RAC 24, commented on by the advisory group consisting of RAC members, then revised accordingly and agreed.

The current document builds on conclusions previously drawn in RAC when the substance was discussed in relation to a restriction proposaland on the RAC opinion on non-classified phthalates DINP/DIDP.

Annex: Reference DNELs derived for DEHP

Annex Reference DNELs for selected substances on Annex XIV of the REACH Regulation (EC) No 1907/2006

SUBSTANCE NAME	EC NUMBER	CAS NUMBER
Bis(2-ethylhexyl) phthalate (DEHP)	204-211-0	117-81-7

Table 1 Overview of reference DNELs for workers, adult (general) population, and children exposed to DEHP derived according to the document

Point of departure for DNEL derivation for DEHP by ECHA			
Rat 3-Gen oral NOAEL in mg/kg/d	**4.80**		
Dosing Regime (Days per Week)	7		
Oral Absorption Percentage	70%		
Derivation for reference DNELs			
	WORKERS	GENERAL POPULATION ADULTS	CHILDREN
Assessment factors			
Interspecies, AS[1]	4	4	4
Interspecies, remaining differences	2.5	2.5	2.5
Intraspecies	5	10	10
Dose Response	1	1	1
Quality of Data Base	1	1	1
Days per week	5	7	7
ORAL			
Absorption percentage	(100%)	100%	100%
NOAEL (corrected)	(not relevant)	3.36	3.36
Reference DNELs[2] ORAL in mg/kg/d	**(not relevant)**	**0.034**	**0.034**
DERMAL			
Absorption percentage	5%	5%	5%
NOAEL (corrected)	94.1	67.2	67.2
Reference DNELs[2] DERMAL in mg/kg/d	**1.882**	**0.672**	**0.672**
INHALATION			
Absorption percentage	75%	75%	100%
Standard respiratory volume in m³/kg bw per day	0.38[3]	1.15	1.15
NOAEC mg/m³ (corrected)	11.0	3.90	2.92
Reference DNECs Error! Bookmark not defined.**[2] INHALATION in mg/m³**	**0.88**	**0.16**	**0.12**

[1] Not to be applied when calculating inhalation DNEC
[2] Not legally binding
[3] The respiratory volume was further adjusted for light work (10/6.7)

Relevance of endpoints

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[4]. 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 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.

DEHP was included on Annex XIV due to its reprotoxic properties. For that reason the DNELs proposed in the present document are only based on reprotoxicity[5]. In this case it is also the most sensitive endpoint, but this may not necessarily be the case with all substances.

Previous discussions on DNELs for DEHP in RAC

During its opinion-making process for a Danish restriction proposal addressing four classified phthalates (*Diethylhexyl phthalate*, DEHP; *Dibutyl phthalate*, DBP; *Di-isobutyl phthalate*, DIBP; and *Benzyl butyl phthalate*, BBP) RAC made an extensive evaluation of the available information related to the hazard profile of the substances. The conclusions related to toxicokinetics, endpoint of concern, identification of a suitable N(L)OAEL, and justification for assessment factors given below have been compiled based on that RAC opinion[6] which was adopted in the Committee's 21st meeting in June 2012.

In addition, oral absorption of phthalates is also addressed in the RAC's opinion on the draft review report of ECHA "Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to Regulation (EC) No 1907/2006 (REACH)" which was adopted in the Committee's 24th meeting in March 2013.

Toxicokinetics and absorption
Following oral administration, phthalates are generally rapidly absorbed from the gastrointestinal tract (probably in monoform). Phthalates can also be absorbed through the lungs, whereas absorption through the skin appears to be limited.

For DEHP, the extent of oral absorption in rats is estimated to be around 60-70%. For humans, RAC considered in their opinion on the restriction proposal addressing four

[4] Article 60(2) states "…an authorisation shall be granted if the risk to human health or the environment from the use of the substance arising from the **intrinsic properties specified in Annex XIV** is adequately controlled".

[5] To the authorisation relevant endpoints refers also section 5 of the document: "How RAC and SEAC intend to evaluate the applications (common approach of RAC and SEAC in opinion development on applications for authorisation, agreed RAC-20/SEAC14, 24/03/2012). Link: http://echa.europa.eu/web/guest/applying-for-authorisation/additional-information

[6] The RAC opinion on the restriction proposal for four phthalates is available here: http://echa.europa.eu/previous-consultations-on-restriction-proposals/-/substance/490/search/+/term

classified phthalates[6] that absorption of DEHP was 70% for adults and 100% in children. However, after revisiting the data, RAC concluded in its opinion of 8 March 2013 on the draft review report of ECHA concerning DINP and DIDP that humans appear to absorb DEHP at 100%. Human volunteer studies with DEHP demonstrate that the amount of radioactivity recovered in urine is dependent on the type and amount of metabolites that are measured in those studies. Measuring all metabolites most likely would result in near to 100% recovery of radioactivity in urine. An unknown amount of excretion via bile contributes further to the absorption estimate.

For all other absorption fractions for DEHP, the values established in the EU-RAR were considered appropriate. Absorption percentages are summarised in Table 2.

Table 2 Consolidated absorption percentages for humans from the previous RAC opinions[6,7,8]

	Oral absorption	Dermal absorption	Inhalatory absorption
DEHP	100%	5%	75% adult, 100% child

Reprotoxicity of DEHP
DEHP is classified as toxic to reproduction on the evidence of adverse effects on the reproductive organs in rats and mice, which are attributed to an anti-androgenic mode of action. When examining the relevant reproduction toxicity studies, RAC recognised that more than one mechanism may have occurred at the same time, leading to several effects which however all seem to follow from an anti-androgenic mode of action. The effects include early marker effects (e.g. on anogenital distance (AGD) and nipple retention), morphological and functional effects (e.g. on testes, epididymes etc.). Although early marker effects may not be adverse *per se*, RAC concluded that in the case of the four phthalates all effects attributable to an anti-androgenic mode of action (be it functional or an early marker) are relevant endpoints, since they are so consistently observed in connection with each other in the available studies. Therefore, the most sensitive of these effects, resulting in the lowest No-Observed-Adverse-Effect Level (NOAEL), was selected for use in the establishment of Derived No-Effect Levels (DNELs).

Selection of the starting point for DNEL derivation
A NOAEL of 4.8 mg/kg bw/day from a three-generation study with dietary exposure of DEHP to rats (Wolfe & Layton, 2003; study summary on p. 413 in EU RAR) was identified. In this study, testicular toxicity (small testes/epididymes/seminal vesicles and minimal testis atrophy) was observed in offspring exposed to 14 mg DEHP/kg bw/day and above as the most sensitive effect attributable to an anti-androgenic mode of action. RAC noted that the same NOAEL had previously been selected in the EU RAR on DEHP and by EFSA when establishing a Tolerable Daily Intake (TDI) of 0.05 mg/kg bw for DEHP. RAC considers the NOAEL of 4.8 mg/kg bw/day to be “conservative”, given the low incidences at the LOAEL.

Assessment factors
In deriving an oral/internal DNEL for DEHP RAC concluded that assessment factors need to be applied for intra- and interspecies differences. Other assessment factors were not necessary.

For intraspecies differences, a factor of 10 (default) was suggested. The same factor of 10 (= 4*2.5) was suggested for interspecies differences. RAC discussed lowering this latter default factor based on information on toxicokinetics (metabolism, distribution) and toxicodynamic data from studies in marmosets. It was felt that this information possibly points to interspecies differences in sensitivity to the reprotoxic effects of phthalates.

[7] EU RAR, p. 482, http://bookshop.europa.eu/en/european-union-risk-assessment-report-pbLBNA23384/
[8] RACs opinion of 8 March 2013 on the draft review report of ECHA concerning DINP and DIDP (to be published)

From the toxicokinetic data available it seemed that there were differences in metabolism and distribution between rats and primates, including humans. Whereas all species hydrolyse the phthalates into the monoform, which is subsequently further metabolised into oxidative metabolites, in contrast to primates, in rats there is no appreciable glucuronidation of the oxidative metabolites. It further appears that, whereas the distribution pattern is the same, rats show higher levels than marmosets of phthalate metabolites in tissues, including testes. In toxicity studies, marmosets appear less sensitive than rats for phthalate toxicity. It has been argued that marmosets are a more appropriate model species than rats to study the reproductive toxic effects of phthalates. These arguments for instance resulted in the use of an interspecies factor of 3 in the risk assessment of DEHP by the FDA, Health Canada and in Japan.

RAC however considered the toxicokinetic differences to be quantitative rather than qualitative, and judged the information on quantitative differences insufficient for providing convincing evidence for a reduced hazard. This is because of the complexity of the (multiple) mechanisms in play for the phthalates toxicity, not all of which may relate to reduced testosterone levels and/or steroidogenesis, and for which the ultimate toxic metabolites are unknown. One of the toxic metabolites is thought to be the mono-form, formed after enzymatic hydrolysis by e.g. lipase. Whereas some studies seemed to indicate that lipase activity is higher in rats than in marmosets, resulting in more toxic metabolites, other studies indicate the opposite or even that lipase activity in humans may be higher than in marmosets and rats. Moreover, studies in rats have shown variable sensitivity to phthalate toxicity depending on the life stage, with rats exposed prenatally and during suckling being much more vulnerable than e.g. sexually mature rats. For marmosets, however, limited data are available for in utero, peri- and neonatal exposure. There is no study with exposure during the entire life cycle such as the multigeneration studies in rats. In fact, there is only one developmental toxicity study (using a single high dose of MBP) with a period of exposure that covers the sensitive window for the programming of the male reproductive system, demonstrating some effects on the testes of neonatal marmosets of which the toxicological significance is unclear. This, combined with the relatively low number of (non-inbred) animals tested in the marmoset studies, makes it difficult to compare the results with those found in (inbred) rats.

All in all, RAC concluded that there is too much uncertainty in the data available to allow a conclusion on humans being less, equally or more sensitive than rats, and thus suggested not to deviate from the default interspecies factor of 10.

Derivation of reference DNELs for DEHP

Based on the previous conclusions in RAC referred to above, DNELs for the adult (general) population, children and workers have been derived for the oral, dermal and inhalation routes. As the DNELs are based on reprotoxicity they are most relevant for protection of pregnant women (and thus foetuses) and very small children. Other groups of the population would however also be protected as reprotoxicity is the most sensitive endpoint for DEHP.

Placenta transfer is supposed to be the same in rats and humans and is not further adjusted for.

Reference DNELs for the adult (general) population

Table 1 gives an overview of the derived reference DNELs for adult consumers exposed to DEHP.

An oral NOAEL in rat of 4.8 mg/kg bw/day for reprotoxicity was identified by RAC from the study of Wolfe et al (study summary on p. 413 in EU RAR[Error! Bookmark not defined.]) in their opinion on the Danish restriction proposal for four classified phthalates.

An oral corrected NOAEL of 3.4 mg/kg bw/day was derived for adults by a correction for differences in oral absorption between rats (70%) and humans (100%).

The oral NOAEL in rat (in mg/kg bw/day) was converted into an inhalatory corrected NOAEC (in mg/m^3) by using a default respiratory volume for the rat corresponding to the daily duration of human exposure followed by a correction for differences in absorption between routes (70% oral absorption in rats, 75% inhalation absorption in humans).

The standard respiratory volume for rats is 0.2 l/min/rat (sRV_{rat}) which corresponds to 0.8 l/min/kg or for 24 h of exposure 1.15 m^3/kg bw. Thus when using the formula below, the corrected inhalatory NOAEC = 4.8 mg/kg bw/day x 1/1.15 m^3/kg bw/24 h x 70/75 = 3.90 mg/m^3.

For <u>general population</u> (in case of 24h exposure/d):

corrected inhalatory NOAEC =

$$\text{oral NOAEL} * \frac{1}{sRV_{rat}} * \frac{ABS_{oral-rat}}{ABS_{inh-rat}} * \frac{ABS_{inh-rat}}{ABS_{inh-human}}$$

$$= \text{oral NOAEL} * \frac{1}{1.15 m^3/kg/d} * \frac{ABS_{oral-rat}}{ABS_{inh-human}}$$

ABS: Absorption; sRV: standard Respiratory Volume

The oral NOAEL rat (in mg/kg bw/day) was converted into a dermal corrected NOAEL (in mg/kg bw/day) by correcting for differences in absorption between routes (70% oral absorption in rats, 5% dermal absorption in humans), resulting in a dermal corrected NOAEL of 67.2 mg/kg bw/day.

Reference DNELs for children

The assumptions are the same as for adults, except that 100% inhalation absorption is assumed for children instead of 75% for adults. The corrected inhalatory NOAEC is thus: 4.8 mg/kg bw/day x 1/1.15 m^3/kg bw/day x 70/100 = 2.92 mg/m^3.

Reference DNELs for workers

DNELs for workers were set using the same basic principles as for the adult (general) population.

The oral NOAEL rat (in mg/kg bw/day) was converted into an inhalatory corrected NOAEC (in mg/m^3) by using a default respiratory volume for the rat corresponding to 8 h duration, followed by a correction for differences in absorption between routes (70% oral absorption in rats, 75% inhalation absorption in humans).

The standard respiratory volume for rats for 8 h exposure is 0.38 m^3/kg bw, which corresponds to an 8 h standard respiratory volume in humans (70 kg) of 6.7 m^3. The respiratory volume was further adjusted to compensate for a higher volume at light work (10 m^3/8 h) in workers.

Thus when using the formula below, the corrected inhalatory NOAEC = 4.8 mg/kg bw/day x 1/0.38 m^3/kg bw/day x 70/75 x 6.7/10 = 7.90 mg/m^3.

For workers (in case of 8h exposure/d):

corrected inhalatory NOAEC = oral NOAEL* $\frac{1}{sRV_{rat}} * \frac{ABS_{oral-rat}}{ABS_{inh-human}} * \frac{sRV_{human}}{wRV}$

= oral NOAEL* $\frac{1}{0.38 m^3/kg/d} * \frac{ABS_{oral-rat}}{ABS_{inh-human}} * \frac{6.7 m^3(8h)}{10 m^3(8h)}$

ABS: Absorption; sRV: standard Respiratory Volume; wRV: worker Respiratory Volume

The NOAEC was further adjusted for an exposure duration of 5 days a week instead of 7 days in the experimental situation (7.87 mg/m^3 x 7/5 = 11.06 mg/m^3).

The oral NOAEL rat (in mg/kg bw/day) was converted into a dermal corrected NOAEL (in mg/kg bw/day) by correcting for differences in absorption between routes, and further correcting for exposure during 5 days a week instead of 7 days a week. The dermal corrected NOAEL = 94.1 mg/kg bw/day.

The default assessment factor of 5 for intraspecies differences was applied when deriving DNELs for workers. It was however noted by RAC that the DNELs for pregnant workers differ from those for pregnant women in the general population due to the differences in intraspecies assessment factors applied (5 versus 10)[9]. The ECHA guidance does not explicitly discuss assessment factors for pregnant women in a working environment. The general principle is that the worker population does not cover the very young, the very old, and the very ill and that therefore an AF of 5 is considered sufficient

[9] The DNELs also differ due to differences in exposure conditions, but this does not lead to different no-effect levels if calculated on a weekly basis.