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APPLICATION FOR AUTHORISATION: ESTABLISHING REFERENCE DNELs FOR 1-BROMOPROPANE (1-BP)

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 relationships for substances prior to receiving applications for authorisation (AfAs). This was approved by RAC as a trial exercise. However, in early 2015, ECHA agreed to continue supporting the practice for Annex XIV substances, recognizing its value to the Authorisation process and its efficiency[1].

The DNELs and dose response relationships so derived are intended 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.

Reference values in the form of DNELs for threshold substances and/or dose response relationships for non-threshold substances (mainly carcinogens) are 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).

Annex: Reference DNELs derived for 1-BP

[1] At the Conference on "*Lessons learnt on Applications for Authorisation*" co-organised by ECHA and the European Commission that took place on 10-11 February 2015.

Annex 1 Reference DNELs derived for 1-BP

Relevance of endpoints

1-bromopropane (1-BP) has been prioritised for Annex XIV listing due to its harmonised classification for reproductive toxicity (fertility and development) in category 1B (H360FD). The reference DNELs proposed in the present document are only based on the reproductive toxicity of 1-BP. It is noted that this is amongst the most sensitive endpoints of the toxicological profile of 1-BP.

Background

According to the support document for the identification of 1-BP as an SVHC, the precise basis on which 1-BP was classified with H360FD (Repr Cat 1B) is unclear[2]. However there are a number of studies reporting reproductive and developmental effects.

An extensive literature search for 1-BP identified a number of reviews documents and peer reviewed publications on the potential reproductive and developmental effects of 1-BP. A review of the REACH databases for 1-BP has revealed 22 aggregated C&L notifications and 4 registration dossiers. Two of the registration dossiers were for intermediate use, one was for full registration at 1000 to 10,000 tonnes per annum (tpa) and one was for full registration at 100 to 1000 tpa. The two full registration dossiers contained toxicological information including studies of reproductive toxicity.

The available data on 1-BP are considered sufficient by RAC to support the derivation of appropriate DNELs for reproductive toxicity.

Consideration of the reproductive toxicity of 1-BP

A number of studies have consistently demonstrated that 1-BP is toxic to reproduction in rodents, impairing both the male and female reproductive systems. Inhalation exposures of 1-BP have produced adverse effects on fertility and pup survival in studies of reproductive toxicity in rats and developmental toxicity in fetuses of rats exposed by inhalation. Individual elements of the reproductive cycle have been investigated in both rats and mice and a range of effects have been observed.

In the 2 generation inhalation study in rats performed by WIL (2001) the $BMDL_{10}$s for several end-points were found in the range 150-350 ppm. Evaluation of the reproductive performance has shown consistent effects on both males (particularly reductions in sperm counts and sperm motility, and increased in abnormal sperm) and females (altered oestrous cycling and reduced maturation of ovarian follicles). The effects were seen in both rats and mice. The most sensitive end-point in the database is a reduction in sperm count and an increase in abnormal sperm in three mouse strains at 50 ppm (Liu et al, 2009). The results of this study are seen at a lower concentration than similar effects in a different strain of mice used by the NTP (2011), where a NOAEL of 125 ppm was identified for reductions in sperm count and motility (sperm abnormalities were not investigated). An in depth investigation of the Liu et al (2009) and NTP (2011) studies (including methodologies, investigations and consistency of results) identified no reasons to discount the Liu et al (2009) results. More detailed information is provided in the contractor's report.

[2] Classifications agreed under the Dangerous Substances Directive (DSD; 67/548/EEC) were later transposed into Classification Labelling and Packaging (CLP; EC 1272/2008) decisions. In the summary record of the Technical Committee on C&L, it is not possible to trace the basis of the original classification decision in any detail.

No investigations of the reproductive toxicity of 1-BP to non-rodents were identified.

There are isolated reports of humans exposed to 1-BP experiencing reproductive problems, but the database is limited and no firm conclusions can be drawn. However, based on the limited information on comparative metabolism and kinetics, there is no reason to believe that the findings in rats and mice are not potentially relevant to humans.

Critical studies for DNEL derivation

The most sensitive and appropriate end-point in the **rat** database is considered to be the BMDL of 150ppm for reduced litter size in the first generation reduced sperm motility in the F_1 parents of the WIL study, as proposed in the TERA (2004) review. The WIL study exposed rats by inhalation for 6h/d, 7 days per week during pre-mating, gestation and weaning over 2 generations at concentrations of 0, 100, 250 or 500 ppm.

From the **mouse** database, the most appropriate point of departure is the LOAEC of 50 ppm from Liu et al for reduction in sperm motility and sperm count and increases in abnormal sperm in 3 different strains of mice. In the Liu et al study, male mice were exposed by inhalation for 8h/d, 7 d/week for 4 weeks to 50, 110 or 250 ppm.

Following discussions at RAC, it was concluded that the most appropriate point of departure for the reference reproductive DNELs for 1-BP was the LOAEC of 50 ppm for sperm effects seen in 3 strains of mice by Liu et al (2009).

Although the Liu et al study only exposed the mice for 4 weeks, this duration is considered adequate to permit evaluation of most effects on sperm (see OECD test guideline 421). Similar effects on sperm have been seen independently of the duration of exposure. There is one study in rats (Banu et al, 2007) where reversibility of sperm effects due to exposures to 1-BP up to 400 ppm was seen, indicating that if early stage sperm were affected, it was not irreversible. Kinetic data indicate 1-BP is rapidly excreted, and accumulation is unlikely. Therefore it is concluded that the 4 week exposure is adequate to cover the sensitive window(s) for effects on sperm produced by exposures to 1-BP and no additional factor is required to extrapolate to lifetime exposures.

The findings in the Liu et al study exhibit a clear dose response relationship. A small number of the effects on sperm were no longer statistically significant in some strains at 50 ppm. This indicates that at 50 ppm the LOAEC is relatively near to a NOAEC. Therefore, the minimum default assessment factor of 3, as defined in the ECHA guidance[3], will be used for extrapolating from a LOAEC to a NOAEC.

Taking account of the generally accepted greater sensitivity of humans, than rodents, to poor reproductive performance due to reduced semen quality / sperm counts it is considered appropriate to base the reproductive DNELs for 1-BP on reduction in sperm count as a sensitive end-point.

Derivation of reference DNELs

For the derivation of DNELs for the oral, inhalation and dermal routes and the application of route-to-route extrapolation, the following route-specific absorption values have been used.

Oral 100% (default)

[3] Guidance on information requirements and chemical safety assessment.
Chapter R.8: Characterisation of dose [concentration]-response for human health (2012)

Dermal 10% (based on *in vitro* study; Frasch et al, 2011)
Inhalation 100% (default)

The following assessment factors (AFs) have been applied to the starting point. For intraspecies and intraspecies differences, the default AFs proposed in the ECHA guidance have been used. For extrapolation of the LOAEC to the NOAEC, an AF of 3 has been selected. There is no need to apply an AF for severity of the effect or for the quality of the database (there is a sufficiently large database for reproductive toxicity including good-quality studies). No additional factor has been applied to extrapolate from a 4 week exposure study as the critical window(s) for effects on sperm is considered to have been covered.

For workers, only inhalation and dermal DNELs have been established. For the general public, inhalation, dermal and oral DNELs have been set. These are summarised in Table 1.

Workers

Inhalation

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

Inhalation absorption is considered to be the same (100%) in mice and humans. No correction is required for absorption.

= 250 mg/m^3

The mouse exposure period was 8 hours and no correction to 8 hour worker exposures is applicable.

= 250 mg/m^3

The ECHA guidance includes a factor for the increased ventilation rate of workers of 0.67.

250 x 0.67 = 167 mg/m^3

The mouse exposure was for 7 days per week, the worker exposure is assumed to be 5 days per week. A correction of 7/5 is applicable.

167 x 7/5 = 234 mg/m^3

Application of assessment factors

The ECHA guidance proposes a minimum default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

234 / 3 = 78 mg/m^3

The allometric scaling factor is not applicable when setting an inhalation DNEL based on an inhalation animal study. The dynamic factor of 2.5 still applies.

78 / 2.5 = 31 mg/m^3

The application of a 5 fold intraspecies factor is recommended in the ECHA guidance for workers.

31 / 5 = 6.2 mg/m^3

Reference DNEL (worker, inhalation, reproduction) = 6.2 mg/m^3 (or 1.25 ppm)

Dermal

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

The mouse exposure period was 8 hours (480 minutes). To obtain a systemic dose, correction is required for the ventilation rate of the mouse of 1.4 L/min/kg bw.

0.25 mg/L x 1.4 x 480 = 168 mg/kg bw/d

No robust data on the comparative extent of inhalation and dermal absorption are available, but a value of 10% is proposed for dermal absorption, based on human *in vitro* data and the extensive evaporative flux of 1-BP. A default value of 100% is applied for inhalation absorption. This gives a dermal topical dose.

168 x 10 = 1680 mg/kg bw/d

The mouse exposure was for 7 days per week. Worker exposures are considered to be 5 days per week. A correction of 7 / 5 is applicable.

1680 x 7 / 5 = 2352mg/kg bw

Application of assessment factors

For the dermal route, the allometric scaling factor of 7 for the interspecies correction applies for mice, together with the 2.5 fold dynamic factor = 17.5

2352 / 17.5 = 134 mg/kg bw

The application of a 5 fold intraspecies factor is recommended in the ECHA guidance for workers.

134/ 5 = 27 mg/kg bw/d

No additional factors are required for study duration or the use of a BMDL.

The ECHA guidance proposes a minimum default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

27 / 3 = 9.0 mg/kg bw/d

Reference DNEL (worker, dermal, reproduction) = 9.0 mg/kg bw/d

Oral

Not required for workers.

	Reference DNELs Workers	
Endpoint	**Inhalation (8-hr)**	**Dermal**
Reproductive toxicity	**6.2 mg/m³**	**9.0 mg/kg bw**

General population

Inhalation

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m³ for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

Inhalation absorption is considered to be the same (100%) in mice and humans. No correction is required for absorption.

= 250 mg/m³

The mouse exposure period was 8 hours and a correction to 24 hour population exposures is applicable.

250 / 3 = 83 mg/m³

The mouse exposure was for 7 days per week, the population exposure is assumed to be 7 days per week. No correction is applicable.

= 83 mg/m³

Application of assessment factors

The ECHA guidance proposes a minimum default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

83 / 3 = 27.6 mg/m³

The allometric scaling factor is not applicable when setting an inhalation DNEL based on an inhalation animal study. The dynamic factor of 2.5 still applies.

27.6 / 2.5 = 11.0 mg/m³

The application of a 10 fold intraspecies factor is recommended in the ECHA guidance for workers.

11.0 / 10 = 1.1 mg/m³

Reference DNEL (population, inhalation, reproduction) = 1.1 mg/m³ (or 0.22 ppm)

Dermal

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

The mouse exposure period was 8 hours (480 minutes). To obtain a systemic dose, correction is required for the ventilation rate of the mouse of 1.4 L/min/kg bw.

0.25 mg/L x 1.4 x 480 = 168 mg/kg bw/d

No robust data on the comparative extent of inhalation and dermal absorption are available, but a value of 10% is proposed for dermal absorption, based on human *in vitro* data and the extensive evaporative flux of 1-BP. A default value of 100% is applied for inhalation absorption. This gives a dermal topical dose.

168 x 10 = 1680 mg/kg bw/d

The mouse exposure was for 7 days per week. Population exposures are considered to be 7 days per week. No correction is applicable.

= 1680mg/kg bw

Application of assessment factors

For the dermal route, the allometric scaling factor of 7 for the interspecies correction applies for mice, together with the 2.5 fold dynamic factor = 17.5

1680 / 17.5 = 96 mg/kg bw

The application of a 10 fold intraspecies factor is recommended in the ECHA guidance for the general population.

96 / 10 = 9.6 mg/kg bw/d

The ECHA guidance proposes a minimum default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

9.6 / 3 = 3.2 mg/kg bw/d

Reference DNEL (population, dermal, reproduction) = 3.2 mg/kg bw/d

Oral

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

The mouse exposure period was 8 hours (480 minutes). To obtain a systemic dose, correction is required for the ventilation rate of the mouse of 1.4 L/min/kg bw.

0.25 mg/L x 1.4 x 480 = 168 mg/kg bw/d

No robust data on the comparative extent of inhalation and oral absorption are available, but default values of 100% are proposed for both routes.

= 168 mg/kg bw/d

The mouse exposure was for 7 days per week. Population exposures are considered to be 7 days per week. No correction is applicable.

= 168 mg/kg bw

Application of assessment factors.

For the orall route, the allometric scaling factor of 7 for the interspecies correction applies for mice, together with the 2.5 fold dynamic factor.

168 / 17.5 = 9.6 mg/kg bw

The application of a 10 fold intraspecies factor is recommended in the ECHA guidance for the general population.

9.6 / 10 = 0.96 mg/kg bw/d

The ECHA guidance proposes a minimum default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

0.96 / 3 = 0.32 mg/kg bw/d

Reference DNEL (population, oral, reproduction) = 0.32 mg/kg bw/d

	Reference DNELs General Population		
Endpoint	**Inhalation (24-hr)**	**Dermal**	**Oral**
Reproductive toxicity	**1.1 mg/m³**	**3.2 mg/kg bw/d**	**0.32 mg/kg bw/d**

Table 1: Overview of derivation of reference DNELs for workers and general population for reproductive toxicity of 1-BP by the inhalation, oral and dermal routes

Point of departure for DNEL derivation by all routes for 1-BP in relation to reproductive toxicity (Liu et al, 2009)		
Mice. Inhalation. 8h/d, 7d/wk for 4 weeks LOAEC	**250 mg/m³ - 168 mg/kg/bd**	
inhalation absorption	100 %	
standard respiratory volume in mouse	1.4 L/kg bw/min	
Derivation of reference DNELs		
	WORKERS	GENERAL POPULATION
Assessment Factors[$]		
Interspecies, Allometric scaling	7	7
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Subacute to chronic	1	1
LOAEC to NOAEC	3	3
Exposure Correction		
Hours/day	1	0.33
5 d/wk exposure for workers vs 7d/wk in animals	1.4	1
INHALATION		
Absorption (%)	100	100
Breathing rate for workers light activity vs rest	0.67	1
Indicative DNEL INHALATION in mg/m³	**6.2**	**1.1**
DERMAL		
Absorption (%)	10	10
Indicative DNEL DERMAL in mg/kg bw/d	**9.0**	**3.2**
ORAL		
Absorption (%)	100	100
Indicative DNEL ORAL in mg/kg bw/d	**-**	**0.32**

*Allometric scaling factor (7 for the mouse) not applied for the derivation of the inhalation DNELs

[$] Justification for selection of assessment factors is given in the contractor's report

References

ECHA (2012). Guidance on information requirements and chemical safety assessment. Chapter R8: Characterisation of dose[concentration]-response for human health.

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NTP (2011). National Toxicology Program technical report on the toxicology and carcinogenesis studies of 1-Bromopropane in F344/N rats and B6C3F1 mice (inhalation studies), August 2011. NTP TR 564. Publication 11-5906.

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