Helsinki, 12 April 2013
RAC/24/2013/09_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 DBP**
Agenda Point:	**7 a) ii. DNEL setting (DBP)**
Action requested:	**For discussion and 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 substance DBP which was reviewed at RAC 24, commented on by the advisory group consisting of RAC members, then revise accordingly and agreed.

The current document builds on conclusions previously drawn in RAC when the substance was discussed in relation to a restriction proposal, as well as on recent discussions related to the DNEL derivation for DEHP.

Annex: Reference DNELs derived for DBP

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

SUBSTANCE NAME	EC NUMBER	CAS NUMBER
Dibutyl phthalate (DBP)	201-557-4	84-74-2

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

Point of departure for DNEL derivation for DBP by ECHA			
Rat developmental toxicity study, oral LOAEL in mg/kg/d	**2**		
Dosing regime (days per week)	7		
Oral Absorption percentage	100%		
Derivation of Reference DNELs			
		GENERAL POPULATION	
	WORKERS	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 (LOAEL to NAEL)	3	3	3
Quality of Data Base	1	1	1
Days per week	5	7	7
ORAL			
Absorption percentage	(100%)	100%	100%
LOAEL (corrected)	(not relevant)	2	2
Reference DNELs[2] ORAL in mg/kg/d	**(not relevant)**	**0.007**	**0.007**
DERMAL			
Absorption percentage	10%	10%	10%
LOAEL (corrected)	28	20	20
Reference DNELs[2], DERMAL in mg/kg/d	**0.19**	**0.07**	**0.07**
INHALATION			
Absorption percentage	100%	100%	100%
Standard respiratory volume in m³/kg bw per day	0.38[3]	1.15	1.15
LOAEC (corrected)	4.94	1.74	1.74
Reference DNECs[2], INHALATION in mg/m³	**0.13**	**0.02**	**0.02**

[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.

DBP 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 DNEL(s) for DBP 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 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.

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 DBP absorption fractions established in the EU-RAR were considered appropriate (Table 1).

Table 1 Absorption percentages for humans used in the RAC opinion[6,7]

[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

[7] EU RAR, p. 482, http://bookshop.europa.eu/en/european-union-risk-assessment-report-pbLBNA23384/

	Oral absorption	Dermal absorption	Inhalatory absorption
DBP	100% adult, 100% child	10%	100% adult, 100% child

Reprotoxicity of DBP
As DEHP and some other phthalates, DBP is classified as toxic to reproduction on the evidence of adverse effects on the reproductive organs in rodents, which are attributed to an anti-androgenic mode of action. When examining the relevant reproduction toxicity studies, RAC recognised that more than one toxic mechanism may have occurred at the same time, leading to several effects which however all seem to follow an anti-androgenic mode of action. 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 the available studies. Therefore, the most sensitive of these effects resulting in the Lowest-Observed-Adverse-Effect Level (LOAEL), was chosen for use in the establishment of Derived No-Effect Levels (DNEL) for DBP.

Selection of the starting point for DNEL derivation
A LOAEL of 2 mg/kg bw/day from a developmental toxicity study with dietary exposure of DBP to rats (Lee et al., 2004) was identified as the starting point for the DNEL derivation based on delayed germ cell development observed in prepubertal rats and mammary gland changes (vacuolar degeneration and alveolar atrophy) in adult male rats exposed perinatally (from gestation day 15 to post-natal day 21) to a dose of 20 mg DBP/kg feed (corresponding to 1.5-3 mg/kg bw/day; as described in Lee et al., 2004).

RAC noted that EFSA used the same study as basis to established the TDI of 0.01 mg/kg bw for DBP, while using an total assessment factor of 200 (2 for LOAEL-extrapolation, 10 for interspecies and 10 for intraspecies extrapolation). RAC also noted that the study was not available at the time when the EU RAR for DBP was prepared.

Assessment factors
In deriving a DNEL for DBP RAC concluded that assessment factors need to be applied for intra- and interspecies differences and for LOAEL-NAEL extrapolation. Other assessment factors were not found to be needed.

For LOAEL-NAEL extrapolation, RAC suggested an assessment factor of 3 for DBP in line with the REACH Guidance (AF of 3 as a minimum and 10 as a maximum). RAC considered a factor 3 more appropriate than a factor of 2, which was the factor applied by EFSA in deriving a TDI for DBP. EFSA judged this factor to be sufficient, given the reversibility of the effects at all dose levels and especially at the LOAEL in the Lee et al. (2004) study, and acknowledging that in several reproductive toxicity studies with longer exposure periods approximately 30-fold higher NOAELs or LOAELs had been determined. Following review of the Lee et al. (2004) study, however, RAC considered the data on reversibility of the effects on germ cell development not sufficiently convincing. Besides, RAC noted differences in sensitivity between animals in the study, as well as the delayed onset of other mammary gland effects and the recovery time of unusual duration.

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.

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 monobutylphthalate) 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 DBP

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 DBP.

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 2 gives an overview of the derived reference DNELs for adult consumers exposed to DBP.

An oral LOAEL in rat of 2 mg/kg bw/day for developmental toxicity was identified by RAC from the study of Lee et al. (2004) in their opinion on the Danish restriction proposal for four classified phthalates. An oral DNEL of 0.007 was derived by RAC for the general population.

The oral LOAEL in rat (in mg/kg bw/day) was converted into an inhalatory corrected LOAEC (in mg/m^3) by using a default respiratory volume for the rat corresponding to the daily duration of human exposure.

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 LOAEC = 2 mg/kg bw/day x 1/1.15 m^3/kg bw/24 h x 100/100 = 1.74 mg/m^3.

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

corrected inhalatory LOAEC =

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

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

ABS: Absorption; sRV: standard Respiratory Volume

The oral LOAEL rat (in mg/kg bw/day) was converted into a dermal corrected LOAEL (in mg/kg bw/day) by correcting for differences in absorption between routes (100% oral absorption in rats, 10% dermal absorption in humans), resulting in a dermal corrected LOAEL of 20 mg/kg bw/day.

Reference DNELs for children

In the case of DBP all assumptions are the same as for adults.

Reference DNELs for workers

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

The oral LOAEL rat (in mg/kg bw/day) was converted into an inhalatory corrected LOAEC (in mg/m^3) by using a default respiratory volume for the rat corresponding to 8 h duration.

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 LOAEC = 2 mg/kg bw/day x 1/0.38 m^3/kg bw/day x 100/100 x 6.7/10 = 3.53 mg/m^3.

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

$$\text{corrected inhalatory LOAEC} = \text{oral LOAEL} * \frac{1}{sRV_{rat}} * \frac{ABS_{oral-rat}}{ABS_{inh-human}} * \frac{sRV_{human}}{wRV}$$

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

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

The LOAEC was further adjusted for an exposure duration of 5 days a week instead of 7 days in the experimental situation (3.53 mg/m^3 x 7/5 = 4.94 mg/m^3).

The oral LOAEL rat (in mg/kg bw/day) was converted into a dermal corrected LOAEL (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 LOAEL = 28 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)[8]. 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.

References:

EFSA Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Material in Contact with Food (AFC) on a request from the Commission related to Di-Butylphthalate (DBP) for use in food contact materials (Question N° EFSA-Q-2003-192),

[8] 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.

adopted on 23 June 2005 by written procedure, EFSA Journal (2005) 242, 1-17

Lee KY, Shibutani M, Takagi H, Kato N, Takigami S, Uneyama C, Hirose M. Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. Toxicology 2004; 203(1-3):221-23, available at http://www.sciencedirect.com/science/article/pii/S0300483X04003385