ECHA
EUROPEAN CHEMICALS AGENCY

Evaluation of new scientific evidence concerning DINP and DIDP

In relation to entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006

Final review report

LEGAL NOTICE

Title: Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006

Reference: ECHA-13-R-07-EN
ISBN: 978-92-9244-001-5
Publ.date: August 2013
Language: EN

If you have questions or comments in relation to this document please send them (quote the reference and issue date) using the information request form. The information request form can be accessed via the Contact ECHA page at:
http://echa.europa.eu/about/contact_en.asp

European Chemicals Agency

Mailing address: P.O. Box 400, FI-00121 Helsinki, Finland
Visiting address: Annankatu 18, Helsinki, Finland

	IUPAC NAME	EC NUMBER	CAS NUMBER
DINP	1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich	271-090-9	68515-48-0
	di-"isononyl" phthalate	249-079-5	28553-12-0
DIDP	1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich	271-091-4	68515-49-1
	di-"isodecyl" phthalate	247-977-1	26761-40-0

Table of Contents

1. Conclusions

ECHA concluded that a risk from the mouthing of toys and childcare articles with DINP and DIDP cannot be excluded if the existing restriction were lifted. No further risks were identified. These conclusions were supported by ECHA's Committee for Risk Assessment (ECHA 2013a,b). Based on the risk assessment in this report, it can be concluded that there is no evidence that would justify a re-examination of the existing restriction on DINP and DIDP in toys and childcare articles which can be placed in the mouth by children (restriction entry 52 in Annex XVII to REACH).

Children
Reasonable worst case RCRs ranging from 1.3 to 2.0 indicate a risk of liver toxicity for children of 0-18 months old from mouthing toys and childcare articles containing DINP or DIDP. Thus, it is concluded that a risk from the mouthing of toys and childcare articles with DINP and DIDP cannot be excluded if the existing restriction were lifted (i.e. in the scenario where DINP or DIDP would be present in toys and childcare articles). This conclusion was supported by RAC (ECHA 2013a,b).

Previously, there had been disagreement between the EU Risk Assessment (EC 2003a) conclusions for DINP and the conclusions drawn on by Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE 2001a) concerning the need for restricting DINP in toys and childcare articles. The reason for the difference in opinions concerning the need of this restriction was resulting from the difference in the selected NOAEL for repeated dose toxicity as a starting point for risk assessment, 15 mg/kg bw/day by the CSTEE versus 88 mg/kg bw/day by the EU Risk Assessment. ECHA re-evaluated the available information and concluded on a NOAEL of 15 mg/kg bw/day, which is in line with the NOAEL selected by other relevant European scientific bodies (CSTEE 2001a; EFSA 2005; SCCP 2007; SCHER 2008; SCENIHR 2008) and the United States (CHAP 2001; US CPSC 2010a).

Concerning DIDP, the EU Risk Assessment (EC 2003b) had concluded that there was a need for restricting DIDP in toys and childcare articles. CSTEE (2001b) did not fully agree with this conclusion.

Since the calculated risk characterisation ratios (RCRs) represent the estimated risk of exposure to DINP or DIDP from toys and childcare articles in case the existing restriction would be lifted, the RCRs give an indication of the risk reduction capacity of the existing restriction. It is assumed that there is at present a very small remaining level of exposure to newborns and infants from consumer articles that are not covered by the existing restriction entry for toys and childcare articles which can be placed in the mouth by children. Thus, current RCRs are assessed to be well below 1.

It is not anticipated that mouthing of erasers containing DINP or DIDP would lead to a considerable risk for children. Furthermore, no risk is expected from combined exposure to DINP and DIDP for children exposed via food and the indoor environment.

Based on the risk assessment in this report, it can be concluded that no further risk management measures are needed to reduce the exposure of children to DINP and DIDP.

Adult consumers
With RCRs of 0.4 in the reasonable worst case use of sex toys, it seems not likely that the use of sex toys with DINP or DIDP would result in a risk. This conclusion is subject to substantial uncertainties with regard to exposure duration and migration rates of the phthalates from sex toys.

Dermal exposure from for instance PVC garments is not anticipated to result in a risk for the adult population.

Exposure from food and the indoor environment are not very significant in the adult population, which is confirmed by the available biomonitoring data.

Based on the risk assessment in this report, it can be concluded that no further risk management measures are needed to reduce the exposure of adults to DINP and DIDP.

2. Background

Entry 52 of Annex XVII to REACH, as amended by Commission Regulation (EC) No 552/2009, restricts DINP, DIDP and DNOP in toys and childcare articles which can be placed in the mouth by children. This entry contains a review clause, which is the basis for the current review report.

52. The following phthalates (or other CAS- and EC numbers covering the substance): (a) Di-'isononyl' phthalate (DINP) CAS No 28553-12-0 and 68515-48-0 EC No 249-079-5 and 271-090-9 (b) Di-'isodecyl' phthalate (DIDP) CAS No 26761-40-0 and 68515-49-1 EC No 247-977-1 and 271-091-4 (c) Di-n-octyl phthalate (DNOP) CAS No 117-84-0 EC No 204-214-7	1. Shall not be used as substances or in mixtures, in concentrations greater than 0,1 % by weight of the plasticised material, in toys and childcare articles which can be placed in the mouth by children. 2. Such toys and childcare articles containing these phthalates in a concentration greater than 0,1 % by weight of the plasticised material shall not be placed on the market. 3. The Commission shall re-evaluate, by 16 January 2010, the measures provided for in relation to this entry in the light of new scientific information on such substances and their substitutes, and if justified, these measures shall be modified accordingly. 4. For the purpose of this entry 'childcare article' shall mean any product intended to facilitate sleep, relaxation, hygiene, the feeding of children or sucking on the part of children.

With regard to the definition of 'childcare articles' in point four of entry 52, the "Guidance Document on the interpretation of the concept "which can be placed in the mouth"" mentions the following: "It is the Commission's interpretation that Directive 2005/84/EC covers the accessible parts of articles such as push chairs, car seats and bike seats which are intended to facilitate sleep and relaxation during transport. The main purpose of pyjamas is to dress children when sleeping and not to facilitate sleep. Pyjamas should therefore be regarded as textiles and, like other textiles, do not fall under the scope of the Directive. Sleeping bags are designed to facilitate sleep, and should therefore fall under the Directive." (European Commission 2006). In addition, the "Questions and agreed answers concerning the implementation of Annex XVII" mentions the following: "… articles which are used for the hygienic care of children such as bathtubs, articles for the bath, bathtub mats, hairbrushes, bath thermometers, or nail cutters are therefore covered by the Entries 51 and 52 and use of phthalates and should conform to the prescriptions of the entries." and "it can be confirmed that mattress protectors are childcare articles as defined in Annex XVII… In conclusion, mattress protectors that can be placed above sheets or that cannot be tightly fixed to the mattress have to comply with the restriction contained in entry 52 of Annex XVII to REACH." (European Commission 2011)

The "Questions and agreed answers concerning the implementation of Annex XVII" clarifies the interpretation of the 0.1% limit as follows: "The threshold of 0.1% is the standard threshold used in Annex XVII. The value of 0.1% has been chosen because it represents a measurable quantity. It is being used to take into account impurities, not to allow the use of certain

substances, e.g. phthalates in toys and childcare articles. One should be aware that in order to plasticise a toy or childcare article concentrations of phthalates of more than 10 per cent are needed.

Different restrictions are applied to each of the two groups of phthalates. The limit value of 0.1% should therefore be applied for each group of phthalates combined, i.e. the concentration of DEHP, DBP and BBP combined should not be higher than 0.1% and the concentration of DINP, DIDP and DNOP combined should also not be higher than 0.1%." (European Commission 2011)

2.1 History of the entry 52

Entries 51 and 52 of Annex XVII have a history dating back to 1998 when the Commission issued a recommendation on phthalates, following concern expressed by the CSTEE about the exposure of children to certain phthalates, in particular to DINP (MOS[1] of 8.8) and to a lesser extent DEHP (MOS of 67) (CSTEE 1998a,b). The Commission recommended Member States to adopt measures required to ensure a high level of child health protection in regard to notably DEHP, DBP, BBP, DINP, DIDP and DNOP in childcare articles and toys intended to be placed in the mouth by children less than three years of age.

In its opinion of November 1998, the CSTEE had revised the MOS values for the six phthalates, concluding that the MOS value for DEHP of 19 raised clear concern and that the MOS value of 75 for DINP raised some concern (CSTEE 1998c).

End 1999, following the opinion of the CSTEE (CSTEE 1998c), a temporary restriction was introduced for the six phthalates DEHP, DBP, BBP, DINP, DIDP and DNOP in the General Product Safety Directive[2] by means of a Commission Decision[3]. Unlike in the present situation, the restriction at the time did not differentiate between the six phthalates. The restriction applied to placing on the market of the toys and childcare articles intended to be placed in the mouth by children of less than three years of age, and that were made of, or in part made of soft PVC containing more than 0.1% (w/w) of one or more of the six phthalates. The temporary restriction was extended more than 20 times until the adoption of Directive 2005/84/EC (see further).

In the following years the CSTEE issued several opinions on EU Risk Assessment reports for the phthalates, and the reports were published (see Table 2.1).

The relevant conclusion of the EU Risk Assessment for DINP relating to toys and childcare articles is the following (not supported by CSTEE 2001a):

[1] A MOS value or "Margin of Safety" is the magnitude by which the N(L)OAEL exceeds the estimated exposure. The MOS approach was used in risk assessments under the "existing substances" legislation (Commission Regulation (EC) No 1488/94). The risk assessor would use the MOS to come to a conclusion on the risk taking into account considerations on variability in the experimental data; intra- and interspecies variation; differences in exposure route, duration, frequency and pattern; dose-response relationship; overall confidence in the database; nature and severity of the effect; the human population to which the quantitative and/or qualitative information on exposure applies. As a simplified comparison between the DNEL/RCR approach under REACH and the MOS approach, one could say that if an AF of 100 was assumed in DNEL derivation (REACH), a MOS below 100 would usually indicate a concern (similarly to an RCR below 1). Based on the MOS and the above considerations, for each population and for each effect, either of the following conclusions were taken:
(i) there is need for further information and/or testing;
(ii) there is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already;
(iii) there is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

[2] Directive 92/59/EEC

[3] Commission Decision 1999/815/EC

> *"**Consumers***
> ***Conclusion (ii)** There is at present no need for further information or testing or risk reduction measures beyond those which are being applied already."* (EC 2003a)

The strategy for limiting the risks for consumers for DINP was formulated as follows:

> *"In the light of the divergent scientific views between the CSTEE and the conclusions of the assessment of the risk for consumers under this Regulation, and taking into account the uncertainties in the evaluation of exposure to DINP from toys and childcare articles, precautionary considerations support the consideration at Community level of proportionate restrictions in Council Directive 76/769/EEC (Marketing and Use Directive) for the use of DINP in toys and childcare articles. Such measures should be reviewed after 3-4 years, in light of further scientific developments."* (Commission Communication 2006/C 90/04)

The relevant conclusion of the EU Risk Assessment for DIDP relating to toys and childcare articles is the following (not fully supported by CSTEE 2001b[4]):

> *"**Consumers***
> ***Conclusion (iii)** There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.*
>
> *This conclusion applies in case DIDP should be used as a substitute for other phthalates in toys because of concerns for hepatic toxicity as a consequence of repeated exposure of infants and newborn babies arising mainly by the oral route from mouthing and sucking toys and baby equipment.*
>
> *Pertaining to reduced offspring survival, due to the uncertainty related to the relevance of this end point for newborns and infants and to the lack of experience in this particular field of transgenerational effect, no formal conclusion could be drawn."* (EC 2003b)

The strategy for limiting the risks for consumers for DIDP was formulated as follows:

> *"to consider at Community level restrictions in Council Directive 76/769/EEC (Marketing and Use Directive) for the use if DIDP in toys and childcare articles."* (Commission Communication 2006/C 90/04)

Table 2.1 Overview of the key dates for the relevant EU Risk Assessment reports

Substance	Last literature search	TCNES review report	CSTEE opinion	Final EU Risk Assessment	Commission communication	Commission recommendation
DEHP	2005	2005	2002/ 2004	2008	2007	2008
DBP	1994*	1999	2001	2003	2006	none
BBP	2003	2001	SCHER HH2005 ENV2006	2007	2008	2008
DINP	2001	2001	2001	2003	2006	none
DIDP	2001	2001	2001	2003	2006	none
DNOP	no EU Risk Assessment available	N.A.	N.A.	N.A.	N.A.	N.A.

*The last full literature survey was carried out in 1994-targeted searches were carried out subsequently

[4] CSTEE concluded "*The RAR suggests conclusion iii) for infants with toys. The CSTEE finds that conclusion iii) is not sufficiently justified and proposes conclusion ii) for liver effects also for infants with toys. However, should the usage of DIDP be similar to that of DINP in children's toys and leading to a similar release rate, the MOS would be 54 (not taking potential species differences in bioavailability into account). If this were to be the situation, the CSTEE would arrive at a conclusion iii) for infants with toys."*

In 2005, the restriction under the General Product Safety Directive was made permanent in the form of an amendment (Directive 2005/84/EC) to the Marketing and Use Directive (Council Directive 76/769/EEC). At this point however, a difference was made between the three classified phthalates DEHP, DBP and BBP (the current entry 51), and the three non-classified phthalates DINP, DIDP, and DNOP (the current entry 52) for reasons of proportionality[5].

The difference between the entries still exists today in Annex XVII of REACH[6]: entry 51 applies to "toys and childcare articles" whereas entry 52 applies to "toys and childcare articles *which can be placed in the mouth by children*".

2.2 First phase of the review: 6 phthalates

Based on the review clause in restriction entries 51 and 52 in Annex XVII to REACH, the Commission requested ECHA on 4 September 2009 to review the available new scientific information for phthalates contained in Annex XVII of REACH (DEHP, DBP, BBP, DINP, DIDP and DNOP) and to evaluate whether there is evidence that would justify a re-examination of the existing restrictions in accordance with Article 69(5) or if applicable Article 68(2) of REACH.

In this request, the European Commission suggested that the highest priority should be given to an evaluation of whether the use of these phthalates in articles intended to be used by children (other than toys and childcare articles), for example school supplies and clothing, poses a risk to children that is not adequately controlled. Thus, the scope of ECHA's review was not limited to the existing restrictions on toys and childcare articles.

ECHA provided the Commission with six review reports containing the results of its work in March 2010. These reports were published on ECHA's web site in July 2010, after being updated on the basis of comments received from CARACAL members and observers in June 2010 (ECHA 2010a,b,c,d,e,f).

In the reports for non-classified phthalates (DINP, DIDP and DNOP), ECHA came to the conclusion that the available new information with regard to the uses of and exposure to these phthalates did not indicate the need for an urgent re-examination of the existing restrictions and suggested that the Commission would wait until the first registration deadline under the REACH Regulation would have been passed before deciding on any further steps in this re-evaluation process. The reports also identified several areas in which further in-depth assessment of the available information would be necessary to draw firm conclusions on the risks related to the use of these substances in articles. The Commission announced its intention to follow this recommendation during the CARACAL meeting held on 15-17 June 2010.

[5] Recitals 11 and 12 to Directive 2005/84/EC read:
"(11) Scientific information regarding di-isononyl phthalate (DINP), di-isodecyl phthalate (DIDP) and di-n-octyl phthalate (DNOP) is either lacking or conflictual, but it cannot be excluded that they pose a potential risk if used in toys and childcare articles, which are by definition produced for children.
(12) The uncertainties in the evaluation of exposure to these phthalates, such as mouthing times and exposure to emissions from other sources, require that precautionary considerations be taken into account. Therefore, restrictions on the use of these phthalates for toys and childcare articles and on the placing on the market of such articles should be introduced. However, the restrictions for DINP, DIDP and DNOP should be less severe than the ones proposed for DEHP, DBP and BBP for reasons of proportionality."

[6] The restrictions in the Marketing and Use Directive were 'transferred' to Annex XVII to the REACH regulation. In accordance with Articles 139 and 141(4) of REACH, the Marketing and Use Directive was repealed with effect from 1 June 2009, the date from which Title VII and Annex XVII of REACH applied. Annex XVII was subsequently amended by Commission Regulation (EC) No 552/2009.

2.3 Second phase of the review: DINP and DIDP

On 14 December 2010, with regard to the entry 52 phthalates (i.e. DINP, DIDP and DNOP), the Commission has requested ECHA to *"review and analyse new scientific information, if any, coming from the registration dossiers with a view to completing the assessment of information already included in the existing review reports and, as appropriate, revise the ECHA conclusions, including the need or not for further actions on these three non-classified phthalates under REACH"*. For the reasons explained in Section 3, the scope in the second phase was limited to a further review of the phthalates DINP and DIDP.

The current report on DINP and DIDP takes the opinion of RAC and the information received during public consultation into account. Furthermore, independent of the public consultation and the opinion forming process in RAC, ECHA has received correspondence and documents from the industry trade organisation the European Council for Plasticisers and Intermediates (ECPI) and ExxonMobil. Comments that were received within the agreed deadline were taken into consideration.

Public consultation
A draft of the current report on DINP and DIDP was subject to a 12-week public consultation, from 7 May to 31 July 2012.

During public consultation, comments were received from authorities in Denmark, Germany, UK and Norway. Extensive comments with 10 supporting documents were received from ECPI. One individual company submitted comments. Furthermore, 6 comments from individuals were received[7]. The comments and ECHA's reaction to the comments are publicly available at

http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

Opinion of RAC
ECHA's Committee for Risk Assessment (RAC) was requested to provide a scientific opinion on the draft review report on DINP and DIDP. The opinion forming process in RAC started on 25 April 2012 with the request for an opinion by ECHA's Executive Director, and was finalised with the adoption of the opinion by RAC on 8 March 2013 (ECHA 2013a,b). The opinion of RAC takes into account the comments of interested parties provided during public consultation.

Following the public consultation, further comments have been submitted by industry stakeholder observers to the Secretariat of RAC in the framework of the opinion forming process. The Secretariat distributed several of those contributions to the committee, subject to the rules laid down by ECHA's 'Code of conduct for observers at ECHA meetings' (ECHA 2008) as well as the 'Rules of procedure for the Committee for Risk Assessment' (ECHA 2012c).

[7] Two out of those comments were individuals sponsored by ExxonMobil, and another two were sponsored by ECPI.

3. Scope of the review

The scope of the second phase was limited to a further review of the information on entry 52 phthalates, i.e. DINP, DIDP and DNOP[8].

In accordance with the recommendations made in the first phase review reports of DINP and DIDP as published in July 2010 (ECHA 2010a,b), ECHA performed an in-depth assessment of the available information on the human health hazard and consumer exposure. Concerning the hazard assessment, in particular repeated dose effects, effects on reproduction, endocrine mode of action and sensitisation were identified for further scrutiny. The exposure assessment focussed on (consumer) exposure of children and adults from direct oral and dermal contact with articles, diet, and the indoor environment. The need to address the risks of combined exposure to DINP and DIDP was also considered.

The EU Risk Assessments for DINP and DIDP were the starting point for the review (EC 2003a,b). The review included a further scrutiny of new information sources that were identified in the first phase review reports (ECHA 2010a,b), information included in the registration dossiers, as well as other information that became available in the course of the review process.

As far as DNOP is concerned, no REACH registration dossier has been submitted to ECHA (last check on 12 August 2013), which gives further support to the information in the ECHA review report on DNOP from July 2010 (ECHA 2010c) that on the one hand there seems to be confusion around the substance identity of DNOP, and on the other hand there seems to be no commercial market in the EU for DNOP[9]. Therefore, ECHA did not conduct any further evaluation of DNOP, and considers that the conclusions drawn in the published review report (ECHA 2010c) are still valid, i.e. that there is no new information available that would justify the re-examination of the current restriction on DNOP.

[8] No further review of the information on entry 51 phthalates (DEHP, DBP and BBP) was considered meaningful. On 14 April 2011, Denmark proposed restrictions on the placing on the market of articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes containing DEHP, DBP, BBP or DiBP. Thus, the Danish authorities had carried out a recent evaluation of the risks for these phthalates, and according to Article 70 of the REACH Regulation, RAC issued an opinion on the proposal. It was not considered meaningful that ECHA would carry out a further review on these substances in parallel to the restriction process.
Moreover, following the inclusion of the four phthalates in the Authorisation List, applicants would need to demonstrate that risks to human health or the environment from the use of the substances are adequately controlled (which includes any risks arising from the article service life). RAC issues an opinion according to Article 64 for any application that will be received.
Lastly, Article 69(2) of REACH requires ECHA to consider whether the use of the four phthalates in articles (including imported articles) poses a risk to human health or the environment that is not adequately controlled.

[9] This is confirmed by the information submitted by INEOS ChlorVinyls (2012) during the public consultation on the draft ECHA review report : *"It is true to state that there has been confusion over the term "DnOP" and with C8 phthalates in general. The substance di-2-ethylhexyl phthalate (DEHP) has been known for many years under the trivial name di-octyl phthalate (DOP). Most references to DOP in the literature do in fact refer to DEHP. Therefore the term DnOP (di-normaloctyl phthalate) is normally taken to refer to the entirely straight chain C8 ester. This is a more unusual – in terms of volume and use – plasticiser although it can give useful properties for certain applications. Given the significant shift from C8 to C9 and C10 phthalates over the past ten years it is not surprising that it has not been registered under REACH".*

3.1 New information on human health hazard and exposure

In general, recent scientific studies seem to have given main focus to DINP as compared to DIDP.

First phase of the review
During the first phase of the review, all information submitted to the Commission by stakeholders by 30 June 2009 was considered as well as additional information that was submitted directly to ECHA in the autumn of 2009. In addition, ECHA performed its own literature search specifically on health hazard properties of DINP, DIDP and DNOP.

Second phase of the review (current report)
In the second phase of the review, additional literature searches were carried out. These were targeted to the hazard properties of DINP and DIDP, in particular for repeated dose effects, effects on reproduction, endocrine mode of action and sensitisation. In addition, a general screening of the literature was carried out by ECHA in order to include the most recent publications. Table 3.1 gives an overview of the relevant literature searches.

Limited information published after the EU Risk Assessments' last literature searches on hazardous properties of DINP and DIDP was referenced in the registration dossiers for DINP and DIDP. In the framework of the review by ECHA, industry submitted new studies for DIDP (Clewell et al. 2011a,b).

Table 3.1 Overview of relevant literature searches with focus on hazard properties

Substance	Last literature search EU Risk Assessment	Final review TECNES	Final EU Risk Assessment	Last literature search 1st phase ECHA review	Last literature search 2nd phase ECHA review
DINP	2001	2001	2003	End 2009	April 2012
DIDP	2001	2001	2003	End 2009	April 2012

The registration dossiers and the public consultation on the draft review report did not bring to light additional uses of DINP and DIDP.

Additional information on exposure available from registration dossiers was limited since DINP and DIDP were not considered to meet the criteria for classification nor were assessed to be PBT or vPvB in the Chemical Safety Reports (CSRs) received by ECHA as part of registration dossiers. As a consequence, these CSRs were not required to contain an exposure assessment nor a risk characterisation. Nevertheless, industry submitted as part of the registrations an assessment specifically for toys and childcare articles "*Review of Recent Scientific Data on Di-isononyl Phthalate (DINP) and Risk Characterisation for its use in Toys and Childcare articles*" (ECPI 2009), and a "*Statement relevant to the re-evaluation of DIDP in toys and childcare articles as required by Directive 2005/84/EC*" (ExxonMobil 2011c). Furthermore, industry voluntarily submitted migration data (TNO 2010; ExxonMobil 2011b).

New data on manufacturing and import, migration rates from articles, biomonitoring and food was collected by Amec Environment & Infrastructure UK Limited on behalf of ECHA under Framework contract No ECHA/2008/02 between ECHA and AMEC Environment & Infrastructure UK Limited (AMEC). The work has been led by COWI, supported by IOM, BRE and AMEC. As part of the data collection, the consultant carried out a targeted stakeholder consultation (see Section 5).

3.2 Targeting and format of the report

ECHA documented the human health hazard assessment and exposure assessment according to part B of the formats for an Annex XV Restriction Report and Chemical Safety Report, though with some adaptations.

The focus of this review was the risk upon exposure with DINP and DIDP for adults and children of 0-6 months, 6-12 months and 12-18 months old. Thus the report is targeted to human health and to consumer exposure.

4. Information on hazard and risk

4.1 Identity of the substances and physical and chemical properties

4.1.1 Name and other identifiers of the substances

	IUPAC NAME	EC NUMBER	CAS NUMBER
DINP	1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich	271-090-9	68515-48-0
	di-"isononyl" phthalate	249-079-5	28553-12-0
DIDP	1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich	271-091-4	68515-49-1
	di-"isodecyl" phthalate	247-977-1	26761-40-0

Note that "iso" in the IUPAC name stands for 'a mixture of isomers' (EC 2003a).

Throughout the report DINP is used as a common name for both EC numbers, unless specified otherwise. Similarly, DIDP is used as a common name for both EC numbers, unless specified otherwise.

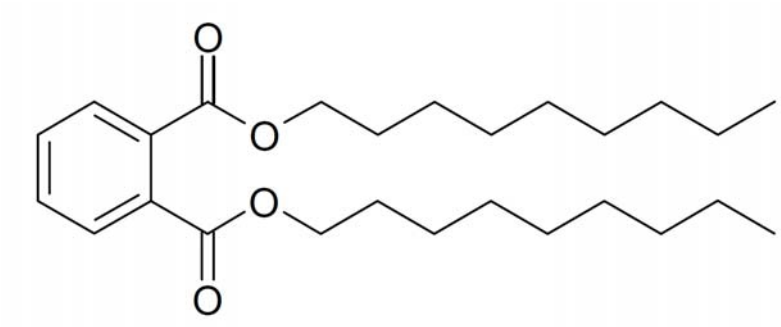
4.1.1.1 DINP

Two different types of DINP are currently on the market:

- DINP-1 (CAS 68515-48-0) is manufactured by the "Polygas" process.
- DINP-2 (CAS 28553-12-0) is n-butene based. (EC 2003a)

The production of a third form DINP-3 (also CAS 28553-12-0) has reportedly been discontinued (EC 2003a).

According to ECPI (2011d), DINP is composed of different alcohol chains depending on the production method. It is a manufactured substance made by esterifying phthalic anhydride and isononanol. Isononanol is composed of different branched C9 alcohol isomers. The two branches on the molecule R1 and R2 are not necessary identical, and are either mainly C_8H_{17} to $C_{10}H_{21}$ (DINP-1) or C_9H_{19} isomers (DINP-2).



R1 (variably branched chain, 9C)

R2 (variably branched chain, 9C)

Figure 4.1 Structure of DINP, CAS No 68515-48-0 (EC 2003a)

DINP-1 (CAS 68515-48-0) contains alcohol groups made from octene, by the "polygas" process (EC 2003a). At least 95 percent of these alcohol groups comprise roughly equal amounts of 3,4-, 3,5-, 3,6-, 4,5-, 4,6-, and 5,6-dimethyl heptan-1-ol (Hellwig et al. 1997 as cited in Babich and Osterhout 2010). DINP-1 is also known by the trade name Jayflex®.

DINP-2 (CAS 28553-12-0) contains alcohol groups made from n-butene, which results mainly in methyl octanols and dimethyl heptanols. DINP-2 is also known by the trade names Palatinol N® and Palatinol DN® (NLM 2009a). DINP-3 (also CAS 28553-12-0) contains alcohol groups made from n-butene and i-butene, resulting in 60 percent methylethyl hexanols. DINPs generally contain 70% or more nonyl alcohol moieties, with the remainder being octyl or decyl (Madison et al. 2000 as cited in Babich and Osterhout 2010).

Although their isomeric composition differs, the different types of DINP are considered to be commercially interchangeable. (Babich and Osterhout 2010)

4.1.1.2 DIDP

DIDP is a complex mixture containing mainly C10-branched isomers (EC 2003b). DIDP was marketed under two CAS numbers. No data on the differences between the types of DIDP has been identified and the EU Risk Assessment (EC 2003b) does not distinguish between the different forms (unlike the Risk Assessment for DINP).

4.1.2 Composition of the substances

4.1.2.1 DINP

The percent composition of the different chain structures of the two forms of DINP is shown in Table 4.1.

Table 4.1 Best estimate of content (%) of the different chain structures of the DINPs (EC 2003a)

	DINP-1	DINP-2
Methylethyl hexanols	5 - 10	5 - 10
Dimethyl heptanols	45 - 55	40 - 45
Methyl octanols	5 - 20	35 - 40
n-Nonanol	0 - 1	0 - 10
Isodecanol	15 - 25	--

4.1.2.2 DIDP

DIDP is a complex mixture containing mainly C10-branched isomers (EC 2003b). The correct structures can only be estimated. Based on nonene (CAS 97593-01-6) isomer distribution analysis and 1H-NMR analysis of isodecyl alcohol, the EU Risk Assessment provides an estimation of key isomeric structures of isodecylalcohol and hence of DIDP, as shown in Table 4.2. The lower ranges do not add up to 100% indicating that the substance may include other chain lengths.

Table 4.2 Best estimates of the different chemical structures of DIDP (EC 2003b)

Longest chain (estimates)	DIDP (CAS 68515-49-1 & CAS 26761-40-0)	Best estimated content (%)
C7	tri-methyl heptanols	0-10
C8	di-methyl octanols	70-80
C9	methyl nonanols	0-10
C10	n-decanol	0

4.1.3 Physicochemical properties

In Table 4.3 and Table 4.4, a summary of the physicochemical properties of DINP and DIDP is given. Note that according to the EU Risk Assessment for DINP (EC 2003a) wide ranges of values were reported, and that therefore, most of the physicochemical data cannot be used to identify the type of DINP being examined.

Table 4.3 Summary of physico-chemical properties of DINP. Taken from the EU Risk Assessment (EC 2003a) and registration dossiers

Property	Value
Melting point	ca. -50°C
Boiling point	> 400°C
Density	ca. 0.975 at 20°C
Vapour pressure	6x10-5 Pa at 20°C
Water solubility	0.6 µg/l at 20°C
Henry's law constant	41.4 Pa.m3/mol
Log Kow	8.8
Flash point	> 200°C
Autoflammability	ca. 380°C
Viscosity[*1]	CAS nr. 28553-12-0 77.6 mm^2/s at 20°C; 27.7 mm^2/s at 40°C CAS nr. 68515-48-0 93 mPa.s at 20 °C

[*1] Values used in the registration dossiers

Table 4.4 Summary of physico-chemical properties of DIDP. Taken from the EU Risk Assessment (EC 2003b)

Property	Value
Melting point	-53 to -39°C (av. -45°C)
Boiling point	> 400°C
Density	0.966 at 20°C
Vapour pressure	5.1x10-5 Pa at 25°C
Water solubility	0.2 µg/l at 20°C
Henry's law constant	114 Pa.m3/mol
Log Kow	8.8
Flash point	> 200°C
Autoflammability	ca. 380°C
Viscosity	ca. 130 mPa.s

4.2 Manufacture and uses

The information on manufacture and uses of DINP and DIDP was collected by Amec Environment & Infrastructure UK Limited, with COWI as subcontractor. For more details the full report can be consulted (COWI 2012).

It has to be noted that it is difficult to make projections for the future situation, since the exact effects of the authorisation requirements on DEHP, DBP, BBP and DIBP on the market for DINP and DIDP and on the import of articles are difficult to predict. In addition, the manufacture and use of DINP and DIDP and the import of articles might be further influenced, depending on the outcome of the restriction proposal on DEHP, DBP, BBP and DIBP that has been submitted by Denmark on 14 April 2011.

4.2.1 Manufacture, import and export

According to ECPI (2011c), about one million tonnes of phthalates are manufactured each year in Europe, of which approximately 93% are used to make polyvinyl chloride (PVC) soft and flexible (ECPI 2011e). ECPI (2011d) indicated that the total consumption of plasticisers in Western Europe is approximately one million tonnes. Calvin (2011) indicated that non-phthalate plasticisers accounted for approximately 16% of the plasticiser market in Western Europe in 2010 (see Table 4.5), and on this basis the consumption of phthalates would be approximately 840,000 tonnes. The difference between manufacturing and EU consumption is quite well in accordance with the data on external trade indicating a net export of C8 (mainly DEHP) and C9/C10 phthalates of approximately 230,000 t/year.

The three phthalates DINP, DIDP and DPHP account for the majority of the C9/C10 phthalates both at global and at an EU level. According to ECPI, the consumption of DINP, DIDP and DPHP (di-2-propylheptyl phthalate), has increased from representing about 50% of total phthalate sales in Europe in 2001 to approximately 83% of the total sales in 2010 (ECPI 2011c). If 83% of the manufacturing of phthalates (as is the case for consumption) is C9/C10 phthalates, the total manufacture of these phthalates corresponds to approximately 830,000 t/year. Figure 4.2 shows that a market shift from DEHP towards DINP, DIDP and DPHP has taken place in the past decade.

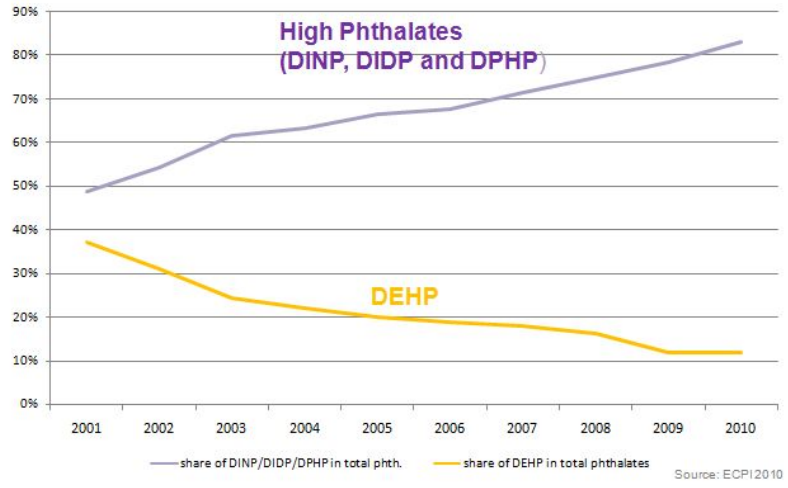


Figure 4.2 Percentage of phthalates sales in Europe compared to other plasticisers (ECPI 2011c)

The total use of plasticisers, including phthalates, has been steady to slightly declining within the EU during the last 10 years, driven by the increasing manufacture of PVC articles outside the EU. While on a global scale producers still foresee an increase in total manufacture and

consumption of plasticisers, consumption within the EU is likely to continue to be steady to slightly declining (ECPI and CEFIC as cited in ECHA 2010a).

Data on manufacturing of DINP and DIDP in the EU, import/export of the substances on their own and an estimate of the import/export of the substances in finished articles are summarised in Figure 4.3. The import/export of the substances on their own may be slightly overestimated as the data from the statistics include all C9/C10 phthalates.

The import/export within articles is quite uncertain, but the export in articles seems to be significantly higher than the imports.

No data on import/export in mixtures have been identified but, as mixtures account for less than 10% of the EU consumption for processing, the import/export in mixtures is considered to be small compared with the import/export in articles. They are thus not expected to have a major influence on the total balance.

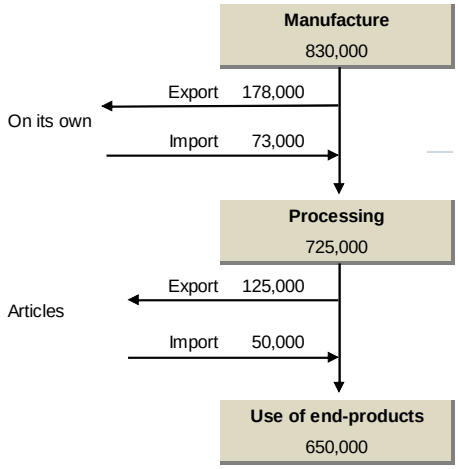


Figure 4.3 Schematic view of the approximate flow of DINP and DIDP in 2010 (COWI 2012)

4.2.2 DINP and DIDP in imported articles

DINP and DIDP may be imported and exported in a large number of articles. In general it is very difficult to obtain specific information on the plasticiser content of the imported articles.

The breakdown of the plasticiser market in Western Europe, USA and Asia is shown in Table 4.5, based on the most recent available estimate for 2010 (Calvin 2011). The total global market for phthalates was estimated at 6 million tonnes, with 1.4 million tonnes in Europe, the Middle East and Africa; 1.1 million tonnes in the Americas and 3.5 million tonnes in Asia (Calvin 2011). Phthalates represent 84% of the global plasticiser market (Calvin 2011).

If the percentages of total plasticiser market shown for the USA are used as a best estimate for the Americas and the percentages for Western Europe are used as best estimates for Europe, the Middle East and Africa, then DIDP and DINP (and other C9/C10 phthalates) should represent about 32% of the global plasticiser market and Asia should represent 39% of the global consumption of the substances. It has been reported that DINP/DIDP represent approximately 30% of the total global consumption of plasticisers (ECHA 2010a) and it is likely that, for countries in the region Europe, the Middle East and Africa outside Western Europe, DINP/DIDP represent a lower percentage than in Western Europe.

Table 4.5 World plasticiser market 2010 (Calvin 2011)

Plasticiser	Percentage of total plasticiser market *1		
	Western Europe	USA	Asia
DEHP	16	19	60
C9/C10 phthalates *2	63	33	21
Linears/other phthalates *3	6	19	9
Non phthalates	16	38	10
Total	100	100	100

*1 The data are indicated to be based on two market reports (SRI,CMAI) and BASF estimates.
*2 Note by COWI (2012): mainly DINP (C9) and DIDP (C10).
*3 Note by COWI (2012): "linears" are linear phthalates such as 911P 9-10-11 linear phthalate.

According to Bisig (2009), in 2008 DPHP/DIDP represented less than 1% of the plasticiser market in Asia, while DINP represented 18% of this market area. For Western Europe and the USA the consumption of DINP was approximately 25% higher than the DPHP/DIDP consumption (Bisig 2009).

For the estimate of import/export of DINP/DIDP in articles it is assumed that DINP/DIDP account for the following percentages of the total plasticiser consumption by region:

- EU, Switzerland, Norway, Iceland: 63%
- The Americas: 33%
- Asia and rest of the world: 21%

Of the import into the EU, 51% of the tonnage of the articles originates from China, whereas only 9% of the imported DINP/DIDP is estimated to originate from China.

Table 4.6 Estimated DINP/DIDP content of EU27-extra traded articles. Average of the years 2008-2010

Product group	Tonnage products t/y		Tonnage plasticiser t/y		Tonnage DINP/DIDP t/y	
	Import	Export	Import	Export	Import	Export
Hoses and profiles	21,572	38,727	3,515	7,501	1,263	4,437
Flooring and wall covering	127,187	231,592	10,569	29,830	2,396	18,993
Film/sheets and coated products	1,164,779	922,288	75,201	68,578	21,505	42,706
Coated fabric and other products from plastisol	283,151	695,235	3,426	5,986	927	3,749
Wires and cables	117,036	153,675	8,183	9,695	2,336	5,780
Moulded products and other	449,756	475,303	63,448	47,006	15,058	29,364
Sum	2,163,482	2,516,820	164,342	168,597	43,485	105,029
Overall total *[1]	2,596,178	3,020,184	197,210	202,316	52,182	126,035

*[1] Some import/export may take place with articles not covered by the assessment e.g. vehicles and electrical and electronic equipment, and the total tonnage imported in these articles are considered to add some 10-30% to the sum. As a best estimate, 20% was added to the sum.

4.2.3 Consumption of DINP and DIDP by use category

The EU risk assessment reports for DINP, DIDP and DEHP applied the same breakdown by application area for the three substances and estimated the consumption for each application area by multiplying the percentage of the total phthalates consumption for the area by the total consumption figures for each of the phthalates.

However, as a consequence of the different properties of DINP and DIDP, some differences in the use by application area are seen as discussed below.

About 95% of DINP is used in PVC applications. The other 5% is used in non-PVC applications such as rubbers, adhesives, sealants, paints and lacquers and lubricants (ECPI 2011d). For DIDP, non-PVC applications are reportedly relatively small, but include use in anti-corrosion and anti-fouling paints, sealing compounds and textile inks (ECPI 2011d).

According to TURI (2006), the price of DIDP in the USA is about 5% higher than the price of DINP. If the same is true in Europe it would be expected that DIDP is mainly used in applications where the substance has some technical advantages compared to DINP. For applications where either DINP or DIDP could be applied (as is often the case) it could be expected that the least expensive of the substances would be used. However, there are other considerations taken into account than simply the price of the substance: e.g., DIDP may have greater weight costs but because of the slightly lower density of the volume cost of the resulting product might be lower (INEOS ChlorVinyls 2012).

The low vapour pressure of DIDP and its higher permanency makes it the preferred plasticizer for applications such as wire and cable formulations where heat aging resistance is required and in areas where emissions of volatile components into the atmosphere during processing is subject to restriction or where good outdoor weathering resistance is required (BASF 2001;

ExxonMobil 2001). Furthermore, DIDP has good resistance to extraction by soapy water (BASF 2001).

In accordance with this, ECPI (2011d) indicated that, due to DIDP's properties of volatility resistance, heat stability and electric insulation, it is typically used as a plasticiser for heat-resistant electrical cords, leather for car interiors, and PVC flooring. DIDP is preferentially used in car interior trims meeting the low fogging thresholds set by car manufacturers, which are usually not met by using DINP or low molecular weight phthalates (ECPI as cited in ECHA 2010b). Høibye et al. (2011) reported that, according to a major manufacturer of cables and wires, DIDP represents 80% of the phthalates used for this application area. Besides the uses in cables, Industry indicated that DIDP is preferably used in extruded and calendered articles (such as profiles, roofing sheets, ponds liners, etc.); however, similarly to DINP, DIDP can also be blended into a paste (plastisol) that is used for coating (production of tarpaulins, synthetic leather, flooring, wall covering, etc.) and rotational moulding (production of certain toys and sporting articles) (ECPI as cited in ECHA 2010a,b).

DPHP is often used as an alternative to DIDP because only minor compound changes are needed to adapt for example wire formulations to DPHP (ECPI 2011d). It similarly matches DIDP performance in automotive applications. Due to its low volatility, DPHP is suitable for higher temperature applications such as wire and cable and automotive interior trim. Its weather resistance makes it a strong candidate for outdoor applications. DPHP boasts better UV stability than most general purpose plasticisers, making it especially suitable for applications like roofing, geomembranes, or tarpaulins.

The total consumption of phthalates by PVC applications area in 1994 is shown in the Table 4.7 below.

Table 4.7 PVC end-use breakdown for all phthalates in 1994 (based on EC 2003b)

Process	Application area	Percentage of total phthalate use for PVC in 1994
Calendering	Film, sheet and coated products	15.7
	Flooring, roofing, wall covering	3.5
Extrusion	Hose and profile	5.3
	Wire and cable	28.7
	Clear, medical, film	7.1
Injection moulding	Footwear and miscellaneous	8.3
Plastisol spread coating	Flooring	10.5
	General (coated fabric, wall covering, etc.)	11.4
Other plastisol applications	Car undercoating and sealants	7.6
	Slush/rotational moulding etc.	1.9

ECPI (2011c) provided an overview on its website of the current use of plasticisers in both PVC and non-PVC applications (Table 4.8). In this breakdown the uses are divided into slightly different groups than used for the 1994 breakdown, but the overall breakdown is not significantly different. For one of the groups included in both breakdowns - wire and cable - the percentage has decreased from 28.7% to 25%, but this difference is considered to be within typical levels of uncertainty for such data.

Table 4.8 Current uses of plasticisers in Europe (ECPI 2011c)

Application are	Percent of total consumption
Wire and cable	25
Extrusions	11
Film and sheet	22
Floor covering	14
Coated fabric	10
Plastisols	9
Other	9
Total	100

Trends
An attempt has been made to project the use of DINP and DIDP in the EU for the year 2015

The consumption of the C9/C10 phthalates (DINP, DIDP and DPHP) in the EU in 2010 is estimated at approximately 670,000 tonnes. DINP and DIDP are assumed to represent the majority, with DPHP accounting for a minor part. If the market trend from the last decade continues over the coming years (disregarding effects from authorisation and possible additional restrictions on DEHP, DBP, BBP and DIBP), the C9/C10 phthalates would represent 100% of the phthalate consumption by 2015. It is however more likely that the trend lines would level off and DEHP would still be used for some applications. The trend will also depend on possible further restrictions on DEHP, DBP, BBP and DIBP as recently proposed by Denmark (ECHA 2011) and on the effects of the authorisation requirements on those substances.

For the year 2015, the consumption of DINP, DIDP and DPHP is assumed to be at some 850,000 tonnes of which 95% is assumed to be used for manufacture of PVC. It will be assumed that the ratio of DINP to DIDP/DPHP is 32:23 (as indicated above for the use in Western Europe in 2010). No data on DPHP consumption are available, but it will here be roughly assumed that DIDP in 2015 would account for 2/3 of the total DIDP/DPHP consumption.

The breakdown of different application areas for the two compounds has been roughly estimated considering the available information on the differences in applications of the substances (Table 4.9). In practice, a new breakdown has been established for DIDP with relatively high percentages for cables and calendering applications. The breakdown for DINP has subsequently been adjusted on the basis of the breakdown for DIDP+DINP and the assumed breakdown for DIDP.

The breakdowns are considered to be “best estimate scenarios” for modelling purposes, but it is not possible to judge how well the estimates reflect the actual situation in Europe.

If the data are combined with the data on import and export of the substances in articles in Table 4.6 (indicating an export of 105,000 tonnes per year in 2008-2010) it can be estimated that export of the substances in articles accounts for 10-20% of the production in the EU.

Table 4.9 Scenario for the breakdown of the use of DINP and DIDP by application area in 2015

Process	Application area	DINP +DIDP		DINP		DIDP	
		Percentage of total	**Consumption (tonnes)**	**Percentage of total**	**Consumption (tonnes)**	**Percentage of total**	**Consumption (tonnes)**
Calendering	Film, sheet and coated products	14.9	109,178	11.5	57,018	22.0	52,140
	Flooring, roofing, wall covering	3.3	24,339	1.6	7,739	7.0	16,590
Extrusion	Hose and profile	5.0	36,856	5.1	25,006	5.0	11,850
	Wire and cable	27.3	199,580	17.3	85,761	48.0	113,760
	Clear, medical, film	6.7	49,373	8.1	39,901	4.0	9,480
Injection moulding	Footwear and miscellaneous	7.9	57,718	9.7	48,249	4.0	9,480
Plastisol spread coating	Flooring	10.0	73,017	13.8	68,299	2.0	4,740
	General (coated fabric, wall covering, etc.)	10.8	79,276	15.5	76,933	1.0	2,370
Other plastisol applications	Car undercoating and sealants	7.2	52,850	10.2	50,498	1.0	2,370
	Slush/rotational moulding etc.	1.8	13,213	2.2	10,845	1.0	2,370
Mixture formulation	Non-PVC applications	5.0	36,600	5.0	24,750	5.0	11,850
Total		100.0	732,000	100	495,000	100	237,000

Note: The values above have been calculated without rounding. The fact that the figures are calculated to the nearest tonne does not mean that they should be interpreted as precise to the nearest tonne.

4.2.4 Concentration of DINP and DIDP in articles

According to information from ECPI, the typical content of DIDP in flexible PVC products is between 25 and 50% (w/w) (ECPI 2011d).

RPA (2000) indicated that in a typical flexible PVC the plasticiser is used at 60 phr[10] (60 parts plasticiser to 100 parts PVC resin) resulting in a typical concentration of approximately 30% (60/200) in the final flexible PVC material considering that fillers (mainly CaCO3) and other additives are typically used at a total of 40 phr. ECPI has for the current report confirmed that these estimates are correct (ECPI 2011f).

Turi (2006) provides the following typical formulations for cable sheathing in the USA: PVC resin (100 parts per hundred resin (phr)), DIDP (55-60 phr), CaCO3 (50 phr), stearic acid (0.25 phr), calcium/zinc stabiliser (4-5 phr) and epoxidised soybean oil (0-5 phr). This corresponds to a DIDP concentration of the final PVC material of approximately 27% (w/w).

Actual analyses of plasticisers in different products demonstrate that, for the same product, often different combinations of plasticisers are found. There are two principal reasons for this:

1) The combination of plasticisers in a PVC material is partly governed by the desired performance characteristics of the plasticised material and partly by the desired process parameters for the manufacturing of the PVC materials. Several plasticisers can be used when a "specialty" plasticiser is used to impart a special property, e.g. cold flexibility or fast fusing (ECPI 2011f). Normally, however, the reason to add general purpose phthalate plasticiser is cost competitiveness: expensive plasticisers will only be used in quantities sufficient to impart the desired technical effect (INEOS ChlorVinyls 2012).
2) Many PVC articles are made up of several layers (e.g., flooring, tarpaulins and synthetic leather) (INEOS ChlorVinyls 2012).

General purpose plasticisers are typically used on their own. A possible explanation for the fact that small concentrations of general purpose plasticisers are reported for products where another general purpose plasticiser is used is that DINP and DIDP have some small amounts of overlapping isomers (phthalate homologues) (ECPI 2011f).

4.2.5 Uses advised against by the registrants

DINP and DIDP shall not be used as substances or in mixtures, in concentrations greater than 0.1 % by weight of the plasticised material, in toys and childcare articles which can be placed in the mouth by children (entry 52 of Annex XVII to REACH).

[10] The unit phr = parts per hundred resin

4.3 Classification and labelling

4.3.1 Classification and labelling in Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation)

There is no harmonised classification for DINP and DIDP according to Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation).

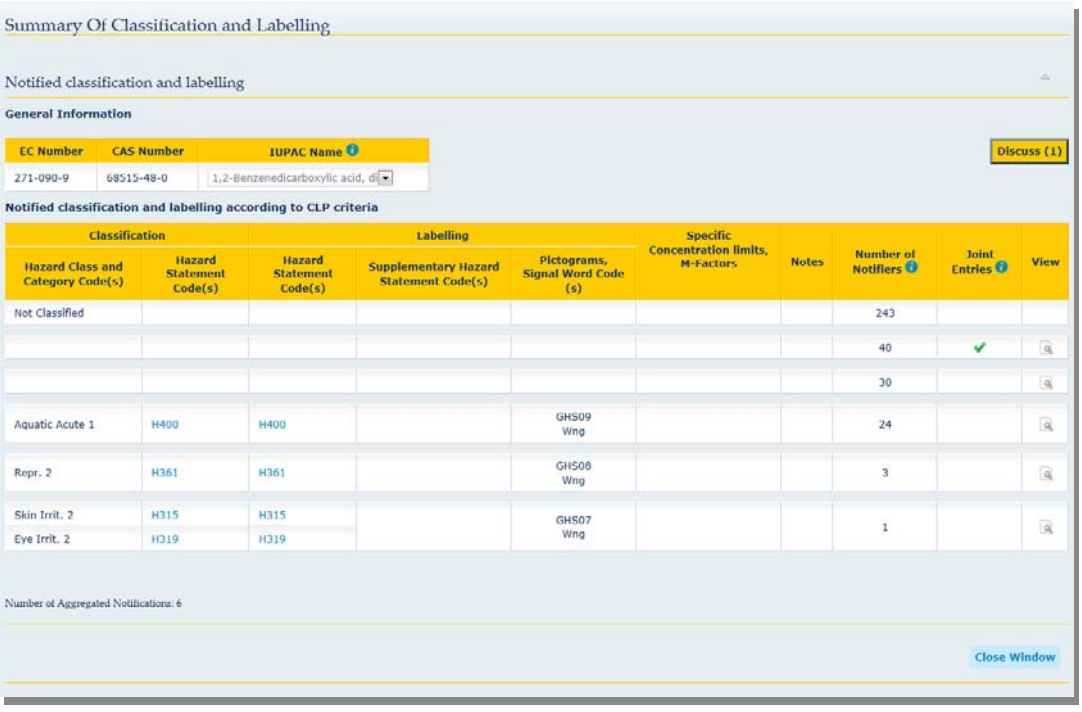
The EU Risk Assessments for DINP and DIDP (EC 2003a,b) had concluded that no classification was required for any endpoint. The Commission Working Group on the Classification and Labelling of Dangerous Substances had agreed not to classify DINP or DIDP for any endpoint (ECB 2000).

4.3.2 Classification and labelling in classification and labelling inventory/Industry's self classification(s) and labelling

The registration dossiers for DINP and DIDP conclude for each endpoint that the classification criteria are not met.

The screenshots below give an overview of the C&L notifications as retrieved on 27 June 2013 from the C&L Inventory database available from ECHA's website.

DINP



Summary Of Classification and Labelling

Notified classification and labelling

General Information

EC Number	CAS Number	IUPAC Name
271-090-9	68515-48-0	1,2-Benzenedicarboxylic acid, di

Discuss (1)

Notified classification and labelling according to CLP criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers	Joint Entries	View
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code (s)					
Not Classified							243		
							40	✔	
							30		
Aquatic Acute 1	H400	H400		GHS09 Wng			24		
Repr. 2	H361	H361		GHS08 Wng			3		
Skin Irrit. 2	H315	H315		GHS07 Wng			1		
Eye Irrit. 2	H319	H319							

Number of Aggregated Notifications: 6

Close Window

Summary Of Classification and Labelling

Notified classification and labelling

General Information

EC Number	CAS Number	IUPAC Name
249-079-5	28553-12-0	bis(7-methyloctyl) phthalate

Discuss (0)

Notified classification and labelling according to CLP criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers	Joint Entries	View
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code (s)					
Not Classified							794	✔	
							30		
Aquatic Chronic 4	H413	H413					28		
Aquatic Acute 1 Aquatic Chronic 1	H400 H410	H410		GHS09 Wng			23		
		H400		GHS09 Wng			1		
Acute Tox. 4 Aquatic Acute 1	H332 H400	H332 H400		GHS07 GHS09 Wng			1		
							1		

Number of Aggregated Notifications: 7

Close Window

DIDP

Summary Of Classification and Labelling

Notified classification and labelling

General Information

EC Number	CAS Number	IUPAC Name
271-091-4	68515-49-1	1,2-Benzenedicarboxylic acid, di

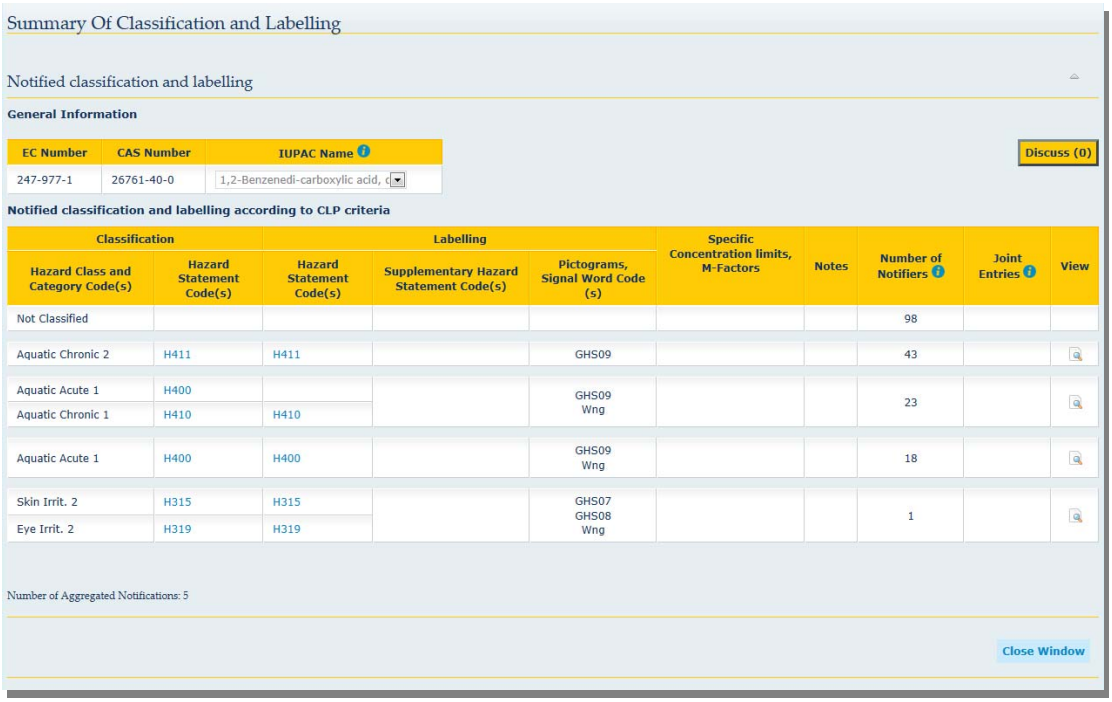
Discuss (0)

Notified classification and labelling according to CLP criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers	Joint Entries	View
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)					
Not Classified							381	✔	
Skin Irrit. 2 Eye Irrit. 2	H315 H319	H315 H319		GHS07 Wng			25		
Eye Irrit. 2	H319	H319		GHS07 Wng			7		

Number of Aggregated Notifications: 3

Close Window



Summary Of Classification and Labelling

Notified classification and labelling

General Information

EC Number	CAS Number	IUPAC Name
247-977-1	26761-40-0	1,2-Benzenedi-carboxylic acid, c

Discuss (0)

Notified classification and labelling according to CLP criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers	Joint Entries	View
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code (s)					
Not Classified							98		
Aquatic Chronic 2	H411	H411		GHS09			43		
Aquatic Acute 1	H400			GHS09 Wng			23		
Aquatic Chronic 1	H410	H410							
Aquatic Acute 1	H400	H400		GHS09 Wng			18		
Skin Irrit. 2	H315	H315		GHS07 GHS08 Wng			1		
Eye Irrit. 2	H319	H319							

Number of Aggregated Notifications: 5

Close Window

4.4 Human health hazard assessment

As stated in section 4.1.1, DINP is used throughout the report as a common name for two different substances: 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich (EC number 271-090-9, CAS 68515-48-0) and di-"isononyl" phthalate (EC number 249-079-5, CAS 28553-12-0).

Both forms of DINP are multi-constituent substances. The differences in toxicology between both substances appear to be small, although only a few studies are available that directly compare the toxicity between the two substances (US CPSC 2010a). The reference of the test compounds in studies has not always been clear: the sample was referred to as "DINP" or with a code name (EC 2003a). In this respect it can be mentioned that there was a second DINP-form with the same CAS number (CAS 28553-12-0), which further complicates a separate assessment. Manufacturing of this form has ceased in 1995 (EC 2003a and US CPSC 2010a). Also results are available from testing with CAS number 71549-78-5, which is believed to be similar to DINP with CAS number 28553-12-0 but was never produced commercially (US CPSC 2010a).

Similarly, DIDP is used throughout the report as a common name for two different substances: 1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich (EC number 271-091-4) and di-"isodecyl" phthalate (EC number 247-977-1).

Several committees, international bodies as well as industry stakeholders have assessed the human health hazards of DINP and DIDP. Relevant findings and conclusions (if any) from the available assessments are reported in each of the endpoint sections in this chapter. In particular the following reports have been taken into account:

Regulatory bodies

Europe

- The EU Risk Assessments on DINP and DIDP (EC 2003a,b)
- The ECHA first phase review reports on DINP and DIDP (ECHA 2010a,b)
- The opinions on the results of the EU Risk Assessment for DINP and DIDP by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE 2001a,b)
- The opinions from the European Food Safety Authority on DINP and DIDP for use in food contact materials (EFSA 2005a,b)
- The opinion of the Scientific Committee on Consumer Products on phthalates in cosmetic products (SCCP 2007)
- The opinion of the Scientific Committee on Health and Environmental Risks (SCHER) on phthalates in school supplies (SCHER 2008)
- The opinion of the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) on the safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk (SCENIHR 2008)

United States

- The report on DINP from the Chronic Hazard Advisory Panel (CHAP) of the United States Consumer Product Safety Commission (US CPSC) (CHAP 2001)
- The Monograph on potential human reproductive and developmental effects of DINP and DIDP by the Centre For The Evaluation Of Risks To

Human Reproduction (CERHR) of the US National Toxicology Program (NTP-CERHR 2003a,b)
- The Revised Technical Review of DINP by the United States Environmental Protection Agency (US EPA 2005)
- The United States Consumer Product Safety Commission (US CPSC) staff assessments of DINP and DIDP (US CPSC 2010a,b)

Stakeholders

Europe
- The Review of Recent Scientific Data on Di-isononyl Phthalate (DINP) and Risk Characterisation for its use in Toys and Childcare articles by the European Council for Plasticisers and Intermediates (ECPI 2009)
- The Endocrine Data Evaluation Report for the phthalates DINP, DIDP and DBP by the European Council for Plasticisers and Intermediates (ECPI 2011a)
- The "Statement relevant to the re-evaluation of DIDP in toys and childcare articles as required by Directive 2005/84/EC" by ExxonMobil (ExxonMobil 2011c)

United States
- The American Chemistry Council Phthalate Esters Panel's comments to EPA's Revised Technical Review of Diisononyl Phthalate (2005)
- The ExxonMobil Chemical Company (ExxonMobil) information submitted to the Chronic Hazard Advisory Panel on Phthalates (CHAP) (ExxonMobil 2011a)

4.4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.4.1.1 DINP

4.4.1.1.1 EU Risk Assessment conclusion

The following cites the 'Summary of toxicokinetics, metabolism and distribution' from the EU Risk Assessment:

"Via GIT, absorption of DINP decreases as dose increases (49% at the low dose of 50 mg/kg and 39% at the high dose of 500 mg/kg eliminated in urine) leading to an estimated absorption of at least 50%. In addition, absorption of the substance seems to be of saturable mechanism, with increasing dose an increasing amount of unabsorbed compound is eliminated (fecal radioactivity associated with parent compound increased from 8% to 41% from the single low to the high dose).

Dermal absorption is very low in rats, most of the unabsorbed dose remained at the skin area at day 7. The maximum percentage of the applied substance being absorbed in 7 days is less than 4%. In humans skin absorption is still lower than in rat as indicated by in vitro comparative studies, when SSARs (steady state absorption rates) were compared (Mint and Hotchkiss, 1993).

Via inhalation, a bioavailability of 75% may be assumed by analogy with DIDP.

In tissues, DINP is mainly recovered in GIT, liver and kidney by oral route whereas following dermal exposure, liver, muscle and adipose tissue contain most of the dose remaining in the body.

DINP metabolites were excreted in urine and to a lesser extent in feces. DINP was de-esterified to the monoester which was further metabolised by side-chain oxidation of the ester group or by hydrolysis to phthalic acid; the formation of oxidation products appeared to increase following the high dose, while hydrolysis to phthalic acid decreased. Repeated dosing caused no accumulation of DINP and/or its metabolites in blood and tissue, but

resulted in increased formation and elimination of the monoester-oxidation products. Small amounts of the major metabolites were also recovered in the testes and fat.

DINP is rapidly eliminated, less than 0.1% of the radioactivity was recovered in tissues after 72 hours. By oral and dermal routes, excretion is shared between urine and faeces. By dermal exposure, biliary excretion is shown.

A transfer through the milk at a low level may be anticipated by analogy with DIDP, for which cross fostering and switch diet studies were conducted in the two-generation study (Exxon, 1997).” (EC 2003a)

4.4.1.1.2 Risk assessments from other international organizations and bodies

CSTEE (2001a) stated the following in its opinion on the results of the Risk Assessment for DINP: “*Bioavailability of DINP in young animals has not been studied. An adjustment factor of 2* [i.e. 100% absorption] *has been used in the RAR for bioavailability of DINP in children 0.5-3 years of age using information from a study with DEHP in rats (Sjöberg et al. 1985). These authors reported a significantly higher AUC, but not Cmax, for MEHP in 25-day old animals compared to 40- and 60-day old animals. However, this experiment was performed at a very high dose of DEHP (1000 mg/kg/d), a dose at which DEHP hydrolysis appears to become saturated in rats. Thus, it is difficult to support the use of the bioavailability adjustment factor of 2 based on the Sjöberg et al. (1985) study.*”. Despite this comment, 100% bioavailability was also assumed by the CSTEE for calculation of oral exposure in children.

The US EPA (2005), US CPSC (2010) and ECPI (2009) descriptions of the toxicokinetics are in line with the EU Risk Assessment (EC 2003a).

4.4.1.1.3 New studies/description key studies

The above summary of toxicokinetics from the EU Risk Assessment (EC 2003a) is based on experimental studies in animals. The metabolism of DINP was not yet investigated in humans at that time.

Humans
Koch and Angerer (2007) described elimination of major DINP metabolites via urine in a study where one human volunteer was dosed 1.27 mg/kg bw (n = 1). The results indicate that as in animals, DINP is fairly rapidly distributed and eliminated in humans. A recovery of 43.6% of the custom synthesised DINP-2[11] was calculated in urine measurements during 48h of four metabolite ‘groups’ of structural isomers[12]. Only 2.2% was recovered as the simple monoester (MiNP), whilst the majority consisted of oxidized isomers (20.2% MHiNP, 10.7% MCiOP, and 10.6% MOiNP –see Table 4.83 for abbreviations). Other possible metabolites (with two or more functional groups or shortened side chains) were not measured. The recovered percentage is thus likely an underestimation of the actual elimination of DINP via urine (Koch and Angerer 2007).

Anderson et al. (2011) studied the kinetics of DINP and DEHP in 10 male and 10 female human volunteers (n = 20). Two dose levels were used of the deuterium labelled DINP and DEHP, which were for DINP 0.78 mg (0.010 mg/kg bw for

[11] CAS No 28553-12-0
[12] Each ‘metabolite’ in Koch and Angerer (2007) is actually a series of structural isomers with the same functional group. Due to the variety of side chains in DINP and the possible oxidations (ω- , ω-1- and β-oxidation) the actual number of oxidised monoester metabolites of DINP can be estimated to be above 100 (Koch et al. 2007).

males and 0.011 mg/kg bw for females) and 7.3 mg (0.090 mg/kg bw for males and 0.107 mg/kg bw for females). A recovery of 32.9 ± 6.4% of the labelled DINP was calculated in urine measurements during 48 h of four metabolites (the same metabolites as in Koch and Angerer). Metabolite half-lives were estimated to be 4-8 h with over 90% excreted in the first 24 h of urine collection. The reported values are similar as in the study by Koch and Angerer (2007), however generally lower. As the authors pointed out, exposure estimates from biomonitoring studies that back-calculated using the Koch and Angerer (2007) estimates lead to slightly lower exposure estimates than when the data from Anderson et al. 2011 is used. Anderson et al. (2011) warned that the results were obtained from Caucasian adults and that caution is to be taken when applying them to other races or to children.

In Silva et al. (2006b) the monoester MiNP was not detected in any of the urine samples of 129 human adults, whereas the oxidative metabolites MHiNP, MCiOP, and MOiNP were detected in nearly all. These findings indicate that the oxidative metabolites are more suitable biomarkers of exposure to DINP than the monoester MiNP.

Table 4.10 Summary of fractional renal excretion (mole basis) of applied dose of deuterium labelled DINP

Metabolite	Percentage of applied dose DINP by time period after application			
	Koch and Angerer (2007) *[1]		Anderson et al. (2011) *[2]	
	0-24 h	0 - 48 h	0-24 h	0 - 48 h
MiNP	2.1	2.2	3.0	3.1
MHiNP	18.4	20.2	11.4	12.3
MOiNP	10.0	10.6	6.3	6.7
MCiOP	9.1	10.7	9.9	10.9
Total	39.6	43.6	30.5	32.9

*[1] The deuterium labelled DINP represented the deuterium analogue to CAS No 28553-12-0 consisting of C9 branched alkyl chains only and in the literature referred to as DINP-2.
*[2] The CAS No of the analogue to the deuterium labelled DINP is not indicated. It is indicated that the deuterium labelled DINP was obtained from BASF, and the analytical standard used D_4-MiNP was said to represent the deuterium analogue of the commercial product Palatinol® N from BASF with CAS No. 28553-12-0. So it is likely that the D_4-DINP was also analogue to the DINP-2 from BASF, CAS No 28553-12-0. However the production process might be different from the industrial process, and the products were said to be purified by silica gel column chromatography.

Rats
Hazleton (1972 as cited in EC 2003a) administered about 2500 mg DINP/kg bw/day over 6 days to albino rats (4 treated, 2 controls). The rats received 5 days 'cold' compound and on the sixth day 'hot' compound by gavage. The amount excreted radioactivity in urine ranged from 8-18%. Considering the high dose and high level of radioactivity recovered in feces, the absorption process was probably saturated (EC 2003a).

Midwest Research Institute (1983 as cited in EC 2003a), also cited as McKee et al. (2002), treated Fischer 344 rats by gavage with a single radioactive dose of 50 and of 500 mg/kg, with recoveries in urine of 49% and 43% respectively (after normalizing to 100% total recovery, which was 99 and 91% at 50 and 500

mg/kg, respectively). In a repeated dose study over 5 days with 50, 150 and 500 mg/kg (gavage), recoveries in urine were 52, 60 and 55 % respectively (after normalizing to 100% total recovery, which was 123, 117 and 115% at 50, 150 and 500 mg DINP/kg bw, respectively). It should be noted that the first four doses were also 'hot' compound which makes the significance of comparison to the administered last dose unclear, even after normalising the results.

From three dermal experiments in rats by Midwest Research Institute (1983), the absorbed dose ranged from 2-4% of the applied dose of DINP (the total recovery was 97-103%). In the preliminary studies the amount dermally absorbed ranged from 4-7%. The data for the interim sacrifices on days 1, 3 and 7 in the three Midwest Research Institute (1983) dermal studies were not reported in EC (2003a) and McKee et al. (2002). Two of the studies used 6 animals with 2 animals sacrificed after dosing on days 1, 3 and 7, leaving 2 animals that were exposed throughout the experiment. In the 3rd experiment 3 animals were used and one animal was exposed throughout the entire experiment. Recovery of radioactivity in feces in the dermal experiments implies excretion in the bile.

Silva et al. (2006a) identified MCiOP as the major metabolite in urine upon a single oral dosing (300 mg/kg) of rats with the two commercial DINPs. Also MHiNP and MOiNP were present. MiNP was only present in low concentrations. The amounts of the individual metabolites differed with the type of commercial DINP dosed, but the same metabolites were identified.

ExxonMobil (2011d) has provided ECHA with summaries of two new studies (study #1 and study #2) conducted at the Hamner Institutes for Health Sciences. Study #1 studied male developmental effects upon foetal exposure to DINP, as well as toxicokinetics. For the results of the study concerning effects on male development, see section 4.6.8.

Once daily, pregnant Spargue-Dawley rats were administered 0, 50, 250, or 750 mg/kg/day DINP via oral gavage on gestation day 12-19. Treated animals were euthanized at 0.5, 1, 2, 6, 12, and 24 hrs after the final (GD 19) dose for measurement of metabolites. The concentrations of MiNP, MCiOP, MHiNP, MOiNP and MiNP-G (monoisononyl phthalate glucuronide conjugation) were measured in maternal serum, liver, placenta, urine and foetal plasma, testes and amniotic fluid. Control animals were sacrificed at 2 and 24 hrs. Cumulative maternal urine was collected at 7 and 24 hrs in dams after the final dose for metabolite analysis.

MCiOP was present at the highest concentration in plasma and tissues, followed in decreasing order by MiNP, MHiNP, MOiNP, and MiNP-G. The half-life for MiNP in maternal plasma was 4hrs at all doses, and 4.5-4.7 hrs in the foetus.

Of the respective doses (50, 250 and 750 mg/kg/day), 54, 47 and 22% was excreted in urine within 24 hrs after the last dose. MCiOP was the major metabolite recovered in urine (76-81%), followed by MHiNP (15-20%) and MOiNP (4%). MiNP and MiNP-G accounted for less than 1 % of the recovered urinary metabolites.

When maternal and foetal kinetic data were evaluated using a PBPK model using DEHP parameters, it provided reasonable predictions of the MiNP plasma data as well as the urinary metabolites data. This indicates that the ADME[13] of DINP is very similar to DEHP.

[13] ADME = absorption, distribution, metabolism and excretion (or elemination).

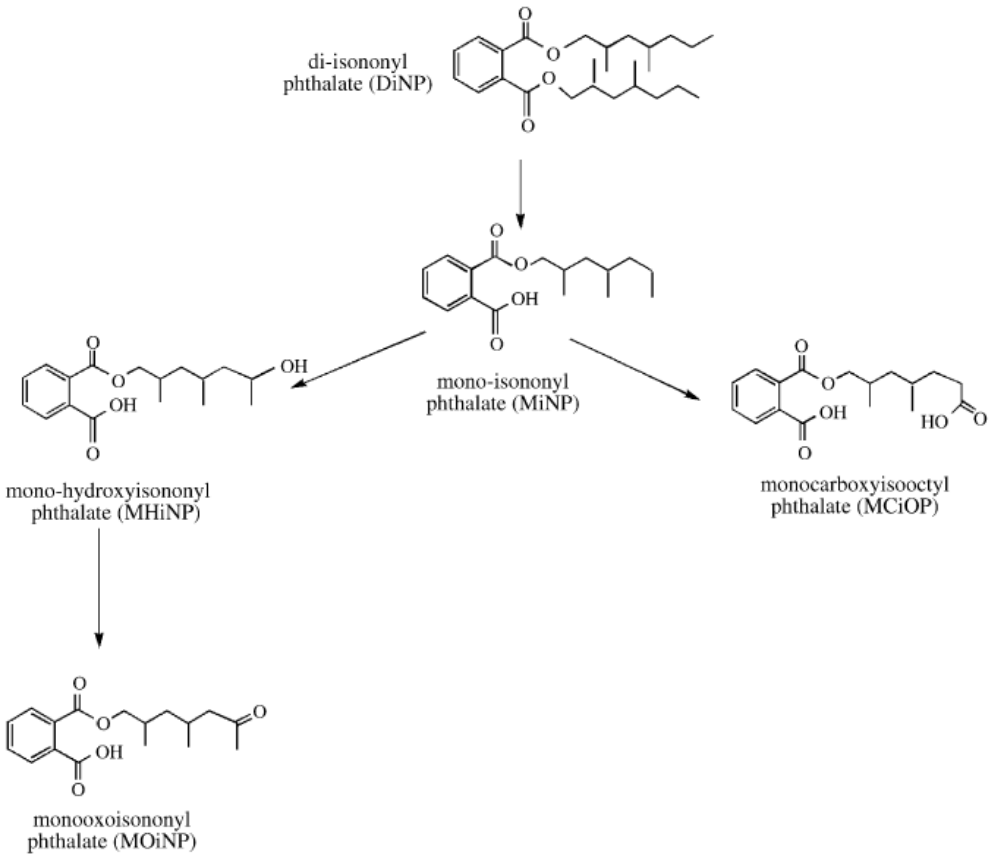


Figure 4.4 Suggested mechanism of DiNP metabolism in rats. The figure shows only one isomer from each metabolite class (from Silva et al. 2006a, used with permission).

4.4.1.2 DIDP

4.4.1.2.1 EU Risk Assessment conclusion

The following cites the 'Summary of toxicokinetics, metabolism and distribution' from the EU Risk Assessment:

"Via GIT, absorption of DIDP decreases as dose increases (56% at the low dose of 0.1 mg/kg, 46% at the mid dose of 11.2 mg/kg and 17% at the high dose of 1,000 mg/kg) and seems to be of saturable mechanism, with increasing dose an increasing amount of unabsorbed compound is eliminated (faecal radioactivity associated with parent compound was increased by a factor two between 0.1 and 1,000 mg/kg).

Via dermal route, absorption is very low (most of the unabsorbed dose remained at the skin area at day 7). DIDP showed a very slow excretion, reflecting a slow dermal uptake process: a possible cutaneous tank may be hypothesised, leading to a progressive systemic release, as indicated by the increased amount of radioactivity eliminated in faeces from day 1 to day 7 (Elsisi et al. 1989). The maximum percentage of absorption may be estimated 4% of applied dose in 7 days by analogy with DINP (Midwest Research Institute, 1983). In humans, skin absorption is still lower than in rat as indicated by in vitro comparative studies, when SSARs (steady state absorption rate) were compared (Mint and Hotchkiss, 1993).

Inhaled DIDP aerosol seems readily absorbed. It can be assumed that a part of insoluble particles are cleared from the nasopharyngeal region and swallowed. In the same way, in the tracheobronchial tree the mucociliary transport system leads deposited particles

upward to the oropharynx where they are swallowed and pass through the GI tract. Thus for the risk characterisation, a 100% absorption may be overestimated and a 75% bioavailability seems realistic.

In tissues, DIDP is mainly recovered in GIT, liver, kidneys, by oral or inhalation route, whereas following dermal exposure, muscle and adipose tissue contain most of the dose remaining in the body. Following inhalation, DIDP content in fat tissue is very low, but remains constant from the end of exposure to the end of the observation period (72 hours).

No parent DIDP or monoisodecyl phthalate (MIDP) but only metabolites (the oxidative monoester derivative and phthalic acid) are excreted in urine. In bile, DIDP was not detected in extracts 24 and 72 hours following dosing. The data on end products suggest a cleavage to the monoester and an alcohol moiety, indicating a metabolic scheme comparable to the one reported for DEHP. In feces the monoester oxidative derivative, MIDP as well as DIDP were detected. It is noticeable that metabolic pathway leading to phthalic acid is saturable, and that consequently monoester elimination is increased.

DIDP is rapidly eliminated and not accumulated in tissues, less than 1% of the radioactivity was recovered in tissues after 72 hours. By oral and inhalation routes, excretion is shared between urine and faeces. By dermal exposure, only faecal elimination was indicated, but considering the low rate of recovery and by analogy with the two other routes and with the DINP behaviour, the same scheme may be anticipated. In addition, results from the two-generation study suggest a possible transfer of DIDP through the milk when dams are exposed by oral route." (EC 2003b)

4.4.1.2.2 Risk assessments from other international organizations and bodies

None of the assessments done by other organisations and bodies present different views or additional relevant information to that of the EU Risk Assessment (EC 2003b) or that presented below under 'New studies'.

4.4.1.2.3 New studies/description key studies

The above summary of toxicokinetics from the EU Risk Assessment (EC 2003b) is based on experimental studies in animals. The metabolism of DIDP has so far not been investigated experimentally in humans, but data from measurements of metabolites in human urine are available.

Humans
Silva et al. (2007a) did not detect the monoester of DIDP (MiDP) in any of the samples in a study with 129 human adults, whereas MCiNP, MHiDP and MOiDP were detected in nearly all of the samples tested. Similar to DINP, these findings indicate that the oxidative metabolites are more suitable biomarkers of exposure to DIDP than the monoester MiDP.

Rats
From a gavage study with radiolabeled DIDP in Sprague-Dawley rats (General Motors Research Laboratories 1983 as cited in EC 2003b), the total absorbed dose was roughly estimated to be 55.6% after 0.1 mg/kg (including 14.3% from bile), 45.9% after 11.2 mg/kg (including 13.8% from bile) and 17.3% after 1000 mg/kg (including 4.7% from bile). This seems to indicate absorption is saturable. The recovered radioactivity from urine and feces was >99%.

Kato et al. (2007) observed rapid clearance of DIDP metabolites in a study with single oral dosing of rats with 300 mg/kg DIDP[14]. The concentration of the major metabolite MCiNP (not to be confused with MCiDP which was detected, but in much lower concentrations) decreased by 90.3% from 24 to 48 h after dosing. The half-life of all metabolites was estimated to be around 14 h. Similarly to DINP, the monoester MiDP was only detected as a minor metabolite in urine. Based on studies with rats and results from human biomonitoring data, the authors identified MCiNP, MHiDP and MOiDP as suitable biomarkers for biomonitoring studies. Detection of oxidative metabolites of diisoundecyl phthalate (DiUdP) and DINP suggested the presence of DiUdP and DINP in DIDP.

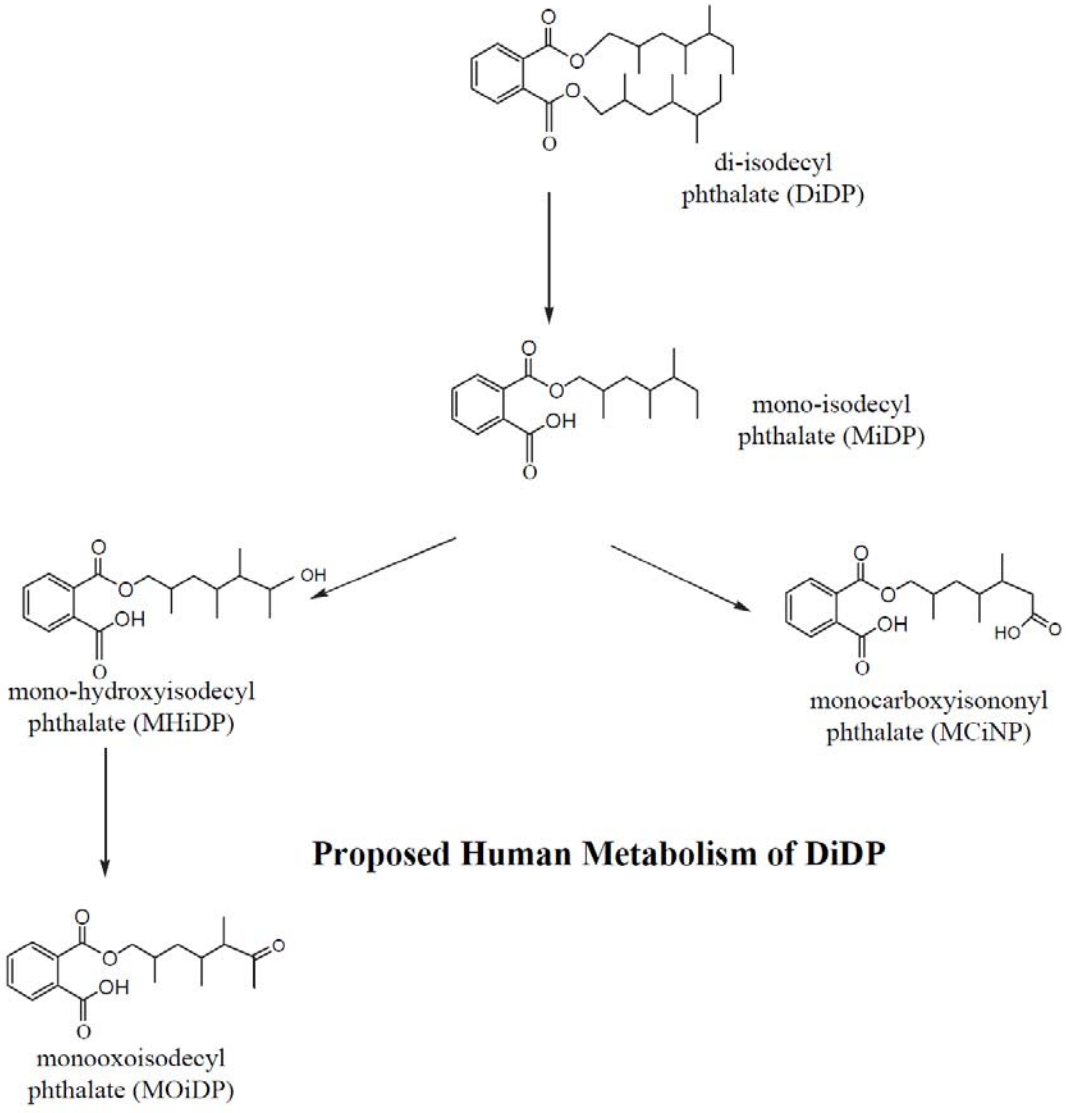


Figure 4.5. Proposed metabolic pathway of DIDP. The figure shows only one isomer from each metabolite class (from Silva et al. 2007a, used with permission).

[14] CAS 68515-49-1

4.4.1.3 Toxicokinetic information from an analogue substance, DEHP

The following cites the supporting document to the opinion of RAC on the draft version of this report (ECHA 2013b):

"Humans
Schmid and Schlatter (1985) studied excretion of DEHP taken orally by 2 volunteers (30 mg or about 0.4 mg/kg) and determined an excretion of 11 and 15% of the dose in urine by measuring 12 DEHP metabolites. DEHP taken by the same volunteers over a period of 4 days at a dose of 10 mg/day (about 0.13 mg/kg/day) resulted in 15 and 25% recovery in urine. The amount recovered for 5 of the 12 metabolites was less than 1%.

Koch et al. (2005) measured 5 metabolites in one human volunteer after doses of 4.7, 28.7 and 650 µg/kg, with recoveries in urine of 66, 65 and 71% respectively (mean of 67%). This is indicative that at these low exposure levels there is no saturation of absorption.

Anderson et al. (2011) studied 10 male and 10 female human volunteers (n = 20) given deuterium labeled DEHP (and DINP, see above) at dose levels of 0.31 mg (0.004 mg/kg for males and 0.005 mg/kg for females) and 2.8 mg (0.034 mg/kg for males and 0.041 mg/kg for females). The recovery in urine was 47% based on measurement of 4 metabolites. Using the same 4 metabolites from the Koch et al. (2005) results, this would in comparison have given 65%. Anderson et al. (2011) noted that the higher results seen in the Koch study can be explained because it is based on a single individual (with results still within the observed standard deviation). The authors also noted that the consequence of the difference is that when calculating exposure from biomonitoring data the conversion factors and therefore the exposure will be slightly higher based on their results.

Kessler et al. (2012) studied 4 male volunteers given 618-665 µg/kg labelled DEHP and found 31% of the dose excreted in urine based on measurement of 3 metabolites. The authors concluded that the results are in line with those from Anderson et al. for the 3 metabolites (29.1 and 33.2%). The results from Koch et al. gave 44.2% excretion in the urine of the 3 metabolites (Kessler et al. 2012). The authors made the same remark as Anderson et al. (2011) regarding the consequences to the estimation of exposure from biomonitoring results in urine.

Animal studies with DEHP
Numerous studies have been performed to study the toxicokinetics of DEHP in different rat strains, and also in non-human primates, mice, hamster, guinea pigs, dogs, miniature pigs. Based on amongst others about 16 kinetic studies with DEHP in rats, RAC concluded in its opinion of 15 June 2012 on the Annex XV dossier proposing restrictions on four phthalates that the absorption of DEHP in rats can be estimated to be 70%.

In a first experiment studying kinetics, Sjöberg et al. (1985) administered 1000 mg DEHP/kg to 25, 40 and 60 days old rats by gavage (9-10 animals per group). The mean AUC of MEHP of 25 day old rats (1213 µg h/ml) was significantly higher than that of the 40 and 60 day old rats (611 and 555 µg h/ml respectively). In a second experiment studying excretion, groups of 25 and 60 day old rats (6 animals per group) were administered 1000 mg 14C-DEHP/kg by gavage. The cumulative excretion of radioactivity was 44% in 25 day old rats and 26% in 60 day old rats. The authors concluded that the observations suggest that the absorption, and therefore exposure, to MEHP and its metabolites was higher in young than in more mature rats.

In Study I of Kurata et al. (2012), groups of 3 and 18 months old marmosets received 100 and 2500 mg/kg 14C-DEHP by gavage (3 animals per group). At the low dose, the cumulative urinary excretion 7 days after dosing was higher in the younger (about 18%) than in the elder animals (13%). At the high dose the younger excreted about 10%, the elder 22% radioactivity in urine. Within one day after the low dose there was no difference between the two age groups (about 10% excretion), whereas at the high dose the excretion in the younger animals was less than in the elder ones (5 versus 15%). Two

hours after dosing radioactivity in blood and bile was more than twofold higher in the younger animals at the low dose and about 40% lower at the high dose. Thus, within one day after the low dose, the younger animals absorb more than the elder ones. At the high dose younger animals show lower radioactivity in urine, bile and blood than the elder ones (about 40% less).

Based on the results from Study II of Kurata et al. (2012), in which 4 week old rats and 3 months old marmosets received 100 mg/kg 14C-DEHP by gavage, there might be large species differences in absorption between rat and marmosets. In rats at 1 day post-dose radio-activity excreted in urine accounted for 58% of the dose, whereas in marmosets this was only 8%."

4.4.1.4 Discussion

The conclusions from the EU Risk Assessment (EC 2003a,b) concerning the toxicokinetics of DINP and DIDP are generally still valid. Studies in animals and humans demonstrate that DINP and DIDP are rapidly absorbed orally and quickly metabolized.

Humans
According to the supporting document to the opinion of RAC on the draft version of this report (ECHA 2013b), human volunteer studies with DEHP clearly demonstrate that the amount recovered in urine is dependent on the type and amount of metabolites that are measured in those studies. It was considered that measuring all metabolites most likely would result in near to 100% recovery of radioactivity in urine and pointed out that an unknown amount of excretion via bile contributes further to the absorption estimate. It was however acknowledged that the studies in humans have not been designed to determine absorption.

RAC (ECHA 2013a,b) concluded that adult humans orally absorb 100% based on read-across from DEHP. This conclusion differs from the EU Risk Assessments (EC 2003a,b) that assumed a bioavailability factor of 50% for calculating internal oral exposure of adults, derived from toxicokinetic data in rats. For newborns and infants a factor of 100% (i.e. twice as much as for rats) was assumed based on a study from Sjöberg et al. (1985) which seemed to show a greater absorption by oral route of DEHP in young rats compared to older ones.

Following the conclusions of the EU Risk Assessments (EC 2003a,b), a bioavailability factor of 75% for inhalation can be assumed for adults and 100% for newborns and infants as a vulnerable subpopulation. The supporting document to the opinion of RAC supported these assumptions, but noted that the absorption of 100% in children could be considered conservative.

Knowledge on metabolites in urine has increased since the EU Risk Assessment and shows that especially the oxidative metabolites MCiOP, MHiNP, and MOiNP can be recovered from urine of both animals and humans. This is of particular relevance for the use of biomonitoring in exposure assessment. For further information on human biomonitoring see section 4.6.8.

Rats
The key result from the Midwest Research Institute (1983 as cited in EC 2003a) was a recovery in urine of 49% in rats treated with a single dose of 50 mg/kg DINP by gavage. The key result from the General Motors Research Laboratories (1983 as cited in EC 2003b) was an estimated oral absorption of 55.6%

(including 14.3% from bile) treated with 0.1 mg/kg DIDP and 45.9% (including 13.8% from bile) treated with 11.2 mg/kg DIDP.

The dermal studies with DINP indicated a dermal absorption of 2-4% (Midwest Research Institute 1983 as cited in EC 2003a). McKee et al. (2002) calculated ratios of urinary excretion and excretion in feces/gastrointestinal tract to come to and assumption of biliary excretion of as much as 60% of urinary excretion. The authors estimated using this assumption that an upper bound of absorption could be estimated to be 75% and 90% in the single dose and repeated dose experiments respectively. However, as description above, the studies and the reporting have limitations. As pointed out also by EC (2003a) it is difficult to precisely estimate the skin absorption from the available data. Thus, assumptions with regard to biliary excretion from these dermal experiments are considered unreliable and should not be used to make quantitative estimates of oral absorption. Indeed, the high biliary excretion is not supported by the observation in the study that the large majority of radioactivity in feces was recovered from unmetabolised DINP and MINP. The metabolite profiling data rather indicates that 12% of radioactivity in feces is in the form of oxidised metabolites, which might result from biliary excretion. Thus biliary excretion might amount to about 6% of the oral dose. With 49% urinary excretion this would total to 55% oral absorption[15]. Also this data is limited as it is based on 1 sample of 1 animal, and potential metabolism by the gut flora makes quantification difficult (as commented by EC 2003b). The main conclusion that can be drawn from the experiments it that indeed there is excretion of radioactivity in the bile.

Thus, when assuming 55% oral absorption (taking into account biliary excretion) after a single dose of 50 mg/kg from Midwest Research Institute (1983 as cited in EC 2003a), and an oral absorption of 55.6% (including 14.3% from bile) after treatment with 0.1 mg/kg DIDP and 45.9% (including 13.8% from bile) after 11.2 mg/kg DIDP, the oral absorption seems to be in the order of 50-55%. The EU Risk Assessment (EC 2003a,b) assumed an oral bioavailability factor of 50% for adult rats. This conclusion is also supported by the opinion of RAC of 8 March 2013 (ECHA 2013). The supporting document to the opinion of RAC (ECHA 2013b) however added that *"As biliary excretion occurs, an unknown percentage of the radioactivity excreted in feces is to be added to the radioactivity excreted in urine to estimate the absorption. The absorption of DINP and DIDP can therefore be assumed to be in the range of 50-70% in the rat."*

As pointed out by EC (2003a), it is difficult to precisely estimate the skin absorption from the available data in rats. Following the conclusions of the EU Risk Assessments (EC 2003a,b), dermal internal exposure for consumers can be derived from a study using dermal contact of rats with a plastic film containing DEHP. The study concluded on a dermal absorption rate of 0.24 µg/cm2/h (Deisinger et al. 1998). This was considered the more relevant study for consumer exposure compared to the rat study that used direct application of DINP to the skin (Midwest Research Institute 1983). Based on evidence that DIDP is 10 times less absorbed through the skin than DEHP (Elsisi et al. 1989) and because of the physico-chemical similarities between DIDP and DINP, a factor of 10 was assumed to extrapolate from DEHP to DINP and DIDP, thus assuming a dermal absorption rate of 0.024 µg/cm2/h.

[15] When using the data in Table 4 of McKee et al. (2002) for single dose 50 mg/kg, 72h after dosing. The results from the repeated dose study cannot be used to reliably estimate oral absorption as the first four doses were also done with 'hot' compound which makes the significance of comparison to the adminsitered last dose unclear.

4.4.1.5 Conclusions

Based on read-across from DEHP, it is assumed that humans orally absorb DINP and DIDP 100%. The oral absorption in adult rats was estimated to be in the order of 50-55%.

A bioavailability factor of 75% for inhalation can be assumed for adults and 100% for newborns and infants as a vulnerable subpopulation.

Based on a study with DEHP (Deisinger et al. 1998), and the assumption that DINP and DIDP are 10 times less absorbed through the skin than DEHP (Elsisi et al. 1989), a dermal absorption rate of 0.024 µg/cm2/h can be assumed.

4.4.2 Acute toxicity

4.4.2.1 DINP

The following cites the 'Summary of acute toxicity' from the EU Risk Assessment:
"Most of the animal studies on acute toxicity were either not available for detailed study or performed prior to establishment of OECD or EU guidelines. However given the consistency of the results for oral, dermal and inhalation exposure, it can be considered that DINP has a low acute oral, dermal and inhalation toxicity. No LD50/LC50 was reported from acute exposure by those routes of exposure. Findings consisted of poor state, respiratory difficulties (laboured respiration, dyspnea) and altered appearance, following oral administration, even at very high level (up to 40,000 mg/kg). Acute inhalation studies, although poorly documented, did not report
any body weight changes, any gross lesions or microscopic alterations of lungs, only slight tearing of the eye and slight clear nasal discharge following aerosol exposure of 4.4 mg/l of air during four hours. Therefore, no classification is indicated according to the EU criteria for acute toxicity." (EC 2003a)

The information and conclusions from the EU Risk Assessment (EC 2003a) are considered valid for this endpoint. It was not considered necessary to actively gather new information for this endpoint.

4.4.2.2 DIDP

The following cites the 'Summary of acute toxicity' from the EU Risk Assessment:
"Most of the animal studies on acute toxicity were either not available as detailed studies or performed prior to establishment of OECD or EU guidelines. However in view of the consistency of the results for all routes of exposure, it can be considered that DIDP has a low acute oral, dermal and inhalation toxicity. No classification is indicated according to the EU criteria for acute toxicity whatever the route of exposure." (EC 2003b)

The information and conclusions from the EU Risk Assessment (EC 2003b) are considered valid for this endpoint. It was not considered necessary to actively gather new information for this endpoint.

4.4.3 Irritation

4.4.3.1 DINP

The following cites the 'Summary of irritation' from the EU Risk Assessment:
"On the whole, DINP may be considered as a very slight skin and eyes irritant, with effects reversible in short time. Thus no classification is indicated according to the EU criteria for those different end points." (EC 2003a)
The information and conclusions from the EU Risk Assessment (EC 2003a) are considered valid for this endpoint. It was not considered necessary to actively gather new information for this endpoint.

4.4.3.2 DIDP

The following cites the 'Summary of irritation' from the EU Risk Assessment:
"Results from animal studies following single skin exposure varying from 5 minutes to 24 hours lead to no or moderate effect, reversible with possible desquamation. Effects on eyes are weak and limited to conjunctiva. There is no indication of upper airways irritation in animal. In humans there is no indication of an irritating potential. Thus no classification is indicated according to the EU criteria for those different end points." (EC 2003b)
The information and conclusions from the EU Risk Assessment (EC 2003b) are considered valid for this endpoint. It was not considered necessary to actively gather new information for this endpoint.

4.4.4 Corrosivity

From section 4.4.3 on irritation, it follows that DINP and DIDP are not corrosive.

4.4.5 Sensitisation

Because many of the experimental studies on sensitisation and other effects on the immune system are conducted with both DINP and DIDP, and the epidemiologic studies do not differentiate between exposures to individual phthalates as exposures most often are not described in detail, the assessment for DINP and DIDP for this endpoint is summarised as a joint section.

4.4.5.1 EU risk assessment conclusion -DINP

The following cites the 'Summary of sensitisation' from the EU Risk Assessment:
"One study conducted according to Buehler gives a positive response after re-challenge, which could lead to a classification according to the EU criteria. Another study conducted according to Buehler (one challenge), gives negative results. The Sqwish Ball® producer reported 5 cases of dermatitis related to misuse of this material, but none of these cases was related directly to DINP. Overall, this provides weak evidence that DINP may cause sensitisation in human. No positive reactions were reported in a RIPT conducted in humans. Sensitising properties have not been demonstrated with any of the phthalates and particularly with DEHP and DBP. Therefore a low sensitising potential can be anticipated. Overall, according to the EU criteria a classification for sensitization properties is not justified with DINP. It should be noted that no experimental data are available for DINP 2 (CAS 28553-12-0). However the same result can be anticipated for both DINP.

Respiratory sensitisation
Pulmonary sensitising properties have not been demonstrated with any of the phthalates and particularly with DEHP and DINP. Therefore a low potential can be anticipated." (EC 2003a)

4.4.5.2 EU risk assessment conclusion -DIDP

The following cites the 'Summary of sensitisation' from the EU Risk Assessment:
One study conducted according to Buehler gives a clear positive response, which should lead to a classification according to the EU criteria. Two other studies conducted either according to Buehler or to Magnusson and Kligman, give negative results with no evidence of irritating effect; these two studies cannot invalidate the previous one since they present some weaknesses in the protocol especially concerning the lack of irritancy at the induction phase. But the marked response obtained in the first Buehler test, normally considered having a low sensibility, is confusing. The strong irritant effect during induction phase, only observed in this assay, is also surprising. In any of the three tests, the DIDP composition is not well established, impurities or additives could explain the discrepancy in results.

No positive reactions were reported in patch test studies conducted in humans. Only one case of dermatitis has been reported in humans. Consequently the evidence that DIDP may cause sensitisation in human is weak.

Moreover sensitising properties have not been demonstrated with any of the phthalates and particularly with DEHP and DBP. A low sensitising potential, if any, can be anticipated. Overall, the weight of evidence is deemed insufficient to justify a classification.

Respiratory sensitisation
Pulmonary sensitising properties have not been demonstrated with any of the phthalates, particularly with DEHP or DBP, and no cases have been reported in humans. Therefore a low potential can be anticipated." (EC 2003b)

Table 4.11 Sensitisation studies with DINP and DIDP from the EU Risk Assessments (EC 2003 a and b) together with the new studies reviewed in this report.

Test Method	Species	Protocol	Results	Test substance	References
Buehler test	Guinea pig Hartley albino	other	DINP:weak sensitiser DIDP: sensitiser	DINP: MRD 92-257 CAS 68515-48-0 DIDP: MRD-92-256	Exxon Biomedical Sciences (1992)
Buehler test	Guinea pig Dunkin Hartley	Directive 84/449EEC, B.6	not sensitising	Jayfex DINP CAS 68515-48-0 Jayflex DIDP	Huntingdon (1994)
Magnusson and Kligman test	Guinea pig	other	not sensitising	DIDP	Inveresk Research International (1981)
Dermatitis	Human	no	dermatitis with child toy	DINP	Brodell and Torrence (1992)
RIPT	Human	other	not sensitizing	DINP: MRD 95-140 CAS 68515-48-0 DIDP	Hill Top Research (1995b)
Irritant and allergic patch test	Human	other	No allergic reactions	DIDP	Kanerva et al. (1996)
Case study	Human	other	A case of allergic contact dermatitis from DIDP in PVC	DIDP	Hills and Ive (1993)
Histamine release in vitro	Human basophils	other	DINP – negative DIDP –	DINP DIDP	Glue et al. (2005)

			possibly weak positive		
Adjuvant effect on IgE, IgG1 and IgG2a	BALB/c mice	other	adjuvant effect on IgE and IgG1 by both substances	DINP DIDP	Larsen et al. (2002)
Adjuvant effect on IgE and interleukins IL-4 and IL-13	BALB/c mice	other	no sensitising effect	DINP	Butala et al. (2004)
Trafficking of antigen-presenting cells (FITC sensitization)	Mice	other	did not enhance sensitisation	DINP	Imai et al. (2006)
An allergic disease, atopic dermatitis-like (AD-like) skin lesions	*Dermatophagoides pteronyssinus* in atopic-prone mice	other	positive	DINP	Koike et al. (2010)
Epidemiolgical: Phthalate exposure and allergy	Human	other	inconclusive	phthalates in PVC	Jaakkola and Knight (2008)
Review of potential to modulate immune and allergic responses	Human, animals	other	association between atopic disease and exposure, causality not demonstrated	DINP DIDP phthalates in PVC	Kimber and Dearman (2010)

4.4.5.3 Risk assessments from other international organizations and bodies

EU bodies

CSTEE 2001a,b

In its opinions on the results of the EU risk assessments for DINP and DIDP (EC, 2003a,b[16]), the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE 2001a,b) agreed with the EU Risk Assessments that an overall evaluation of the available information indicated that a classification for sensitising properties was not justified for DINP and DIDP.

[16] The CSTEE reviewed earlier drafts of the EU Risk Assessments for DINP and DIDP (EC 2003a,b).

4.4.5.4 New studies

After the EU Risk Assessment, the effect of DINP and DIDP and other phthalates (i) on pro-inflammatory response and histamine release (inflammatory part of allergic diseases) in vitro, (ii) as adjuvants in in vivo mouse models, (iii) on allergic skin lesions in a mouse model, and (iv) as a respiratory sensitizer in a mouse model have been studied. In addition, reviews of epidemiologic studies have been published. Some of the mechanistic studies and a summary of the epidemiologic literature (based on review articles) were included in this report to provide a general update of the current views on the immunomodulatory potential of phthalates. Information on sensitisation for DINP, DIDP and other phthalates is often contradictory, fragmentary or inconclusive. Sensitisation was therefore not the main focus of the assessment, and the section should not be regarded as an exhaustive assessment of the available literature, in particular not of the epidemiologic studies.

The EU Risk Assessments (2003a,b) did not evaluate the epidemiologic information related to PVC exposure that is included in the present report although several studies were available at that point in time.

Adjuvant activity
In the context of hypersensitisation an adjuvant means a substance or material which can enhance immune responsiveness without itself being an antigen. Adjuvants are used in vaccines to induce broader immune responses and to enhance immune responses to poorly immunogenic antigens. They can accelerate, prolong or enhance a specific immune response, by modifying B- and T-cell responses thorough various receptor types. Substances known to have adjuvant properties include aluminium containing substances, lipopolysaccharides, saponins, emulsions and cationic liposomes (Mastelic et al. 2010, Kimber 2010).

Pro-inflammatory response in vitro
DINP[17] modified the pro-inflammatory response in an in vitro study using the human monocytic cell line (THP-1) as a model for macrophages. A statistically significant increase in TNFα production at the highest concentration (10 µM) and a dose dependant reduced phagocytosis at all concentrations tested (0.2-10 µM) was observed (Bennasroune et al. 2012). The authors concluded that although the substances examined alter the immune response in vitro, in vivo studies are necessary to elucidate the effects.

Histamine release in vitro
The role of phthalates, including DINP and DIDP, in the inflammatory part of allergic disease was investigated using a basophil histamine release assay (Glue et al. 2005). Basophils were obtained from human blood, incubated with phthalates (5, 50 and 500 µM), and stimulated with anti-IgE, calcium ionophore or an allergen (cat hair extract) after which histamine release was measured. None of the phthalates induced histamine release per se. Histamine release was obtained only after crossbinding the high affinity IgE receptor on the basophils by stimulation with anti-IgE antibodies. The strongest effect was seen for phthalates with eight carbon atoms in the side chain, e.g. DEHP, while phthalates with four, nine or ten carbon atom side chain length (such as DINP and DIDP) had none or low inducing effect on histamine release. While the results for DINP were clearly negative, DIDP showed a weak positive response at the middle of the three concentrations tested.

[17] DINP purchased from Sigma; no specification on CAS number.

In vivo adjuvant studies in mice

Larsen et al. (2002) investigated the adjuvant effect of DBP, DNOP[18], and DINP and DIDP by injecting different phthalate concentrations (resulting in doses of 100, 10, 1 and 0.1 µg per animal) together with ovalbumin (model antigen) or ovalbumin alone subcutaneously in BALB/c mice. The serum levels of ovalbumin-specific immunoglobulin E (IgE), IgG1 and IgG2a were determined. Allergic asthma and allergic rhinitis are Type I allergies and are mediated by a T-helper cell type 2 (Th2) response. The response is characterised by increased IgE production in humans and IgE and/or IgG1 production in mice. A positive adjuvant effect was considered to occur if a statistical increase in antibody production occurred in a test group as compared to the ovalbumin control group together with a dose-response relationship.

DINP significantly elevated the IgE and IgG1 levels after one booster with ovalbumin in the 10 µg dose group, but not at the 100 µg dose. The decrease in antibody production at the highest dose levels was presumed to be caused by an immunosuppressive effect. DINP also significantly increased the IgG1 levels in a concentration-dependant manner after a second booster (at 10 and 100 µg) as well as IgE levels (at 100 µg), indicating adjuvant activity.

DIDP significantly elevated the levels of IgE after one booster with ovalbumin at 100 µg. The effect of DIDP on IgG1 production gave ambiguous results as none of the DIDP treated groups showed responses higher than the corresponding control group. However, when compared to the results of a cumulated ovalbumin control group DIDP enhanced IgG1 levels after a second booster. The authors concluded that DIDP may have weaker adjuvant potential than DINP. A comparison revealed that DEHP and DINP were stronger adjuvants than DBP, DNOP and DIDP.

Imai et al. (2006) studied the ability of phthalates to enhance sensitisation to fluorescein isothiocyanate (FITC). Mice were sensitised with 160 µl FITC (0.5% w/v) dissolved in a 1:1 acetone:phthalate solution applied epicutaneously on day 0 and day 7 (amount phthalate per mouse is thus 80 µl applied twice). Controls were FITC in acetone, acetone only, and acetone with DBP only. The authors did not indicate how long the solution remained on the skin. Sensitisation was evaluated as ear swelling after a challenge with 20 µl solution of FITC (0.5%) in A/DBP. Di-butyl phthalate (DBP) and di-n-propyl phthalate (DPP) strongly enhanced the ear-swelling, while di-methyl phthalate (DMP) and di-ethyl-phthalate (DEP) were less effective and di-(2-ethylhexyl) phthalate (DEHP) and DINP did not show any effect. DIDP was not studied. According to the authors, one possible mechanism of the adjuvant effect of phthalates esters could be enhanced trafficking of antigen-presenting cells (Langerhans cells and/or myeloid dendritic cells) from skin to draining lymph nodes although other mechanisms might be involved as well.

Skin lesion aggravation in a mouse model

In another mice study (Koike et al. 2010) the effect of DINP on an allergic disease, atopic dermatitis-like (AD-like) skin lesions, was examined. The skin lesions were induced with *Dermatophagoides pteronyssinus (Dp)* in atopic-prone mice. DINP was administered to mice intraperitoneally on days 2, 5, 9 and 16 after *Dp* treatment with doses up to 150 mg/kg of DINP. The mice were evaluated for ear thickening, histological findings, protein levels of cytokines/chemokines (IFN-γ, IL-4, IL-5, IL-13, eotaxin and thymic stromal lymphopoietin (TSLP)) in the ear tissue, and for levels of Ig and histamine in serum.

[18] CAS: 117–84–0, obtained from Tokyo Kasei Organic Chemicals, Japan.

In addition, the effect of DINP on splenocytes and bone marrow derived dendritic cells were examined in vitro. To evaluate whether DINP affects the phenotypes and function of bone marrow derived cells (BMDC) in NC/Nga mice, the production of TH_1/TH_2 cytokines and chemokines, the expression of cell surface molecules, and antigen- presenting activity of BMDCs after exposure to DINP in vitro was measured. The cells exposed to DINP (0 – 100 µM) were evaluated for phenotype and function.

DINP aggravated skin lesions, increased histamine levels in serum, and increased the expression of eotaxin and eotaxin-2 in ear tissue. It also increased the numbers of thymic stromal lymphopoietin (TSLP) and dendritic cells in the ear tissue. In in vitro studies, antigen-presenting activity of bone marrow cells (BMDCs), interleukin-4 production in splenocytes and splenocyte proliferation were increased. DINP did not affect Ig levels. DINP exposure for 24 hr significantly increased the production of TH_2 but not TH_1 chemokines. The authors conclude that DINP aggravated the AD-like skin lesions by a mechanism which might be partly mediated through the TSLP-related activation of dendritic cells and by direct or indirect activation of immune cells.

Respiratory sensitisation in a mouse model

To address the hypothesis that exposure to phthalates may contribute to childhood asthma Butala et al. (2004) investigated the respiratory sensitisation potential of DINP and other phthalate esters in a B6C3F1 mice IgE model. The objective of the study was to evaluate the respiratory sensitizing potential of four phthalates (DEHP, DINP, DIHP and BBP). Mice were treated topically with the respective phthalate (2x50 µl undiluted phthalate per animal 5 times weekly for 2 weeks) after which total serum IgE and the levels of interleukin 4 (IL-4) and IL-13 proteins and their mRNAs were determined in auricular lymph nodes. The cytokines studied are mediators of bronchial asthma. The levels of serum IgE, IL-4 and IL-13 proteins as well as levels of IL-4 and IL-13 mRNAs in the phthalate-treated animals were all at similar levels to that of the controls. The authors concluded that it was unlikely that the studied substances would produce antibody-mediated respiratory allergy and would thus not contribute to childhood asthma.

Discussion on adjuvant effects

Some phthalates, at specific doses and applied via certain routes of administration, have been shown to modulate immune and inflammatory processes in a diverse pattern, ranging from potentiation to no effects or at higher doses possibly even inhibition of immune and inflammatory responses (Larsen et al 2002).

Effects on IgG1 and/or IgE antibody responses were observed both with DINP and DIDP when administered via subcutaneous exposure (Larsen et al. 2002). Adjuvant effects (increase in antibody production) were observed at intermediate doses, and possible immuno-suppressive effects at higher doses might have led to the lower observed antibody production in the same experiment. This is suggestive of a bell-shaped dose-response relationship (Larsen et al. 2002).

DINP increased pro-inflammatory cytokine (TNFα) production at rather high concentration in vitro but reduced phagocytosis also at lower concentrations indicating alteration of immune parameters (Bennasroune et al. 2012). DINP and DIDP did not show any (or very limited in the case of DIDP) histamine release in vitro, indicating that these substances might be less efficient in stimulating the inflammatory part of allergic diseases than some other phthalates (Glue et al. 2005). However, atopic dermatitis-like skin lesions were aggravated by DINP in a

mouse model (Koike et al. 2010), and DINP was shown to increase serum levels of histamine in this study. DINP did not enhance sensitisation to FITC and therefore, unlike some other phthalates did not seem to have an effect on trafficking of antigen-presenting cells from skin sites (Imai et al. 2006). Taken together at least DINP seem to be able to aggravate atopic inflammatory processes under certain exposure conditions (intraperitoneally), even if it may be less potent than some other phthalates.

The levels of serum IgE, IL-4 and IL-13 proteins, and IL-4 and IL-13 mRNAs in a respiratory sensitisation model in mice were not elevated by exposure to DINP and some other phthalates which may indicate that these phthalates are not effective in contributing to antibody-mediated respiratory allergy (Butala et al. 2004). These results are in accordance with the conclusions of the EU Risk Assessments for DINP and DIDP (EC 2003a,b) that DINP (and DIDP) lack sensitising potential.

Summary of epidemiological information

New information from human studies is available which is relevant for assessing the role of phthalates in development of asthma and allergic disease (Table 4.12 and Table 4.13).

Jaakkola and Knight (2008) reviewed the epidemiological literature obtained from a Medline search from 1950 through May 2007 to evaluate the role of exposure to phthalates from PVC-containing products in the development of asthma and allergies. Several of the epidemiological studies investigated the relationship of exposure of adults at home or at work to PVC containing materials and the respiratory or asthma symptoms. They found that increased risk of asthma symptoms in those individuals that were in buildings showing signs of dampness-related degradation of DEHP in PVC flooring. The study by Norbäck et al. (2000) showed that there was a reduction in the prevalence of respiratory, nasal and conjunctival symptoms when indoor pollutants were reduced. There was also a correlation between the presence of plastic wall coverings at work and an increased risk of asthma (Jaakkola et al. 2006).

Kimber and Dearman (2010) conducted a literature review on epidemiological studies related to phthalate exposure through PVC exposure. They concluded that heated PVC fumes can contribute to development of asthma in adults. Hot-wire cutting of PVC film has been linked to asthma and other respiratory symptoms. However, the majority of these studies were not adjusted for confounders.

Based on the analysis by Kimber and Dearman (2010) and Jaakkola and Knight (2008), the epidemiologic studies in children show associations between indicators of phthalate exposure and risk of asthma and allergies. Kimber and Dearman (2010) also evaluated a case–control study among Bulgarian children (Kolarik et al. 2008) which provides further evidence for a relation between DEHP concentration in house dust and the risk of wheezing, rhinitis, and/or asthma which was not available to Jaakkola and Knight (2008) at the time of their review. Kimber concluded that the available studies demonstrated an association between exposure to phthalates and the worsening of respiratory symptoms, such as bronchial obstruction or wheeze, or the development of atopic diseases (rhinitis or eczema) in children. Following adjustment for various confounders, risks of the various respiratory outcomes were reported to be increased in the presence of plastic materials in the home environment. An association, but not a causal link, was found between DEHP exposure and the respiratory symptoms.

Jaakkola and Knight (2008) concluded that there is only scarce information about the emission rates of phthalates from interior surface materials in normal indoor environmental conditions. Phthalates migrate from PVC tiles to house dust and inhalation of particles containing phthalates is a plausible route of exposure. Heating or burning PVC materials releases phthalates and other combustion products into indoor and ambient air. There is evidence that dampness enhances degradation of PVC flooring, resulting into a release of phthalates to the indoor air.

Only a few studies examine indoor air and home dust concentrations of phthalates. DEHP has been found to be the predominant phthalate species in total suspended dust (mean 64 µg/100 mg dust) in homes of children 0–2 years of age. In the 38 samples of sedimented dust, DEHP accounted for a mean of 69% of the total amounts of phthalates in total dust (Jaakkola and Knight 2008).

Table 4.12 Summary of epidemiological studies exploring the relationships between exposure to phthalates in PVC materials and the risk of asthma and allergy. Data are from the review by Jaakkola and Knight (2008).

Reference, location	Study design	Study population	Exposure	Outcomes	Results	Comment
Polakoff et al. (1975), USA	Cross-sectional study	17 meat wrappers: 21 office personnel and store clerks as a reference group	Inhalation exposure to pyrolysis products of PVC film; assessment based on job category (meat wrappers exposed) and questionnaire information	Symptoms, signs based on questionnaire information; pre- and postshift spirometry: FVC, FEV_1, PEF, FEF_{25}, FEF_{50}, FEF_{75}, FEF_{90}	Exposed had a higher prevalence of cough ever (47.1% vs. 23.8%), work-related shortness of breath (23.5% vs. 0%), wheezing (5.9% vs. 0%), eye watering and itching (17.6% vs. 9.5%), nasal and pharyngeal symptoms (29.4%vs.4.8%), allergies (11.8% vs. 9.5%), and decline over shift in FEV1 ($p < 0.05$) and FEF50 ($p < 0.05$)	Frequency matching of reference group but no adjustment for potential confounders
Falk and Portnoy (1976), Houston, TX, USA	Cross-sectional study	145 meat wrappers; 150 checkers and 150 meat cutters as a reference group	Inhalation exposure to pyrolysis products of PVC film; assessment based on job category and interview information	Symptoms, signs based on questionnaire information	Symptom prevalences in exposed vs. checkers and cutters: shortness of breath (16% vs. 4% and 4%; $p < 0.05$), wheezing (12% vs. 5% and 7%; NS), chest pain (17% vs. 5% and 7%; $p < 0.05$), bronchitis (31% vs. 19% and 13% $p < 0.01$) pneumonia (36% vs. 27% and 9%; NS), and pleurisy (33% vs. 16% and 9%; $p < 0.01$)	Frequency matching of reference group but no adjustment for potential confounders
Andrasch et al. (1976), Portland, OR, USA	Cross-sectional study	96 meat wrappers	Inhalation exposure to pyrolysis products of PVC film; assessment based on job title (meat wrappers exposed) and questionnaire information	Symptoms and signs based on questionnaire information (response rate, 58%); and on bronchial provocation test to PVC fumes and price-label adhesive fumes for 14 workers	69% had work-related respiratory, mucosal, or system symptoms; 3 of 11 workers developed a mean decrease of 25% in FEV1 after exposure to PVC fumes; 9 of 13 workers developed a 49% decrease in FEV1 and 40% decrease in FVC after exposure to price-label adhesive fumes	77% of symptomatic workers reported improvement on weekends and during vacations; no adjustment for potential confounder

Brooks and Vandervort (1977), Ohio, USA	Cross-sectional study	44 workers in retail food industry: 24 exposed meat wrappers; 20 office workers and store clerks as a reference group	Inhalation exposure to pyrolysis products of PVC film and thermoactivated price-label adhesive fumes	Symptoms and signs based on questionnaire information, spirometry (FVC, FEV_1, MMF, VC_{50}, and VC_{25})	Exposed vs. reference: cough, 37% vs. 10%; dyspnea, 29% vs. 10%; wheezing, 12% vs. 0%; asthma/allergy, 17% vs. 5%; nasal symptoms, 14% vs. 0%; no differences between pre- and postshift lung function tests	Exposed attributed symptoms to PVC film fumes rather than price-label adhesive fumes; no adjustment for potential confounders
Eisen et al. (1985), Boston, MA, USA	Cohort study	83 workers in the retail food industry: 40 exposed to hot-wire or cool-rod fumes, and 43 as a reference	Inhalation exposure to pyrolysis products of PVC film; assessment based on job title: meat wrappers, meat cutters, and delicatessen product workers	Change in FEV1 over time (mL/year)	No difference in FEV1 change between the exposed and reference group; interaction term "hot-wire exposure* asthma/allergy," 76 mL/year, $p < 0.06$	Workers with asthma or allergy may be more susceptible; adjusted for age, smoking, and asthma/allergy
Markowitz (1989), Plainfield, NJ, USA	Cross-sectional study	39 workers in a PVC processing plant: 20 exposed employed as machine attendants and calendar operators, 19 unexposed	Exposed to PVC thermal degradation products and phthalic acid esters	Symptoms, signs based on questionnaire information, bronchial provocation test, specific serum IgGs and IgEs, spirometry (VC, FEV1, FEF50, FEF75)	Exposed vs. reference: conjunctivitis, 25% vs. 0% ($p < 0.02$); rhinitis, 20% vs. 10%; unspecific bronchial hyperreactivity, 25% vs. 5%; dry cough, 45% vs. 0% ($p < 0.001$); asthma, 10% vs. 0%; one positive reaction in bronchial provocation; one exposed had IgG against phthalic anhydride; no differences in lung function parameters	No adjustment for potential confounders
Nielsen et al. (1989), Denmark	Cross-sectional study	39 workers in a PVC processing plant: 20 exposed employed as machine attendants and calendar operators, 19 unexposed	Exposed to PVC thermal degradation products and phthalic acid esters	Symptoms, signs based on questionnaire information, bronchial provocation test, specific serum IgGs and IgEs, spirometry (VC, FEV1, FEF50, FEF75)	Exposed vs. reference: conjunctivitis, 25% vs. 0% ($p < 0.02$); rhinitis, 20% vs. 10%; unspecific bronchial hyperreactivity, 25% vs. 5%; dry cough, 45% vs. 0% ($p < 0.001$); asthma, 10% vs. 0%; one positive reaction in bronchial provocation; one exposed had IgG against	Adjustment for age, height, and smoking habits

					phthalic anhydride; no differences in lung function parameters	
Norbäck et al. (2000), Sweden	Cross-sectional study	87 workers in four hospitals: 50 residing in exposed buildings and 37 residing in reference buildings	Two exposed buildings with signs of dampness-related degradation of DEHP in PVC flooring and presence of 2-ethyl-1-hexanol in indoor air; two reference buildings	Doctor-administered questionnaire on presence of asthma symptoms, wheezing, and/or attacks of breathlessness	Exposed (yes/no): asthma symptoms, AOR, 8.6 (95% CI, 1.3–56.7)	Adjusted for sex, age, atopy, current smoking, building dampness at home and at work
Tuomainen et al. (2004), Finland	Repeated cross-sectional study before and after intervention	Office building with 148 workers: first survey, 92 participants; second survey, 115 participants	Before intervention: damp and damaged PVC flooring, 1–3 µg 2-ethyl-1-hexanol per cubic meter of air	Questionnaire information on symptoms and perceived air quality	Index office vs. national rates: eight new cases of asthma in 4 years, 9.2 times more than expected	Intervention included removal of floor coverings, adhesives and smoothing lay
Jaakkola et al. (2006), southern Finland	Population-based incident case–control stud	521 new cases of asthma (21–63 years of age), and 932 population controls	Questionnaire information on presence of plastic wall paper and flooring in the home	Standardized clinical diagnosis of asthma based on history, bronchial challenge, and PEF monitoring	Asthma AOR (95% CI): plastic wall materials at work, < 50% surface vs. none, 1.26 (0.49–3.22); ≥ 50% surface vs. none, 2.43 (1.03–5.75); PVC flooring at work, 1.13 (0.84–1.51)	Adjusted for sex, age, education, smoking, ETS, other surface materials at home and at work

Table 4.13 Summary of the five epidemiologic studies on the relations between exposure to phthalates and PVC materials and the risk of asthma, allergy in children reviewed by Jaakkola and Knight (2008).

Reference, location	Study design	Study population	Exposure	Outcomes	Results	Comment
Jaakkola et al. (1999), Oslo, Norway	Cohort-based matched case–control study	Children 0–2 years of age: 251 cases of bronchial obstruction and 251 one to-one matched controls	Blinded investigator assessment: presence of PVC flooring and a quantitative PVC index (range, 0–8)	Case defined as two or more episodes with symptoms and signs of bronchial obstruction or one episode lasting > 1 month	AOR (95% CI): PVC flooring, yes/no, 1.89 (1.14–3.14); PVC index, Q3 vs. Q2 & Q1, 1.34 (0.78–2.30); PVC index, Q4 vs. Q2 & Q1, AOR, 2.71 (1.50–4.91)	Adjustment for other surface materials, sex, parental atopy, having siblings, daycare attendance, breast-feeding, exposure to ETS, dampness problems, maternal education, family income

Jaakkola et al. (2000), Espoo, Finland	Population-based cross-sectional study	2,568 children 1–7 years of age	Questionnaire information on presence of plastic wall or flooring material in the home	Questionnaire information on the presence of asthma, allergic rhinitis, respiratory symptoms, infections	AOR (95% CI), plastic wall material (yes/no): asthma, 1.52 (0.35–6.71); rhinitis, 1.20 (0.36–3.97); wheeze, 3.42 (1.13–10.4); cough, 2.41 (1.04–5.63); phlegm, 2.76 (1.03–7.41); nasal congestion, 0.95 (0.33–2.71); nasal excretion, 0.90 (0.32–2.57)	Adjusted for sex, age, highest parental education, single guardian, daycare center attendance, pets, ETS, dampness Problems
Bornehag et al. (2004a, 2004b), Varmland, Sweden	Population-based cross-sectional study	10,851 children 1–6 years of age	Questionnaire information on presence of PVC, dampness, and mold	Questionnaire information on doctor-diagnosed asthma, rhinitis and respiratory symptoms	AOR (95% CI) for PVC flooring (yes/ no): asthma, 0.98 (0.77–1.24); rhinitis, 1.09 (0.91–1.30) For water leakage (yes/no), asthma,1.23 (0.96–1.58); rhinitis, 1.35 (1.12–1.62) For PVC and leakage (yes/no), asthma, 1.48 (1.11–1.98); rhinitis, 1.22 (0.96–1.55)	Evidence of an interaction between PVC flooring and water leakage on asthma; adjusted for sex, age, allergic symptoms in family, smoking in household
Bornehag et al. (2004b), Varmland, Sweden	Population-based prevalent case–control study	198 cases of persistent asthma, rhinitis, or eczema and 202 population controls; 106 asthma and 79 rhinitiscases and 177 controls	Trained investigator assessment of PVC flooring and bedroom dust concentrations of DEHP, BBzP, and four other phthalates	Baseline and 2-yearfollow-up surveys; medical examination and case verification	Q4 vs. Q1, AOR (95% CI) BBzP concentration for asthma, 1.87(0.92–3.81); rhinitis, 3.04 (1.34–6.89); eczema, 2.56 (1.24–5.32); DEHP for asthma: AOR (95% CI), 2.93 (1.36–6.34); rhinitis, COR,1.55 (0.73–3.28); eczema, COR, 1.50 (0.76–2.96)	Adjusted for sex, age, smoking at home, type of building, construction period, flooding
Jaakkola et al. (2004), nine cities, Russia	Cross-sectional study	5,951 children 8–12 years of age	Questionnaire information on recent installation of surface materials and furniture	Questionnaire information on current asthma, current wheezing, any allergy	AOR (95% CI), "linoleum"/ PVC flooring (yes/no): past 12 months for asthma, 1.13 (0.44–2.04); wheeze, 1.36 (1.00–1.86); allergy, 1.31 (1.05–1.65) Earlier for asthma, 1.39 (0.67–2.77); wheeze, 1.21 (0.99–1.59); allergy, 1.22 (1.04–1.45)	Adjustment for age, sex, preterm birth, low birth weight, parental atopy, maternal smoking in pregnancy, exposure to ETS, mother's and father's education

4.4.5.5 Conclusion

In general, phthalates (including DINP and DIDP) lack intrinsic sensitising potential. It has however been suggested that phthalates could be one possible contributor to the increasing prevalence of atopic (IgE-mediated) allergic diseases and asthma in Europe and other Western countries as they have adjuvant potential. Especially DEHP has been implicated in this regard. Available epidemiological data also provide some evidence that exposure to phthalates may be associated with increased risk of development of allergies and asthma, but the general lack of detailed exposure information limits the use of such studies for risk assessment purposes. Even if an association has been shown between exposure to phthalates and asthma and allergic disease a causal relationship remains to be established. The precise role of different phthalates in the aetiology of allergic airway diseases and asthma also remains unclear.

Several studies have been performed in mice to examine the adjuvant effects of phthalates on immune responses, most commonly on antibody or cytokine levels. It has been shown that many phthalates, including DINP and DIDP, can affect serum levels of IgG1 and IgE if mice are exposed via the subcutaneous or intraperitoneal routes. For DINP data also indicate a potential to aggravate atopic inflammatory processes. DIDP is so far less studied. It can be concluded that both DINP and DIDP share at least some of the adjuvant properties demonstrated for phthalates and an effect on atopic responses in humans cannot be excluded.

4.4.6 Repeated dosed toxicity

4.4.6.1 DINP

4.4.6.1.1 EU Risk Assessment conclusion

The following cites the 'Summary of repeated dose toxicity' from the EU Risk Assessment:

"The liver is a target for chronic toxicity. Repeated-dose studies performed to assess the peroxisomal proliferation potential of DINP, reveal that DINP acts as a peroxisomal proliferator in rodents as well as DEHP or DIDP.

It is now well-accepted that peroxisome proliferation is specific to rodents and in the monkey study (Huntington Life Science, 1998) the data obtained following oral administration of DINP for 13 weeks provide no evidence that the compound caused induction of peroxisome proliferator. The NOAEL of 500 mg/kg/d from the marmoset and cynomolgus monkey studies clearly indicates that monkeys and subsequently probably men are far less sensitive than rodents to peroxisome proliferation and its relative liver effects.

Indeed, it has been established that peroxisome proliferators exhibit their pleitropic effects due to activation of PPARα and that PPPARα is expressed only at low level in humans, explaining the absence of significant response of humans to the action of peroxisome proliferators.

Nevertheless, for liver effects, a NOAEL of 88 mg/kg/d was assumed from a well-conducted chronic / carcinogenicity rat study according to GLP (Aristech, 1994), based on liver toxicity at higher doses consisting of hepatic biochemical changes (increased ALT, AST), of liver weight increase in both sexes concurrently with histopathological findings. This NOAEL defined in rats for chronic toxicity on liver may be used for risk characterisation since based on liver changes unrelated to specific peroxisome proliferation effects.

For kidney effects a NOAEL of 88 mg/kg/d, derived from the above study (Aristech, 1994) and based on increased kidney weights in both sexes, is used for the risk characterisation. Effects on the rat kidneys were described in the majority of the rat studies as slight to

moderate changes in the kidney weight with sometimes modifications of the physiological parameters more marked in males (increases of blood urea and/or blood creatinine concentrations, proteins in urine and decrease of the specific gravity). Histologically, there was an increase in frequency/severity of chronic progressive nephropathy at quite low doses, but specifically in males. Histological features are consistent with the specific male rat nephropathy irrelevant to humans, namely alpha 2u globulin nephropathy, also hypothesised in the DIDP risk assessment where in some repeated dose studies, mineralisation of the renal papilla (Aristech, 1994), or sporadic kidney neoplasms (Lington, 1997) are observed in male rats. It is assumed that accumulation of protein droplets occurs rapidly, whereas continued chemical treatment results in additional histological changes in male rats: papillary mineralisation and atypical hyperplasia, leading to renal adenomas or carinomas on prolonged exposure. Moreover Caldwell (Caldwell et al. 1999b) demonstrated by immunohistochemical techniques that exposure to DINP results in a dose-dependent alpha 2u-globulin accumulation in male rat kidneys (Exxon, 1986) and is likely the mechanism for kidney tumours seen only in male rats administered high dietary levels (1.2%) of DINP (Aristech, 1994).

In mice, there was also progressive nephropathy observed at tremendous doses: tubular nephrosis at 20,000 ppm (5,700 mg/kg/d) in a 13-week study (Hazleton, 1992) and granular pitted/rough kidneys in female mice at 8,000 ppm (1,900 mg/kg/d) in a chronic/carcinogenicity study (Aristech, 1995). In dogs renal effect was observed at the high dose of 2% (2,000 mg/kg/d), and consisted of hypertrophy of kidney tubular epithelial cells in few animals in the 13-week study (Hazleton, 1971). No kidney effects were reported in monkeys up to 2,500 mg/kg/d in a 13-week study (Huntington life Sciences, 1998).

Haematological effects: in rats, in some studies, slight anemia was described but this finding was not clearly treatment-related. In other studies, there were slight increases of leukocytes, as previously, this effect was not clearly treatment-related.

Concerning effects on reproductive organs in adult rats, in the 2-year study with Fischer 344 rats (Exxon, 1986) there was a statistically significant increase in relative testis weights at the high dose of 0.6% (307 mg/kg/d in males) associated with a slight, but not statistically significant, increase (13%) of absolute testis weight. In some sub-acute and sub-chronic studies with Fischer 344 rats (Bio/dynamics 1982a; b; c; Hazleton, 1991a) relative testis weights were statistically significantly increased with or without concurrent increase of absolute testis weights and decrease of body weights at quite high doses (about 1,500 mg/kg/d in one week study, about 700 mg/kg/d in 13-week studies).

In mice, a NOAEL of 1,500 ppm (276 mg/kg/d) can be derived from a 104-week study (Aristech, 1995c) based on testicular weight decrease observed from 4,000 ppm (742 mg/kg/d) and is used for the risk characterisation. In addition, in 4-week and a 13-week repeated-dose mouse studies, slight decreases of testis weight were observed accompanied by the presence of abnormal / immature sperm forms in the epididymes at doses of 6,500 mg/kg/d and 5,700 mg/kg/d, respectively (25,000 and 20,000 ppm). In those mouse studies (4-week and 13-week) effects were noted in uterus (hypoplasia and absence of endometrial glands) and in ovaries (absence of corpora lutea suggesting an arrest of ovulation) at doses of 20,000 ppm and 25,000 ppm.

It should be noted that in the 13-week study in monkeys (Huntingdon Life Sciences, 1998) no changes were reported in testis weight and testis microscopic examination. In addition, there were no treatment-related changes in estradiol and testosterone concentrations assessed. In conclusion, for effects on the liver and kidneys, a NOAEL of 88 mg/kg/d is determined in rats regarding results found in a chronic / carcinogenic study (Aristech, 1994). For reproductive organs, a NOAEL of 276 mg/kg/d can be derived from a mouse study. These NOAELs will be used for the risk characterisation. The effects seen in the repeated dose toxicity tests do not justify classification Xn R48 according to the EU classification criteria." (EC 2003a)

Commentary to the EU Risk Assessment
In the EU Risk Assessment several repeated dose studies were evaluated (Table 4.14), and a NOAEL of 88 mg/kg/day was determined based on non-peroxisome proliferation related liver effects in a 2-year dietary chronic/carcinogenicity study in rats (Aristech 1994, also reported by Butala et al. 1996 and Covance 1998). It is not clearly stated in the EU Risk Assessment why it chose the NOAEL of 88 mg/kg bw/day based on the Aristech study over the lower (15 mg/kg) from the other relevant long term toxicity study by Exxon (1986).

Table 4.14 Summary of repeated dose toxicity studies (from EC 2003a)

Protocol, animal, substance	NOAEL	LOAEL	Reference
One-week prechronic oral study, rat (68515-48-0)		2% (1,700 mg/kg) increased kidney, liver weights, macroscopic liver changes decreased cholesterol, triglycerides at 2%	Bio/dynamics (1982a)
2-week Study, rat (CAS 68515-48-0)		25 mg/kg DOS activity (peroxisome proliferation), 1500 mg/kg liver weight increased	Hüls (1992)
2-week Study, rat (CAS 28553-12-0)	25 mg/kg bw/day DOS activity	75 mg/kg/d DOS activity peroxisome proliferation, 1,500 mg/kg/d increased liver weights	Hüls (1992)
2-week or 4-week Studies, rat (CAS not specified)	1000 ppm in rats	12000 ppm increased in liver weight, PBOx, DNA synthesis. Inhibition GJIC	Smith et al. (1999; 2000)
3-week Study, rat (CAS 68515-48-0)		0.6% (607-639 mg/kg bw/day) increased liver weights, lauric acid 11 and12-hydroxylase, decreased cholesterol, triglycerides	Bibra (1985)
4-week Study, rat, (CAS 28553-12-0)		0.2% (125 mg/kg/d) increased catalase at 0.2% increased CAT activity at 0.2%	Midwest Res. Inst. (1981a)
13-week Study, rat (CAS 68515-48-0)	150 mg/kg bw/day	500 mg/kg bw/day increased kidney, liver weights with	Hazleton (1971b)

		hepatocytic hypertrophy	
13-week Study, rat (CAS 68515-48-0)	0.1% (77 mg/kg bw/day)	227 mg/kg bw/day increased kidney, liver weights decreased cholesterol levels from 0.3%	Bio/dynamics (1982b)
13-week Study, rat (CAS 68515-48-0)		0,3% (201 – 251 mg/kg bw/day) increased kidney, liver weights, decreased triglycerides and urine chemistry changes	Bio/dynamics (1982c)
13-week Study, rat (CAS 28553-12-0)		152 – 200 mg/kg bw/day decreased triglyceride levels at 3000 decreased alimentary peripheral fat deposits in hepatocytes at 3,000 ppm	BASF (1987f)
13-week Study, rat (CAS 28553-12-0)		176-218 mg/kg bw/day increased liver and kidney weight at 2,500 ppm	Hazleton (1991a)
13-week Study, rat (CAS not specified)	1000 ppm (60 mg/kg bw/day)	1,000 ppm (60 mg/kg bw/day) increased incidence of mononuclear cell infiltration and mineralisation of the kidneys in male 3,000 ppm (180 mg/kg bw/day) slight signs of anemia in males, increased relative kidney weight and slight decreased of globulin in females	Hazleton (1981a)
Chronic toxicity 2-year Study, rat (CAS 68515-48-0)	**0.03% (15-18 mg/kg bw/day)**	**0.3% (152-184 mg/kg bw/day) increased liver and kidney weights**	Exxon (1986) Hazleton (1986a); **Lington et al. (1987); Lington et al. (1997)**

		increased incidence of non-neoplasic changes	
2-year Study, rat (CAS 68515-48-0)	**1500 ppm (88-103 mg/kg bw/day)**	**6000 ppm (358-442mg/kg bw/day) increased kidney weights in both sexes; histopathological findings in males; liver toxicity (increased ALT, AST values, liver weights and histopathological findings)**	**Aristech (1994)** **Aristech (1995b); Covance (1998); Moore (1998a); Butala et al. (1996)**
2-year study, rat, (CAS 71549-78-5, similar to DINP, commercial name Santicizer 900)		500 ppm (27-33 mg/kg bw/day) minimal to slight focal hepatocellular necrosis in treated males.	Bio/dynamics (1986)
2-week or 4-week studies, mice, (CAS not specified)	500 ppm	6000 ppm hepatic changes increased in liver weight, PBOx, DNA-synthesis; Inhibition GJIC	Smith et al. (1999; 2000)
4-week, mice Study, (CAS 28553-12-0)	3000 ppm (635 mg/kg bw/day)	635-780 mg/kg/d increased liver weight (absolute and relative) at all doses6000 ppm (1300 mg/kg bw/day) decreased absolute/relative testes weight	Hazleton (1991b)
13-week Study, mice, (CAS 28553-12-0)	for liver effect 1500 ppm (365 mg/kg bw/day) 4000 ppm (972 mg/kg bw/day)	4000 ppm (972 mg/kg bw/day).Enlarged liver increased absolute and relative liver weight 10000 ppm (2,600 mg/kg bw/day)decreased (absolute) epididymis and testes weight	Hazleton (1992)

2-year Study, mice, (CAS 68515-48-0)	500 ppm (90.3 mg/kg bw/day) 1500 ppm (276 mg/kg bw/day)	1500 ppm (275-335 mg/kg bw/day) increased kidney and liver weights 4000 ppm (742 mg/kg bw/day) decreased absolute and relative (to brain weight) testis weight	Aristech (1995c); Covance (1998); Moore (1998b)
13-week Study, dog, CAS 68515-48-0		37 mg/kg bw/day increased AST in females. Increased liver weight	Hazleton (1971a)
2-week Study, *cynomolgus* monkey, (CAS not specified)	500 mg/kg bw/day	No changes in body weight, organ weights, urinalysis, haematology, clinical chemistry, no inflammation or necrosis in the liver, kidney and testes, no change in hepatic peroxisomal β-oxidation or replicative DNA synthesis. No effect on GIJC *in vitro*.	Hall et al. (1999)
13-week Study, marmoset, (CAS not specified)	500 mg/kg bw/day	2500 mg/kg bw/day minor changes: decreased body weight, decreased body weight gain	Huntington Life Sciences (1998)
6 week study, rabbit, dermal	0.5 ml/kg (500 mg/kg)	2.5 ml/kg/d slight or moderate erythema and slight desquamation	Hazleton (1969)

4.4.6.1.2 Risk assessments from other international organizations and bodies

EU bodies

CSTEE 2001

In its opinion on the results of the EU Risk Assessments for DINP (EC 2003a[19]), the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE

[19] The CSTEE reviewed an earlier draft of the EU Risk Assessment for DINP (EC 2003a).

2001a) concluded as follows: "*The RAR uses this NOAEL [88 mg/kg bw/day] for risk characterisation purposes because liver pathology unrelated to peroxisome proliferation was seen in this study. However, in the Exxon study (Lington et al. 1997) using Fischer 344 rats, there was a dose-related increase in relative organ weights of liver and kidney in both males and females with a clear NOAEL of 15(males)-18(females) mg/kg/d. In addition to the increased liver and kidney weights at the LOAEL of 152(females)-184(males) mg/kg/d, males had increased incidences of spongiosis hepatis and serum levels of alkaline phosphatase and transaminases. Spongiosis hepatis, which is a focal degeneration of parasinusoidal cells, presumably not related to peroxisome proliferation, was also seen in males in the Aristech study (Moore, 1998). The NOAEL/LOAEL for spongiosis hepatis are the same in the two studies as for the increases in liver and kidney weights. The RAR does not use the NOAEL/LOAELs for spongiosis hepatis for risk characterisation.*

After the RAR was finalised, the Chronic Hazard Advisory Panel on DINP of the US Consumer Product Safety Commission has reported its risk characterisation using spongiosis hepatis as the critical endpoint […]. The CPSC have calculated the benchmark dose corresponding to a 5% response for this effect to be 12 mg/kg/d based on the Exxon study and 15 mg/kg/d on the Aristech study [sic[20]]. The CSTEE finds the approach applied being scientifically sound and supports the use of the benchmark dose for spongiosis hepatis as the starting point of the risk characterisation."

EFSA 2005

The European Food Safety Authority (EFSA) did not carry out a new extensive risk assessment to come to its opinion on use of DINP in food contact materials (EFSA 2005a). EFSA concluded that the pivotal toxicological effect for DINP was considered to be the hepatic changes seen in various studies. The NOAEL/LOAEL for spongiosis hepatis in the two studies (Aristech 1994 and Exxon 1986) was said to be the same as for the increases in liver and kidney weights. The Panel referred to the two-year chronic toxicity study in rats of Exxon (1986), and the increased incidence of spongiosis hepatis, accompanied by increased serum levels of liver enzymes and increases in absolute and relative liver and kidney weights in both sexes. The Panel agreed to use the NOAEL of 15 mg/kg/day from the Exxon study based on non-peroxisomal proliferation-related chronic hepatic and renal effects in establishing a tolerable daily intake (TDI) of 0.15 mg/kg bw.

SCCP 2007

The Scientific Committee on Consumer Products (SCCP) has not carried out a new hazard assessment on DINP. In the opinion of the SCCP on ten phthalates in cosmetic products (SCCP 2007), a TDI for DINP of 0.15 mg/kg/day derived by EFSA was used. EFSA had used a NOAEL of 15 mg/kg/day and an uncertainty factor of 100.

SCHER 2008

The Scientific Committee on Health and Environmental Risks (SCHER) has not carried out a new hazard assessment on DINP. In the opinion of SCHER on phthalates in school supplies (SCHER 2008), a TDI of 0.15 mg/kg bw/day was assumed on the basis of a NOAEL of 15 mg/kg bw/day for non-peroxisome

[20] The value of 15 mg/kg bw/day was the BMD_{05} value obtained with pooled Exxon (1986) and Aristech (1994) data using the maximum likelihood estimate (MLE) method and with the 88 mg/kg bw/day outlier omitted. The BMD_{05} value obtained with Aristech (1994) data only using the maximum likelihood estimate (MLE) method and with the 88 mg/kg bw/day outlier omitted was 69 mg/kg bw/day.

proliferation-related chronic hepatic and renal effects, and an uncertainty factor of 100.

SCENIHR 2008

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has not carried out a new hazard assessment on DINP. In the opinion of SCENIHR on the safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk (SCENIHR 2008), the publicly available information (published papers) and information submitted by stakeholders were considered. The SCENIHR pointed to the differences in opinion concerning the selection of the appropriate starting point for repeated dose toxicity between the EU Risk Assessment on DINP (EC 2003a) and the CSTEE opinion (CSTEE 2001a), but SCENIHR's opinion does not state which of the NOAELs they actually supported (both the 15 and 88 mg/kg bw/day are presented in the comparison table of DEHP with alternatives, although the latter value was between brackets).

The United States

CHAP 2001 and US CPSC 2010a

The Chronic Hazard Advisory Panel (CHAP) of the United States Consumer Product Safety Commission risk assessed DINP (CHAP 2001). The conclusions concerning repeated dose effects were essentially taken over in a renewed assessment on DINP by the United States Consumer Product Safety Commission staff (US CPSC 2010a). The CHAP (2001) used spongiosis hepatis as the critical endpoint.

The CHAP (2001) made a statistical data analysis on the data of both studies, which was supportive to the idea that the differences seen in the incidences of spongiosis hepatis between the studies could be explained by the sample frequency (see also the discussion below). The CHAP considered that in addition the larger number of animals used in the Exxon study might have had contributed. The CHAP derived a 5^{th}-percentile benchmark dose (BMD_{05}) of 12 mg/ kg bw/day based on the Exxon study and determine an ADI of 0.012 mg/kg bw/day by applying an uncertainty factor of 100 (see also section 4.4.11).

US EPA 2005b

In September 2000, the United States Environmental Protection Agency (US EPA) proposed to include DINP in the list of chemicals under the so-called Section 313 of the Emergency Planning and Community Right-to-Know Act, supported by a Technical Review (US EPA 2005a). In response to comments on the proposal and the Technical Review, US EPA revised its hazard assessment for DINP. The Revised Technical Review of DINP (US EPA 2005b) identified the liver and kidney as the target organs for DINP-induced toxicity. Spongiosis hepatis was considered as the most sensitive non-cancer response.

According to US EPA (2005b), there is general agreement that spongiosis hepatis develops from the perisinusoidal (Ito) cells of the liver whereas the peroxisome proliferating-induced liver tumors and the other toxic effects of DINP on the liver involved hepatocytes. In the absence of information that clearly indicates a species-specific mode of action for development of spongiosis hepatis, the occurrence of this lesion in rats was assumed to be relevant to humans.

US EPA argued that the apparent differences in spongiosis hepatis observed in the two rat studies (Aristech 1994 and Exxon 1986) may have reflected differences in the range of doses tested and that the methodological differences

between the studies may account for the greater incidence of foci of cellular alteration and foci of spongiosis hepatis observed in the Exxon (1986) study.

US EPA identified a NOAEL of 15 and 88 mg/kg bw/day from resp. Exxon (1986) and Aristech 1994, "*based on indications of serious liver damage (i.e. statistically significant increased incidence of spongiosis hepatis and increased liver weight and liver enzyme activities) in male rats chronically exposed to DINP for two years*". US EPA did not indicate which value of the two studies would be preferred for risk assessment.

Concerning kidney effects, the agency stated that renal tumors appear to be due to alpha-2u-globulin nephropathy which is not considered relevant to humans. It was claimed that nevertheless an increased incidence and severity of nephropathy in female mice (Aristech 1995c as cited in EC 2003a) and increased kidney weight in female rats (Aristech 1994) could not be explained by an alpha-2-globulin mode of action. It is not clear which NOAELs US EPA would assume for kidney effects.

Industry

ECPI 2009
In its "Review of Recent Scientific Data on Di-isononyl Phthalate (DINP) and Risk Characterisation for its use in Toys and Childcare articles", the European Council for Plasticizers and Intermediates (ECPI) used a NOAEL of 88 mg/kg bw/day for risk assessment based on the Aristech 1994 study (ECPI 2009). ECPI claimed that "*spongiosis hepatis, is a spontaneous degenerative change seen in aging rats without a counterpart in human hepatic pathology and therefore is not relevant to the assessment of risk in children*". The ECPI concluded that consequently the use of spongiosis hepatis is not an appropriate endpoint for the selection of the NOAEL. ECPI based the selection of the NOAEL of 88 mg/kg bw/day on "*liver toxicity at higher doses consisting of hepatic biochemical changes (increased ALT, AST), of liver weight increase in both sexes concurrently with histopathological findings*" and based on increased liver kidney weights in both sexes in the Aristech 1994 study. Furthermore, ECPI argued that based on dose spacing used in the Aristech 1994 and studies, the 'true' NAEL (No Adverse Effect Level) would lie between 15 and 152 mg/kg bw/day (a NOAEL of 15 and LOAEL of 152 mg/kg bw/day in the Exxon 1986 study and a NOAEL of 88.3 and LOAEL of 359 mg/kg bw/day in the Aristech 1994 study).

ACC 2005
The American Chemistry Council Phthalate Esters Panel (ACC 2005) submitted a series of comments in reaction to the US EPA proposal and its Technical Review (see US EPA 2005a,b above).

With respect to repeated dose toxicity the following can be noted.

ACC argued that there is no evidence that spongiosis hepatis occurs in humans, but that there would be evidence on the contrary. It was stated that the literature had not reported spongiosis-like lesions in humans (with the exception of Bannash and Zerban (1997)) and no spongiosis hepatis or similar lesions were reported in an investigation of nearly 200 diseased human livers. ACC considered spongiosis hepatis as merely a histological observation with unknown pathological consequences. ACC attributed liver enlargement to peroxisome proliferation, and thus claimed it would not be relevant for humans. Liver enzyme changes were claimed not to have been linked to any pathological changes and would thus not

themselves be toxicological effects in rats, let alone humans. In addition primate data was argued not to show adverse liver effects.

In relation to renal toxicity, ACC argued that no kidney weight changes were seen in studies with primates, and claimed that other kidney effects were either of speculative toxicological significance or rodent-specific. ACC concluded that the kidney-related observations did not provide a basis for concluding that DINP can reasonably be anticipated to cause kidney toxicity in humans.

Table 4.15 Summary of NOAEL/LOAEL and BMD values determined by different organisations and bodies for DINP

NOAEL/ BMD	LOAEL (mg/kg bw/day)	Critical effects at LOAEL	**Organisa-tion/body**	Study description	Study reference
NOAEL 88 (mg/kg bw/day)	358	Increased liver and kidney weights in both gender Increased ALT and AST in both gender Liver histopathology in males (Enlargement and/or granular/pitted/rough changes)	**EC 2003 ECPI 2009**	2-year dietary, rat (F344), doses: 0, 29, 88, 359, 733 mg/kg bw/day for males and 0, 36, 109, 442, 885 mg/kg bw/day for females	Aristech (1994)
NOAEL 15 (mg/kg bw/day)	152	Non-peroxisomal proliferation-related chronic hepatic and renal effects	**EFSA 2005** SCCP 2007 SCHER 2008	2-year dietary study, rat (Fisher 344), dose levels 0, 15, 152, 307 mg/kg bw/day for males and 0, 18, 184, 375 mg/kg bw/day in females	Exxon (1986)
BMD (5%) **12**	not relevant	Spongiosis hepatis	**CHAP 2001 CSTEE 2001a**	2-year dietary study, rat (Fisher 344), dose levels 0, 15, 152, 307 mg/kg bw/day for males and 0, 18, 184, 375 mg/kg bw/day in females	Exxon (1986)

4.4.6.1.3 New studies/description key studies

ECHA did not identify new repeated dose toxicity studies with DINP. ECHA has however re-assessed the two key repeated dose toxicity studies with DINP (Aristech 1994 and Exxon 1986, see Table 4.16) as part of the review and therefore the studies are described here. For completeness, another 2-year study is described briefly since the study seems to give some additional weight of evidence to the spongiosis hepatis findings in the two key studies (Bio/dynamics study 1986).

Table 4.16 The key repeated dose toxicity studies with DINP

Study type	**Dosing**	**NOAEL**	**Effects**	**References**
Chronic toxicity 2-year dietary rat (Fisher 344)	CAS 68515-48-0 dietary concentrations of 0, 0.03, 0.3 and 0.6% (w/w) M ca. 0, 15, 152, 307	0.03% (15-18 mg/kg bw/day)	0.3% M Increased: - incidence of spongiosis hepatis; - serum levels of liver	**Exxon (1986)** Also referred to as: Hazleton (1986a); Lington et al. (1987); and

guideline: equivalent or similar to OECD Guideline 452 GLP compliant	mg/kg bw/day F ca. 0, 18, 184, 375 mg/kg bw/day Dose groups n = 220 (110/sex)		transaminases (1.5-2x); - relative and absolute spleen weights (61%). M and F Increase of absolute and relative liver (11-19%) and kidney weights (5-10%). Other histopathological findings indicating liver toxicity.	Lington et al. (1997)
Chronic toxicity 2-year dietary rat (Fisher 344) guideline: equivalent or similar to OECD Guideline 452 GLP compliant	CAS 68515-48-0 dietary concentrations of 0, 0.05, 0.15, 0.6 and 1.2% and high dose (1.2%) recovery group M ca. 0, 29, 88, 359, 733 mg/kg bw/day, high dose recovery group 637 mg/kg bw/day F ca. 0, 36, 109, 442, 885 mg/kg bw/day, high dose recovery group 774 mg/kg bw/day Dose groups n = 70-85/sex and a recovery high-dose group of 55/sex	0.15% (88-103 mg/kg bw/day)	0.6% M Increased incidence of spongiosis hepatis. M and F Increased: - serum levels of liver transaminases; - absolute and relative liver and kidney weights. Other histopathological findings indicating liver toxicity.	**Aristech (1994)** Also referred to as: Aristech (1995b); Covance (1998); Moore (1998a); and Butala et al. (1996)

Exxon 1986 (key study)
In the 2-year dietary study by Exxon (1986) a NOAEL for chronic hepatic and renal effects of 0.03% could be set, corresponding to doses of 15 and 18 mg/kg/day for males and females, respectively. See Table 4.16 for a description of the main study facts. The NOAEL was based on slightly decreased survival in females, increased incidence of spongiosis hepatis (See Table 4.17) and increased serum levels of liver transaminases in males (1.5-2x), increase of absolute and relative liver (11-19%) and kidney weights (5-10%) in both sexes and an increase of relative and absolute spleen weights (61%) in males at 0.3%.

The liver weight increases in the mid dose males were 11-19% with no significant increase at 18 months. In the high dose males, the liver weight increases were 10-30% with the increase getting smaller towards the end of the study. The kidney size increases were 5-10% in the mid dose, without a significant increase at 18 months. The kidney weight increase was 8-20% in the high dose males. In females, the liver weight enlargement in the mid dose was 11 to 16% and about 30% in the high dose group. The mid dose kidney weight increase was 7-10% and at high dose were 9-14%.

The rats had enlarged livers and a small increase in liver enzymes (ALT, AST, ALP) induced by dietary DINP during periods up to 18 months. There was an absence of histopathological lesions and (2) clinical chemistry tests for the liver

were not increased to more than 1.5 to 2 – fold in the mid and high dose groups when compared to the control animals. ALT and ALP were elevated in the mid dose only at month 24 in males. AST was increased in the mid dose at 6 and 12 months but not at 24 months and in males only. In the high dose males, AST was increased at 6, 12 and 18 but not 24 months. High dose ALT values were elevated at 6 and 18 months but not at 12 or 24 months. Male AP was increased at 6 and 24 months males only.

Table 4.17 Incidence of selected liver lesions in the Exxon (1986) study (table 5-2 from US CPSC 2010a)

Lesion	Percent DINP in Feed			
	0	**0.03**	**0.3**	**0.6**
Males				
Number examined	81	80	80	80
Focal necrosis	10	9 (0.51)	16 (0.13)	26 (0.0018)
Spongiosis hepatis	24	24 (0.55)	51 (1.2×10^{-5})	62 (7.3×10^{-10})
Hepatopathy associated with leukemia[c]	22	17 (0.25)	34 (0.030)	33 (0.043)
Centrilobular to midzonal hepatocellular enlargement	1	1 (0.75)	1 (0.75)	9 (0.0084)
Females				
Number examined	81	81	80	80
Focal necrosis	13	11 (0.41)	19 (0.15)	21 (0.082)
Spongiosis hepatis	4	1 (0.18)	3 (0.51)	4 (0.63)
Hepatopathy associated with leukemia	16	18 (0.42)	24 (0.093)	33 (0.0025)
Centrilobular to midzonal hepatocellular enlargement	1	0 (0.50)	0 (0.50)	11 (0.0024)

[a] All deaths includes terminal sacrifice and spontaneous deaths.
[b] Numbers in parentheses are Fisher's exact p-values for pair–wise comparisons with controls.
[c] Increased mortality observed in mid- and high-dose rats of both sexes was principally due to monocytic leukemia. Leukemia was also diagnosed in 3 mid-dose males and 2 high-dose males and in 1 control, 2 mid-dose and 3 high-dose females where death was ascribed to other causes. The leukemia was associated with a variety of hepatic alterations including regenerative nodules, focal necrosis and spongiosis hepatis. Other hepatocellular changes were included under the term "hepatopathy associated with leukemia." (Exxon 1986).

Aristech 1994 (key study)
In the 2-year dietary study by Aristech (1994) a NOAEL of 0.15% could be set, corresponding to doses of 88 and 109 mg/kg bw/day for males and females, respectively. See Table 4 for a description of the main study facts. The NOAEL was based on hepatic biochemical changes (increased ALT and AST), absolute

and relative liver and kidney weight increases in both sexes and other histopathological findings indicating liver toxicity. There was also a clear increase of spongiosis hepatis in males at the two highest doses, i.e. at ca. 359 and 733 mg/kg bw/day (see Table 4.18).

The study by Aristech (1994) concluded that of the non-neoplastic findings, liver and kidney were the target organs. The increase of liver weights (absolute and relative) and liver transaminase levels (ALT and AST) in both sexes from the mid-high dose of 0.6% associated with histopathological evidence (enlargement and/or granular/pitted/rough changes) of liver toxicity led to the same NOAEL as that for kidney effects based on increased absolute/relative weights in both sexes from the mid-high dose of 0.6%, from week 79 up to termination.

Table 4.18 Incidence of selected liver lesions in the Aristech (1994) study (table 5-3 from US CPSC 2010a)

Lesion	Percent DINP in Feed					
	0	**0.05**	**0.15**	**0.6**	**1.2**	**1.2[b]**
Males						
Number examined	80	50	50	65	80	50
Individual cell degeneration/necrosis	0	0	0	1 (0.45)	5 (0.0029)	0
Focal necrosis	3	1	0	0	3 (0.69)	4 (0.27)
Spongiosis hepatis	5	5	2	14 (0.0068)	21 (0.0051)	9 (0.037)
Diffuse hepatocellular enlargement	0	0	0	0	37 ($3.1x10^{-14}$)	0
Increased cytoplasmic eosinophilia	0	0	0	0	43 ($4.4x10^{-17}$)	0
Females						
Number examined	80	50	50	65	80	50
Individual cell degeneration/necrosis	0	0	0	0	1 (0.50)	0
Focal necrosis	1	3 (0.17)	4 (0.078)	4 (0.13)	7 (0.034)	3 (0.17)
Spongiosis hepatis	0	0	0	1 (0.45)	2 (0.25)	0
Diffuse hepatocellular enlargement	0	0	0	0	52 ($6.6x\ 10^{-22}$)	0
Increased cytoplasmic eosinophilia	0	0	0	0	45 ($4.3x\ 10^{-18}$)	0

[a] Numbers in parenthesis are Fisher's exact p-values for pair-wise comparisons with controls.
[b] Treated for 78 weeks, followed by a 26 – week recovery period.

Bio/dynamics study 1986 (supportive study)
The study was conducted with a substance referred to as "Santicizer 900" (also referred to as DINP-A). The CAS number was reportedly not available from the study (EC 2003a).

The CHAP (2001) mentions the following in this respect: *"Although Santicizer 900 (CAS# 71549-78-5) was never commercialized, samples were analyzed in Germany (BASF AG). According to Mr. Patrick Harmon of BASF, "Santicizer 900 is chemically similar to the current BASF product Palatinol(R) N and to other DINPs such as CAS# 28553-12-0 that are produced from isononanol made via the dimerization of butene" (Harmon, personal communication to the US CPSC, 2000)."*

No distinction is made in the EU Risk Assessment between the different types of DINP (EC 2003a). The differences in toxicology between CAS 68515-48-0 and CAS 28553-12-0 DINP substances appear to be small, although only a few studies are available that directly compare the toxicity between the two substances (US CPSC 2010a). There is no 2-year chronic toxicity study available to confirm the similarity in toxicology.

The EU Risk Assessment for DINP reads-across results from DIDP, and vice-versa (EC 2003a,b). DINP and DIDP are said to be structurally similar by the industry Chemical Safety Report(s) for DIDP. The Chemical Safety Report(s) for DIDP and DINP use read-across from one another.

In the light of the above acceptance by both authorities and industry to use read-across between different forms of DINP and between DINP and DIDP, it seems unreasonable to disregard a 2-year chronic toxicity study carried out with a substance that is reportedly chemically similar to CAS 28553-12-0 (DINP-2). Nevertheless, ECHA acknowledges the limitations and therefore only considers the study as supportive evidence for the effects seen in the two key studies for repeated dose toxicity carried out both with CAS 68515-48-0 (DINP-1), in particular the significantly elevated spongiosis hepatis incidences at the mid (43% incidence) and high dose (46% incidence) in males. See Table 4.19 for a description of the study.

Table 4.19 Description of the Bio/dynamics (1986) study

Study type	Dosing	NOAEL	Effects	References
Chronic toxicity 2-year dietary Sprague Dawley CD rats guideline: not indicated in EC (2003a) or CHAP (2001) GLP : not indicated in EC (2003a) or CHAP (2001)	Santicizer 900 (CAS 71549-78-5 according to CHAP 2001) dietary concentrations of 0, 500, 5 000, 10 000 ppm M ca. 0, 27, 271 and 553 mg/kg bw/day F ca. 0, 33, 331, 672 mg/kg bw/day Dose groups n = 70/sex/dose level	27 mg/kg bw/day	Spongiosis hepatis was significantly elevated at the mid and high dose in males. In males, the incidence of focal necrosis was significantly elevated at the low and high doses, while the mid dose was non-significantly elevated.	Bio/Dynamics (1986)

Table 4.20 Incidence of selected liver lesions in the Bio/dynamics (1986) study (table 5-4 from US CPSC 2010a)

Lesion	Percent DINP in Feed			
	0	0.05	0.5	1.0
Males				
Number examined	70	69	69	70
Focal necrosis	5	17 (0.0042)	11 (0.086)	23 (0.0001)
Spongiosis hepatis	16	11 (0.89)	30 (0.0079)	32 (0.0036)
Females				
Number examined	70	70	70	70
Focal necrosis	10	15 (0.19)	7	10 (0.60)
Spongiosis hepatis	4	3	6 (0.38)	11 (0.051)

[a] Numbers in parenthesis are Fisher's exact p-values for pair-wise comparisons with controls.

4.4.6.1.4 Discussion

In both the Aristech (1994) and Exxon (1986) studies statistically significant increases in the incidences of spongiosis hepatis, serum levels of liver transaminases, liver and kidney weights were observed at the LOAEL determined in the studies (358 and 152 mg/kg bw/day respectively). As discussed in section "New studies/description key studies", the spongiosis hepatis seen at 271 mg/kg bw/day in male rats in the Bio/dynamics study (1986) can be considered to be supportive to the two key studies.

In both key studies the incidence of spongiosis hepatis in females was similar and low for all control and treated doses. Spongiosis hepatis is observed as a clear male effect in both studies, which is consistent with the observed male predilection for spongiosis hepatis upon exposure to xenobiotics (see Box 1). The increased incidence of spongiosis hepatis was clearly treatment related and dose-dependent in both the Aristech (1994) and Exxon (1986) study.

On request by Chemical Manufacturers Association, both key studies were peer reviewed by Dr. Deborah Bannas and by a panel of pathologists (Pathology Working Group, PWG) with the purpose to determine the incidence of lesions, amongst others spongiosis hepatis (EPL 1999).Table 4.21 presents the consensus diagnose of the Pathology Working Group (PWG) for spongiosis hepatis incidences. Table 4.22 gives a more detailed report of the spongiosis hepatis incidences reported by the PWG. In the recovery group (637 mg/kg bw/day) of the Aristech study, the incidence of spongiosis hepatis was 20% (about twice the incidence in the control group, but not statistically significant).

Table 4.21 Spongiosis hepatis (males) in Exxon (1986) and Aristech (1994) as determined by consensus in the PWG (EPL 1999)

Study	Dose (mg/kg bw/day)							
	0	15	29	88	152	307	359	733
Exxon (1986)	27%	30% NOAEL			64% LOAEL	77,5%		
Aristech (1994)	11%		12%	6% NOAEL			33% LOAEL	47%

Table 4.22 Incidence of spongiosis hepatis at terminal sacrifice as reported by the Pathology Working Group (EPL 1999) (Table VI-2 from the CHAP 2001)

Study	Group No.	Dietary Concentration (mg kg $^{-1}$ d^{-1})	Sex	Number of Rats	Number of Rats with Spongiosis Hepatis	Incidence (%)
Exxon (1986)	1	0	M	81	22	27.2
			F	81	4	4.9
	2	0.03% (M: 15; F:18 mg kg $^{-1}$ d^{-1})	M	80	24	30.0
			F	81	1	1.2
			F	81	1	1.2
	3	0.30% (M: 152; F:184 mg kg $^{-1}$ d^{-1})	M	80	51	63.8**
			F	80	3	3.8
	4	0.60% (M: 307; F:375 mg kg $^{-1}$ d^{-1})	M	80	62	77.5**
			F	80	4	5.0
Aristech (1994)	1	0	M	55	6	10.9
			F	55	0	0
	2	0.05% (M: 29; F:36 mg kg $^{-1}$ d^{-1})	M	50	6	12.0
			F	50	0	0
	3	0.15% (M: 88; F:109 mg kg $^{-1}$ d^{-1})	M	50	3	6.0
			F	50	0	0
	4	0.60% (M: 359; F:442 mg kg $^{-1}$ d^{-1})	M	55	18	32.7**
			F	55	1	1.8
	5	1.2% (M: 733; F:885 mg kg $^{-1}$ d^{-1})	M	55	26	47.3**
			F	55	2	3.6
	6	1.2% Recovery (M: 637; F:774 mg kg $^{-1}$ d^{-1})	M	50	10	20.0
			F	50	0	0

** P.L. ≤ 0.01, one –tailed Fisher's exact test (Babich and Greene 2000)

If both the Exxon and the Aristech studies would have been conducted under exactly the same conditions, the dose response could have been expected to be the same in both studies. Consequently, the true NAEL (No Adverse Effect Level) could be argued to be somewhere between 88 and 152 mg/kg/day. The combined dose levels of male rats are 0, 15 (NOAEL Exxon), 29, 88 (NOAEL Aristech), 152 (LOAEL Exxon), 307, 359 (LOAEL Aristech) and 733 mg/kg bw/day (see Table 4.22). Thus, the next dose level after the NOAEL of 88 mg/kg bw/day from the Aristech study is the 152 mg/kg bw/day from the Exxon study, where effects on liver and kidney were observed. A similar dose spacing argument has been followed by ECPI (2009). The PWG (EPL 1999) seems to have followed the same reasoning when proposing a NOAEL of 1500ppm in male rats (88 mg/kg bw/day from Aristech) and 3000ppm in females (184 mg/kg bw/day from Exxon).

However, the Exxon and the Aristech studies were not conducted under exactly the same conditions.

As can be seen from the Table 4.21 and Table 4.22, the incidences of spongiosis hepatis were uniformly less in the Aristech (1994) study as compared to the Exxon (1986) study. Both studies used Fischer 344 rats, and used the same type of DINP (DINP-1) although from two different suppliers.

According to the PWG, different sampling of the liver lobes was used in the two studies. The Exxon (1986) study evaluated 4-5 liver sections, whereas the Aristech (1994) study only examined 1-2 sections. The PWG noted that this makes comparison of the two studies difficult (EPL 1999). CHAP (2001) suggested that the differences between the studies can be explained by the different sampling of the liver lobes in the two studies since the chance of finding a microscopic lesion such as spongiosis hepatis in an affected liver increases with the amount of liver sections examined.[21] According to CHAP (2001), the Exxon (1986) study would have had roughly four times greater chance of observing spongiosis hepatis if present.

CPSC staff, with CHAP input, conducted a statistical data analysis (Babich and Greene 2000 in CHAP 2001). Statistical comparison on Aristech (1994) data scaled to 4 slides (Figure 4.6), and another comparison with Exxon (1986) data scaled to one slide (Figure 4.7) showed no statistical significant difference between the data sets. This indicates that if the studies would have used the same liver sampling methodology, the dose response would have been the same. In other words, as the Exxon study used more liver slides, it is considered more sensitive than the Aristech study.

[21] This observation is shared by ECPI (2012d): *"From the PWG report, it is apparent that the number of sections evaluated greatly influence the incidence of spongiosis hepatis observed.".*

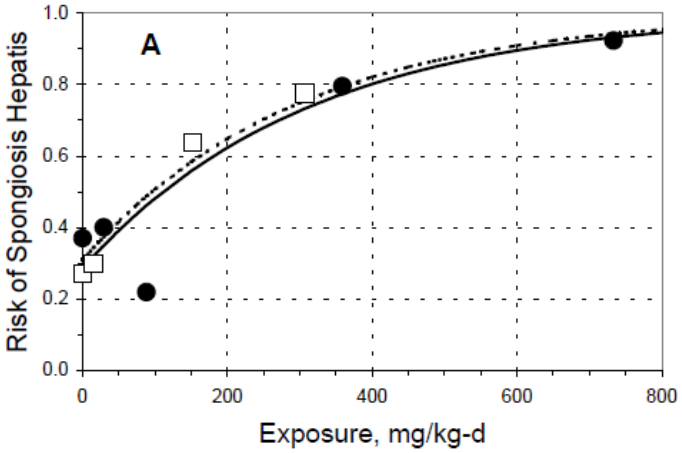


Figure 4.6 Data shown correspond to an assumption of 4 slides per liver, using data from Exxon (open squares) and Aristech data scaled to 4 slides/liver (solid circles). The pooled data from both studies were fit to multistage-polynomial models using nonlinear least-squares (solid curve). The dashed curve represents the pooled data without Aristech observation at 88 mg/kg bw/d (figure reproduced from the CHAP 2001).

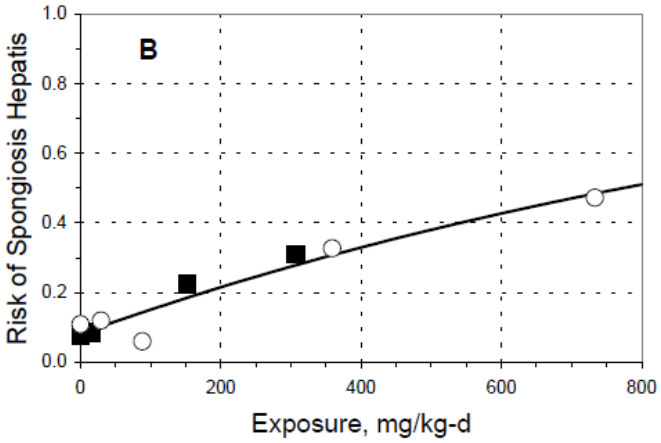


Figure 4.7 Data shown correspond to an assumption of 1 slide per liver, using data from Exxon scaled to 1 slide/liver (solid squares) and unmodified data from Aristech (open circles). The pooled data from both studies were fit to multistage-polynomial models using nonlinear least-squares (solid curve). The dashed curve represents the pooled data without Aristech observation at 88 mg/kg bw/d (figure reproduced from the CHAP 2001).

Furthermore, the 88 mg/kg bw/day dose group in the Aristech (1994) study could be seen as an outlier with its incidence of spongiosis hepatis being lower than that of the control, and this data point did not fit the dose response curve of the pooled data of the Exxon and the Aristech studies (CHAP 2001). This is an additional argument against the selection of a NOAEL of 88 mg/kg bw/day for repeated dose toxicity.

Regarding the choice between benchmark dose approaches or NOAELs the Chapter R.8 of the ECHA Guidance on information requirements and chemical safety assessment gives the following advice:

"*Advantages of this approach* [BMD approach] *over the NOAEL are:*

- the Benchmark dose is derived using all experimental data and reflects the dose-response pattern to a greater degree;
- the Benchmark dose is independent of predefined dose levels and spacing of dose levels;
- the Benchmark approach makes more reasonable use of sample size, with better designs resulting in higher Benchmark doses.

A disadvantage of this new method [BMD approach] *is the uncertainty with respect to the reliability of the approach in case results are obtained from toxicity studies performed according to the requirements defined in current guidelines […]. For the derivation of reliable dose-response relationships, the classical study design of three dose groups and a vehicle control group is far from ideal, especially if one considers the unfavourable possibility that in a particular experiment, adverse effects may be identified only at the highest dose level.*

An improved benchmark model fit would be possible by increasing the number of dose groups without changing the total number of animals in the test. However, such a change in study design would generally no longer allow a proper derivation of a NOAEL. Thus, in practice, the NOAEL and the benchmark concepts appear to be incompatible.

The BMD can be used in parallel to derivation of a NOAEL or as an alternative when there is no reliable NOAEL. In addition, the Benchmark dose (BMD) approach is, when possible, preferred over the LOAEL-NAEL extrapolation".

Several benchmark doses (BMD) have been calculated using different methods and input data (CHAP 2001). Spongiosis hepatis incidence data were fit to polynomial (multistage) and lognormal (log probit) dose-response models with pooled and unpooled data and with or without the 88 mg/kg bw/day group of the Aristech (1994) study. The polynomial model gave somewhat lower benchmark doses than the lognormal model. With all models, better fits were obtained with the Exxon (1986) data and fits to the Aristech (1994) data improved substantially when the data point at 88 mg/kg bw/day was omitted.

Exxon (1986) data pooled with Aristech (1994) data (after upscaling the incidences of spongiosis hepatis to the expected outcome if four slides per liver had been assessed) gave a BMD_{05} of 15 mg/kg bw/day when using the maximum likelihood estimate (MLE) method and omitting the 88 mg/kg bw/day group. This result is comparable to a BMD_{05} of 12 mg/kg bw/day (MLE) using the Exxon (1986) data only[22]. CHAP (2001) used the latter approach to set an ADI of 0.120 mg/kg bw/day using a combined uncertainty/adjustment factor of 100.

The liver is not a homogeneous organ, and therefore a very small number of samples is not representative for the whole liver. So if one accepts that the difference in the observed incidence of spongiosis hepatis is largely resulting from the difference in sample method, and that it is justified to multiply (upscale) the Aristech incidence data from one slide to predict the incidences that would have occurred if four slides would have been assessed, the pooled BMD_{05}-level of 15 mg/kg bw/day is indeed supportive to the NOAEL of 15 mg/kg bw/day identified

[22] ECHA guidance Chapter R.8 mentions the following in respect to how a BMD5 copares to a NOAEL: *"A BMD calculated as the lower confidence limit of the dose that produces a response of 5% (BMD5) has, on average, been proposed to be comparable to a NOAEL (WHO, 2000). If other BMD indicators are used, e.g. a BMD10, it should be considered on a case-by-case basis whether an additional dose-response assessment factor is needed.".*

from the Exxon study. The CSTEE (2001a) supported the use of both the 12 and 15 the mg/kg bw/day BMD for spongiosis hepatis as the starting point of the risk characterisation.

Taking the ECHA guidance into account it was concluded that a NOAEL approach would be the most appropriate in the present evaluation. The Exxon study contains only three dose groups, and to allow for pooling of the Exxon and the Aristech dataset (as was done by the CHAP 2001) several assumptions have to be made. The pooled BMD_{05}-level of 15 mg/kg bw/day may however be supportive to the NOAEL of 15 mg/kg bw/day identified from the Exxon study.

4.4.6.1.5 Conclusion

A NOAEL of 15 mg/kg bw/day with a LOAEL of 152 mg/kg bw/day (Exxon 1986) and a NOAEL of 88 mg/kg/day with a LOAEL of 359 mg/kg bw/day (Aristech 1994) were identified in the two key repeated dose toxicity studies based on statistically significant increases of incidence of spongiosis hepatis together with other signs of hepatotoxicity.

Considering the dose spacing in those studies, in particular the Exxon study with 152 mg/kg as the next higher dose, the true NAEL (No Adverse Effect Level) could be argued to be somewhere between 88 and 152 mg/kg/day. However, there were differences in methodology between both studies: the Exxon (1986) study evaluated 4-5 liver sections, whereas the Aristech (1994) study examined 1-2 sections. Also, the 88 mg/kg bw/day dose group in the Aristech (1994) study could be seen as an outlier.

As a result of the methodological difference, the Exxon (1986) study was considered the most appropriate to use. Thus a NOAEL of 15 mg/kg bw/day was selected for repeated dose toxicity of DINP. This conclusion was supported by RAC (ECHA 2013a). RAC however noted that the NAEL could be higher given the large dose spacing in the Exxon study.

4.4.6.2 DIDP

4.4.6.2.1 EU Risk Assessment conclusion

The following cites the 'Summary of repeated dose toxicity' from the EU Risk Assessment:

"The target organ for oral sub-acute and sub-chronic DIDP toxicity in animals (rodent and dog) appears to be the liver (increased liver weights and significant changes in liver proliferator peroxisome enzyme activities in rodent). It is clear that NOAELs derived from rat studies are related to peroxisome proliferation liver effects, which are generally considered to be species-specific. Humans are very likely far less sensitive than rats.

In dogs, a NOAEL of 15 mg/kg/d is identified in a 13-week oral study (Hazleton Laboratories, 1968b) for effects in the liver (swollen and vacuolated hepatocytes at higher doses). In spite of the limitations underlined, it is proposed to consider this result in the risk characterisation. Indeed, the dog appears to be, in this case, a more relevant species for human risk assessment: dog is considered not responsive or refractory to peroxisome proliferation. It should be noticed that this study was only considered from a qualitative point of view in the NTP draft monograph
(NTP, 1999).

Since the dog study cited above is not very reliable, a NOAEL of 60 mg/kg/d is identified in rats from a standard 90-day study based on increased relative liver weight in female rats at the higher dose (BASF, 1969). Changes in kidney weights are also observed in repeated dose toxicity tests but in a non-consistent way and with no concurrent histopathological changes. Renal damages are only observed in the two-generation study (about 12 weeks) from 100-200 mg/kg/d, but only in male rats and a strong presumption of a specific male rat effect is assumed.

The effects seen in the repeated dose toxicity tests do not justify classification Xn with R48 according to the EU classification criteria." (EC 2003b)

<u>Commentary to the EU risk assessment</u>

The studies used for NOAEL setting in the EU Risk Assessment (2003) are subchronic and pre-guideline/non-GLP studies. Since the peroxisome proliferation effects in the liver of rodents are generally seen as species-specific, dog was considered to be a more relevant species for human risk assessment. A NOAEL of 15 mg/kg bw/day was set on the basis of the dog study by Hazleton (1968b). However, because of the limitations of the dog study, a NOAEL of 60 mg/kg bw/day from a 90-day rat dietary test was considered in addition (BASF 1969). Both studies were taken further in the risk assessment, i.e. risk characterisation was carried out for both NOAELs separately.

Table 4.23 Summary of repeated dose toxicity studies from the EU Risk Assessment for DIDP (EC 2003b).

Species	Substance Purity/dose	Treatment	Clinical Signs	Biochemistry/ Haematology	Microscopy	NOAEL	Reference
Young Rat Fisher 344	DIDP 99.84% pure 0-0.3-1.2-2.5%	21 days in diet	No change	↓ serum triglycerides and cholesterol (1.2 and 2.5% males) ↑ cyanide – insensitive palmitoyl – CoA oxidation 1.2%-2.5% (female and male) ↑ 11 and 12 – hydroxylation of lauric acid 0.3-1.2-2.5% (males) ↑ 12 hydroxylation of lauric acid 2.5% (females)	↓ hepatocyte cytoplasmic basophilia 1.2 and 2.5% ↑ eosinophilia (2.5%) No testicular change	0.3% (304 mg/kg/d for males) (264 mg/kg/d for females)	BIBRA (1986)
Rat Fisher 344	0.020-0.05-0.1-0.3-1%	28 days in diet	-	↑ cyanide insensitive palmitoyl CoA oxidation from 0.1%	No testicular atrophy	0.05% (57 mg/kg/d)	Lake et al. (1991)
Rat Sprague Dawley	Palatinol Z 5,000 and 10,000 ppm	28 days in diet	No change	No Change	No change	5,000 ppm (600 mg/kg/d for males) (1,100 mg/kg/d for females)	BASF (1969a)
Rat Sprague Dawley	**Palatinol Z 800-1,600-3,200 & 6,400 ppm**	**90 days in diet**	**No change**	**No Change**	**No change**	**3,200 ppm (200 mg/kg/d for males)*[1] 800 ppm (60 mg/kg/d for females)**	**BASF (1969b)**
Rat	DIDP-FDA Grade 0.05-0.3-1%	90 days in diet	No change	No Change	No change	0.3% (200 mg/kg/d)	Hazleton (1968)
Dog (beagle)	**0.05 - 0.3 - 1%**	**90 days in diet**	**No change**	**No Change**	**Swollen and vacuolated hepatocytes from 0.3%**	**0.05% (15 mg/kg/d)**	**Hazleton (1968)**

*[1] Note that according to CSTEE 2001b the EU Risk Assessment was inconsistent, and the use of relative liver weights (as for females) instead of absolute weights would have led to a LOAEL of 55 mg/kg bw/day in male rats.

4.4.6.2.2 Risk assessments from other international organizations and bodies

EU bodies

CSTEE 2001b
In its opinion on the results of the EU Risk Assessment for DIDP (EC 2003b[23]), the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE 2001b) argued that on the basis of an overall evaluation of the repeated dose studies a NOAEL based on increased liver weight was most likely to be in the range of 25 mg/kg bw/day. This conclusion was based on the considerations below.

Concerning the apparent sex difference in the rat study that was used for selection of the NOAEL in the EU Risk Assessment (EC 2003b), with a NOAEL of 60 mg/kg bw/day in females and 200 mg/kg bw/day in males (BASF 1969b), the CSTEE noted that *"[i]t is not clear why in male rats the NOAEL is based on absolute liver weight and in females on relative liver weight, especially since relative liver weights were increased at all dose levels tested in male rats. If relative liver weights are used also in males a LOAEL of 55 mg/kg bw/day is derived."*

Concerning the dog study (Hazleton 1968b), the CSTEE pointed to the small number of animals used, and to the conclusions of the NTP-CERHR Expert Panel that it was not possible to derive a NOAEL for this study. CSTEE thus assumed a LOAEL of 77 mg/kg bw/day and 88 mg/kg bw/day for male and female dogs, respectively, thus indicating a NOAEL of approximately 25 mg/kg bw/day.

The CSTEE concluded that the LOAEL of 77-88 mg/kg bw/day from the dog study, a NOAEL of 60 mg/kg bw/day (female rats) and a LOAEL of 55 mg/kg bw/day (male rats based on relative liver weight), and a NOAEL of 57 mg/kg bw/day from an additional 28-day study in rats (Lake et al. 1991) justified using a NOAEL of 25 mg/kg bw/day for risk characterisation.

EFSA 2005b
The European Food Safety Authority (EFSA) did not carry out a new extensive risk assessment to come to its opinion on use of DIDP in food contact materials (EFSA 2005b). In a previous opinion of the Scientific Committee for Food (SCF), a group Tolerable Daily Intake (g-TDI) for DIDP and DINP of 0.15 mg/kg/day was set based on peroxisome proliferation in rodent liver. However, since there is a scientific consensus that liver peroxisome proliferation in rodents is not relevant for human risk assessment, and the dog was considered to be a species with low sensitivity to peroxisome proliferation, it was concluded that the NOAEL of 15 mg/kg bw/day from the dog study (Hazleton 1968b) should be used in the risk assessment. Making use of this NOAEL and of an uncertainty factor of 100, a TDI (not a g-TDI) of 0.15 mg/kg bw was derived for DIDP.

SCCP 2007
The Scientific Committee on Consumer Products (SCCP) did not carry out a new hazard assessment on DIDP to form its opinion in 2007. In the opinion of the SCCP on ten phthalates in cosmetic products (SCCP 2007), a TDI for DIDP of 0.15 mg/kg bw derived by EFSA was used.

SCHER 2008
The Scientific Committee on Health and Environmental Risks (SCHER) did not carry out a new hazard assessment on DIDP to form its opinion in 2008. In the opinion of SCHER on phthalates in school supplies (SCHER 2008), a TDI of 0.15 mg/kg bw was assumed on the basis of a NOAEL of 15 mg/kg bw/day for liver effects in dogs (considered as a non-sensitive species to peroxisome proliferation), and an uncertainty factor of 100.

[23] The CSTEE reviewed an earlier draft of the EU Risk Assessment for DIDP (EC,2003b).

The United States

US CPSC 2010b
The toxicity review on DIDP carried out by the United States Consumer Product Safety Commission staff (US CPSC 2010b) considered the liver as a target in subchronic studies. This conclusion was established on the observed increased liver weight, increased peroxisomal enzyme levels and histological changes (swelling and vacuolation of hepatocytes). Based on systemic effects, the US CPSC considered DIDP as a probable toxicant (according to the definition of "toxic" under the Federal Hazardous Substance Act).

US CPSC used a NOAEL of 15 mg/kg bw/day (Hazleton 1968b) and a safety factor of 100 (10 for animal to human extrapolation, and 10 to account for sensitive populations) to derive an ADI of 0.15 mg/kg bw for liver effects.

US CPSC regarded the kidney also to be affected by DIDP, considering the kidney weight increases. The lowest LOAEL was 13.36 mg/kg bw/day for females and 17.37 for males from a study by Cho et al. (2008). US CPSC derived these LOAELs[24] to calculate an ADI of 0.13-0.17 mg/kg bw for kidney effects, using a safety factor of 100.

Comment
Note that the US CPSC 2010b did not yet take account of the corrigendum concerning the dose-levels to Cho et al. 2008, leading to higher dose-levels in that study, namely 21.86, 110.25 and 479.20 mg/kg bw/day for males and 22.92, 128.18 and 619.59 mg/kg bw/day for females (Cho et al. 2010).

Industry

ExxonMobil 2011c
In a "Statement relevant to the re-evaluation of DIDP in toys and childcare articles as required by Directive 2005/84/EC" issued by ExxonMobil in 2009 as part of the registration dossier (ExxonMobil 2009) it was argued that the 90 day rat study (BASF 1969b) was the correct study to use for the derivation of the DNEL. After the upwards correction (Cho et al. 2010) of the dose levels from a new carcinogenicity study (Cho et al. 2008), the statement by Exxon was updated (ExxonMobil 2011c, not part of the registration dossier). The updated statement proposed that not the 90 day rat study, but rather the study by Cho et al. would be used in the DNEL derivation. ExxonMobil (2009) assumed a NOEL (No Observed Effect Level) of 2000 ppm, corresponding to 110.25 and 128.18 mg/kg bw/day for males and females respectively. In the registration dossier, the Cho et al. (2008) *"study is rated a "2" because it used appropriate test methods but no information is available concerning application of a test guideline or compliance with GLP"* (ExxonMobil 2009).

In ExxonMobil (2011c) the dog study (Hazleton 1968b) was not considered appropriate for risk assessment. It was reported that the REACH registration dossier had attributed a reliability code of "3" (Not reliable) to the study accompanied by the statement that *"[t]he study is rated a "3" because it was conducted prior to the development of test guidelines and GLP, used only 3 animals per sex per dose and no statistical evaluation was conducted*".

[24] It is unclear why US CPSC did not derive NOAELs (in stead of LOAELs) of 3.03 and 4.13 mg/kg bw/day as starting points for the ADI derivation considering the uncorrected dose estimates of 0, 0.53, 3.03, 13.36 for females and 0, 0.85, 4.13, 17.37 mg/kg bw/day for males.

Table 4.24 Summary of NOAEL/LOAEL values determined by different organisations and bodies for DIDP

Organisation/ body	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical effects at LOAEL	Study description	Study reference
EC 2003b* EFSA 2005 US CPSC 2010b SCCP 2007 SCHER 2008	**15**	75	Liver weight increase accompanied by swollen and vacuolated hepatocytes at higher doses	90 days dietary, dog (Beagle), 3 males and 3 females per group, doses: approx. 0, 15, 75, 300 mg/kg bw/day for both sexes.	Hazleton (1968b)
EC 2003b*	**60****	120	Dose-related increase of relative liver weights	90-day dietary, rat (Sprague Dawley), doses: 0, 55, 100, 200, 400 mg/kg bw/day for males and 0, 60, 120, 250, 500 mg/kg bw/day for females	BASF (1969b)
CSTEE 2001b**	**25**	/	Liver effects	Combination of the results from three studies	Hazleton (1968b) BASF (1969) Lake et al. (1991)
US CPSC 2010b	**/**	**13.36-17.37**	Significant increase in relative kidney weight in males and females	2 year dietary, rat (Fischer 344), doses: **0, 0.85, 4.13, 17.37** mg/kg bw/day for males and **0, 0.53, 3.03, 13.36** mg/kg bw/day for females***	Cho et al. (2008)
Exxon 2011c	**NOEL 110 mg/kg bw/day**	**/**	Peroxisome proliferation	2 year dietary, rat (Fischer 344), doses: **0, 21.86, 110.25, 479.20** mg/kg bw/day for males and **0, 22.92, 128.18, 619.59** mg/kg bw/day for females	Cho et al. (2008) as corrected by Cho et al. (2010)

*The EU Risk Assessment studies took both the rat and the dog studies further in the risk assessment.
**Note that according to CSTEE 2001b the EU Risk Assessment was inconsistent, and the use of relative liver weights (as for females) instead of absolute weights would have led to a *LOAEL* of 55 mg/kg bw/day in male rats.
*** Note that the US CPSC 2010b did not yet take account of the corrigendum concerning the dose-levels to Cho et al. 2008, leading to much higher dose-levels in that study, namely 21.86, 110.25 and 479.20 mg/kg bw/day for males and 22.92, 128.18 and 619.59 mg/kg bw/day for females (Cho et al. 2010).

4.4.6.2.3 New studies/description key studies

BASF 1969b
BASF (1969b as cited in EC 2003b) conducted a 90-day rat dietary study (n = 20/sex/dose, in control group n = 10/sex) with Palatinol Z (DIDP CAS number 26761-40-0) in concentrations of 800, 1600, 3200 and 6400 ppm (equivalent to about 55, 100, 200 and 400 mg/kg bw/day in males and 60, 120, 250 and 500 mg/kg bw/day in females).

In male rats, from day 77 onward there was a slight lag in body weight development in the 1600, 3200 and 6400 groups with normal food uptake. This finding was still present after the 21-day reversibility period (6400 ppm group).

In males, absolute liver weight was increased in all experimental groups but significantly only at the highest dose (12.19 versus 9.31 g in controls), relative liver weight was significantly higher in all experimental groups but without dose-effect relationship and still statistically significantly elevated at the end of the 21-day post observation period. In female absolute liver weights were significantly increased at the two highest doses (6.04 and 6.94, respectively, versus 5.20 g in controls), and not statistically at 1600 ppm but by 9.8% (5.71 vs. 5.20 g). However, this increase was dose-related, and relative liver weights significantly increased from 1600 ppm.

In males relative kidney weights were increased (statistically significant) in all treated group but without dose-effect relationship. In females relative kidney weights were increased only at 1600 and 3200 ppm but not at 6400 ppm.

No pathological changes were observed.

The EU Risk Assessment derived a NOAEL of 200 mg/kg bw/day for males based on increased absolute liver weight. However, as pointed out by CSTEE (2001b), a LOAEL of 55 mg/kg bw/day could be derived based on relative liver weights.

In females, a NOAEL of 60 mg/kg bw/day can be assumed based on dose-related increase of relative liver weights.

Hazleton 1968b
Hazleton (1968b as cited in EC 2003b) performed a 13-week study with Beagle dogs (n = 3/sex/dose) with dietary DIDP levels of 0.05% (about 15 mg/kg bw/day), 0.3% (about 75 mg/kg bw/day) and 1% (about 300 mg/kg bw/day).

Three dogs in the highest diet level showed slight to moderate body weight losses, these findings did not appear to be related to decreased food consumption except for one animal. All clinical laboratory values were generally within accepted limits and comparable between all groups. Gross necropsy examinations did not reveal any consistent compound-related alterations.

A dose-related increase in the mean liver weights was observed: 212; 220; 287 versus 190 g in females and 248; 274; 317 versus 253 g in males at 0.05, 0.3 and 1%, respectively. As a consequence of the individual variations and the small numbers of animals used (3/sex/dose), no statistics were available. The increased liver weights were however accompanied by minor microscopic changes: slight to moderate swelling and vacuolation of hepatocytes revealed by microscopic examination at 0.3% and 1%. There was a lack of a significant dose-response in severity and number of animals affected for these microscopic changes (which is not surprising considering the low number of animals used). Liver enzyme activities (ALT and AST) and BSP clearance were not modified suggesting a that hepatocellular injury was minimal.

A NOAEL of 15 mg/kg bw/day can be derived for this study on the basis of hepatic effects. However, the large limitations of the study need to be emphasised.

Cho et al. 2008, 2010

In a 2-year rodent carcinogenicity study on DIDP (CAS No. 26761-40-0), assessing also non-neoplastic systemic effects (Cho et al. 2008 and the corrigendum Cho et al. 2010) Fischer 344 rats were fed diets containing 0.04, 0.2 or 0.8% DIDP (n = 52/sex/group). The average daily doses for male rats of 21.86, 110.25 and 479.20 mg/kg bw/day and for females 22.92, 128.18 and 619.59 mg/kg bw/day. Table 4.25 and Table 4.26 show the results as presented in Cho et al. (2008). The study is non-guideline and non-GLP, but ECHA considers the study to be relevant, reliable (Klimisch code 2 "reliable with restrictions") and adequate (in line with ExxonMobil 2009).

Relative kidney and liver weights of both males and females were significantly increased in the high dose group.

Histopathological changes of the liver included spongiosis hepatis which was not present in the control animals but observed at a low but statistically significant incidence in all male treatment groups. It was not reported how many sections per liver were examined.

A decrease in altered cell foci in the liver was observed in male rats at the mid and high dose and in females in the high dose group. Liver necrosis was significantly increased in the 0.8% exposed males and females. Microgranuloma of the liver was increased in the 0.04, 0.2 and 0.8% males but without a clear dose response. Effects that occurred only in the highest dose group of males included increased oval cell hyperplasia, hypertrophy and peliosis (multiple cyst-like, blood-filled cavities). In females, liver microgranulomas and altered cell foci were decreased in the highest dose group.

Hyaline cast, interstitial nephritis and chronic progressive nephropathy of the kidney were decreased in the 0.8% exposed females compared to the control. Male kidneys had increased mineralisation and interstitial nephritis in the highest dose group. Inflammation of the kidney was increased in the 0.04 and 0.2% treatment groups in the females but not in the highest dose group.

The relative kidney and liver weights of both males and females exposed to 0.8% were significantly increased.

The incidence of extramedullary hematopoiesis in the spleen was decreased in the 0.2 and 0.8% exposed females and 0.8% exposed males when compared to the control. C-cell hyperplasia in the thyroid gland was increased in the 0.04 and 0.2% exposed female and 0.2% male treatment groups. Inflammation of the prostate and hyperplasia were increased in the 0.04 and 0.2% in males, respectively. There was also degeneration of the prostate in the lowest dose group. Medullary hyperplasia of the adrenal glands was increased in the 0.04 and 0.2% males.

No information on clinical chemistry or blood analyses was reported.

Table 4.25 Incidence of non-neoplastic lesions in male rats exposed to DIDP for 2 years (Cho et al. 2008).

	Control	0.04%	0.2%	0.8%
Number examined	**49**	**48**	**49**	**39**
Adrenal glands				
Cortical hyperplasia	3[a] ((6.1)[b])	2(4.2)	0* (0.0)	0* (0.0)
Medullary hyperplasia	0(0.0)	10** (20.8)	6** (12.2)	0(0.0)
Kidney				
Mineralization	0 (0.0)	1(2.1)	1(2.0)	13** (33.3)
Interstitial nephritis	2(4.1)	2(4.2)	5(10.2)	7** (17.9)

Liver				
Fatty change	4(8.2)	6(12.5)	1(2.0)	0* (0.0)
Altered cell foci	27(55.1)	19(39.6)	18* (36.7)	3** (7.7)
Oval cell hyperplasia	1(2.0)	3(6.3)	2(4.1)	6* (15.4)
Hypertrophy	0(0.0)	0(0.0)	1(2.0)	4* (10.3)
Microgranuloma	1(2.0)	5* (10.2)	6* (12.2)	4* (10.3)
Necrosis	3(6.1)	7(14.6)	5(10.2)	8* (20.5)
Peliosis	1(2.0)	0 (0.0)	2(4.1)	4* (10.3)
Spongiosis hepatis	0 (0.0)	3* (6.3)	3* (6.1)	5** (12.8)
Prostate				
Degeneration	10(20.4)	20* (41.7)	16(32.7)	8(20.5)
Hyperplasia	4(8.2)	11* (22.9)	12* (24.5)	6(15.4)
Inflammation	5(10.2)	7(14.6)	11* (22.4)	8(20.5)
Spleen				
Extramedullary hematopoiesis	9(18.4)	5(10.4)	5(10.2)	2* (5.1)
Red pulp hyperplasia	3(6.1)	1(2.1)	0* (0.0)	1(2.6)
Thyroid gland				
C-cell hyperplasia	14(28.6)	8(16.7)	7* (14.3)	11(28.2)

*,**Significantly different ($P < 0.05$ and $P < 0.01$) from the vehicle control group by the poly-3 test.
a Number of animals with lesion.
b Values between brackets are expressed as percentage.

Table 4.26 Incidence of non-neoplastic lesions in female rats exposed to DIDP for 2 years (Cho et al. 2008).

	Control	0.04%	0.2%	0.8%
Number examined	**49**	**47**	**47**	**40**
Kidney				
Hyaline cast	6[a] ((12.2)[b])	11(23.4)	8(17.0)	1* (2.4)
Inflammation	0(0.0)	4* (8.5)	4* (8.5)	0(0.0)
Interstitial nephritis	6(12.2)	3(6.4)	3(6.4)	1* (2.5)
Chronic progressive nephropathy	9(18.4)	4(8.5)	10(21.3)	0* (0.0)
Liver				
Altered cell foci	31(63.3)	26(55.3)	27(57.4)	17* (42.5)
Inflammation	2(4.1)	8* (17.0)	11** (23.4)	3(7.5)
Microgranuloma	10(20.4)	6(12.8)	12(25.5)	3* (7.5)
Necrosis	2(4.1)	4(8.5)	6(12.8)	9** (20.9)
Spleen				
Extramedullary hematopoiesis	15(30.6)	11(23.4)	3** (6.4)	5* (12.5)
Thyroid gland				
C-cell hyperplasia	15(30.6)	24* (51.1)	25* (53.2)	10(25.0)

*,**Significantly different ($P < 0.05$ and $P < 0.01$) from the vehicle control group by the poly-3 test.
a Number of animals with lesion.
b Values between brackets are expressed as percentage.

Cho et al. 2011
After a dose ranging study of 4 weeks in rasH2 wild-type mice, a 26-week carcinogenicity study was conducted on CB6F1-Tg rasH2 mice dietary exposed to 0.1, 0.33 and 1% of DIDP (CAS No. 26761-40-0). Wild-type mice were administered diets containing the vehicle control, and 1% DIDP (n = 15/sex/group). The daily intake was not reported. The rasH2 mouse is a hemizygous transgenic mouse carrying the human prototype c-Ha-ras gene with its own promoter/enhancer. The rasH2 mouse is sensitive to genotoxic and non-genotoxic carcinogens and is considered to be a promising model for short-term carcinogenicity studies (Kanno 2003).

Amongst others, relative liver and kidney weights of both males and females were significantly increased in the high dose group of the transgenic mice and in the liver of both sexes and in the kidney of females of the wild-type mice. Liver weights were significantly increased in the mid-dose males as well.

The liver showed significantly higher incidences in parenchymal inflammation, diffuse hepatocyte hypertrophy with eosinophilic granules (significant in all dose levels and in both sexes), focal necrosis, pigmented hepatocytes/Kupffer cells or prominent Kupffer cells in rasH2 and wild-type mice. These effects are seen in males and/or females starting from the low dose group or higher.

In the kidney a higher incidence of tubular basophilia and tubular hyperplasia in the high dose male rasH2 and in the male wild-type mice was observed.

The incidence of other spontaneous lesions was reported to be within the historical ranges.

4.4.6.2.4 Discussion

The EU Risk Assessment recognized that both the dog (Hazleton 1968b) and rat (BASF 1969) studies used for risk characterization had several shortcomings (EC 2003b). Both studies were subchronic studies, and were non-guideline and non-GLP. In addition, the dog study used only 3 animals per group. Furthermore, the liver effects seen in short term studies in the rat might be attributed to peroxisome proliferation which is generally seen as rat-specific (see Box 1 for more details).

From a second 90-day rat study by Hazleton (1968a as cited in EC 2003b) a NOAEL of 0.3% (approx. 200 mg/kg bw/day) could be derived (LOAEL of 1%, approx. 650 mg/kg bw/day). As the NOAEL of 200 mg/kg bw/day in the Hazleton (1968a) study is higher than the LOAEL in the BASF (1969) study (120 mg/kg/day), it is the BASF study that determines the overall NOAEL for a study of that duration in the rat. It could be noted that furthermore the Hazleton study used 10 animals per dose group versus 20 in the BASF study, and 3 dose levels versus 4 dose levels respectively.

The supporting document to the opinion of RAC on the draft version of this report (ECHA 2013b) did not consider the Hazleton study appropriate for DNEL calculation. This is consistent with the approach in the EU RAR for DIDP (EC 2003b). Industry argued that the 90 day rat study (Hazleton 1968a) should be used in addition to determine DNELs for DIDP as it was conducted with the substance which is produced commercially within the EU today (CAS number 68515-49-1). ECHA (2013b) did not consider this argument to be convincing, noting that read-across between the two forms of DINP and between the two forms of DIDP is general practise both by industry and by regulatory authorities, and furthermore, imported articles might contain either form of DIDP.

Due to the shortcomings of the dog study (Hazleton 1968b) and the 90 day study in rat (BASF 1969), the new 2-year rodent carcinogenicity study on DIDP by Cho et al. (2008, 2010) might be chosen as the key study for repeated dose effects. Spongiosis hepatis was the most

sensitive effect in this study and was significantly increased in all male treatment groups. In the high dose group also several other significant changes in the kidney and liver were observed (479.20 for males and 619.59 mg/kg bw/day for females). There was no spongiosis hepatis observed in the control or female animals. Spongiosis hepatis was thus observed as a clear male effect, which is consistent with the findings for DINP and with the observed male predilection for spongiosis hepatis upon exposure to other xenobiotics (see Box 1).

Spongiosis hepatis was observed in 3/48 (6.3%) animals in the lowest exposure group, in 3/49 (6.1%) animals in the mid dose group and in 5/39 (12.8%) animals in the high dose group. The incidences observed in the Exxon (1986) and Aristech (1994) were uniformly higher (including controls) than in the Cho et al. (2008, 2010) study, although all three studies were 2-year studies using Fischer 344 rats. It is not clear from the Cho et al. publications how many sections per liver were examined, and whether perhaps different criteria were used to diagnose spongiosis hepatis. Historical control data from the Cho et al. laboratory and the Japanese Charles River breeder was not reported.

Of 9 NTP carcinogenicity studies with F344 rats reported by Karbe and Kerlin (2002), the control incidences were 0-34%. An additional study by David (2000) reported a control incidence of spongiosis hepatis of 3/80 (4%) in F344 rats. In the 2 year studies on DINP the incidences of spongiosis hepatis in the untreated Fischer rats were 24/81 (30%) animals (Exxon 1986) and 5/80 (6%) (Aristech 1994). The fact that the incidences observed in Cho et al. are within the historical control range and could perhaps be due to chance findings casts some doubts on the reliability of these findings.

On the other hand, it can be argued that historical control data from NTP studies has limited relevance for evaluating the 0% incidence of spongiosis hepatis in the control of the Korean study by Cho et al. (2008, 2010): the uniformly low incidences seen in the study might be a consequence of e.g. the different breeder, possible differences in diagnosis criteria, possible differences in amount of liver sections taken[25]. Thus, it could be argued that the statistical significance of the study is a relevant finding that is related to the dose. Moreover, a zero control incidence might not be a deviating finding, as the range of historical controls was reported to be 0-34% from 12 studies with F344 rats, of which 1 showed a zero control incidence. Furthermore, the observation of spongiosis hepatis after treatment with DIDP can be expected to be treatment related as the structurally related substance DINP clearly induced spongiosis hepatis at comparable dose levels.

RAC (2013a) was of the opinion that as it can be questioned whether the LOAEL in the Cho et al. (2008, 2010) study is dose related and considering that the relevance of spongiosis hepatis for humans has been questioned (see Box 1), it was not recommended to exclusively use the Cho et al. study to identify the repeated dose NOAEL for DIDP. Instead, RAC (2013a) proposed

[25] There is scientific consensus that concurrent control group is the most appropriate and accurate in determining whether proliferative lesions are treatment related as opposed to historical control data (Keenan et al. 2009). According to EMEA (2002 as reported in Keenan et al. 2009): *"When HCD are used, they should be from the same strain of animal and the same testing laboratory, performed during the five years prior to the study in question. If data from the literature are considered to be informative, they may also be added."*
Keenan et al. (2009) pointed out that *"Study design–related parameters such as laboratory, species/strain, route of administration, vehicle, feed, feeding practices, study duration, and housing have a potential to impact study outcomes and control findings. These parameters should be considered when selecting the appropriate studies for the HCD.".* Moreover *"Pathology practices, including necropsy and trimming procedures and application of diagnostic criteria, can impact study data and HCD. HCD are best if these factors are standardized. HCD from the laboratory that conducted the study under review will likely be more comparable than HCD compiled from several laboratories."*
Directive 91/414/EEC (plant protection products) specifies that *"Where submitted, historical control data should be from the same species and strain, maintained under similar conditions and should be from contemporaneous studies."*

to use the NOAELs from the 90 days studies in dogs (Hazleton 1968b, NOAEL 15 mg/kg bw/day) and rats (BASF 1969, NOAEL 60 mg/kg bw/day) in addition.

4.4.6.2.5 Conclusion

As the three key studies with DIDP each have certain limitations, a weight of evidence approach using a LOAEL of 22 mg/kg bw/day based on spongiosis hepatis in a 2-year study in rat (Cho et al. 2008, 2010), a NOAEL of 15 mg/kg bw/day based on hepatic effects in a 90 days study in dogs (Hazleton 1968b) and a NOAEL 60 mg/kg bw/day[26] based on in a 90 days study in rats (BASF 1969b) could be followed as recommended by RAC (ECHA 2013a,b).

[26] As pointed out by CSTEE (2001b), a LOAEL of 55 mg/kg bw/day could be derived based on relative liver weights in males in the 90 days study in rats in stead of a NOAEL of 60 mg/kg bw/day (BASF 1969b). Thus, using a NOAEL of 60 mg/kg bw/day is a less cautious approach. This was not discussed by RAC (2013a,b).

Box 1 Liver pathology related to DINP and DIDP

Liver pathology related to DINP and DIDP

The liver is an important target organ for DINP and DIDP toxicity. In this section addresses some of the most common histopathological findings together with some considerations regarding mechanism.

Spongiosis hepatis

Spongiosis hepatis is observed as a treatment related effect in long term toxicity studies conducted with DINP and DIDP (Exxon 1986; Aristech 1994; Cho et al. 2008, 2010). Spongiosis hepatis was also identified as a treatment related effect in a chronic study with rats with DEHP (NOAEL of 2500 ppm or 147 mg/kg bw/day, David et al. 2000) and with several other xenobiotics (see 'Substances that induce spongiosis hepatis' below). As this effect is predominantly found in aging male rats and certain fish species, the relevance of spongiosis hepatis for humans has been discussed. A second issue concerns whether or not spongiosis hepatis should be regarded as a pre-neoplastic lesion. A brief update is given here of some of the recent literature related to spongiosis hepatis, including the publication by Karbe and Kerlin (2002), the comments on this review by Bannash (2003), and the response to Bannash's comments by Karbe and Kerlin (2004).

Terminology

The terms and interpretations of spongiosis hepatis used by different authors are not consistent. Karbe and Kerlin (2002) recommended using the terms spongiosis hepatis and cystic degeneration as synonyms and strongly objected to the term "spongiotic pericytoma" (Kerlin and Karbe 2004). Bannash (2003) however defended the use of the latter term.

The International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice (INHAND) uses the term spongiosis hepatis and cystic degeneration as synonyms (Thoolen et al. 2010).

Prevalence

Spontaneous as well as xenobiotic-induced spongiosis hepatis is found in aging rats of some strains with some male predilection (Thoolen et al. 2010; Ettlin et al. 2010). It has also been found in medaka (a teleost fish) after exposure to toxicants (e.g. Reddy et al.1999; Hobbie et al. 2011)). It is less common in mice and has not been described in dogs and non-human primates (Thoolen et al. 2010; Ding et al. 2010; Karbe and Kerlin 2002). According to a literature search in Pubmed performed in September 2010 there is no description of spongiosis hepatis in any other animal species (with the exception of the human cases described below).

Histopathology

Spongiosis hepatis appears to be derived from altered perisinusoidal (Ito) cells and was described by Bannasch in 1981. Ito cells are also called hepatic stellate cells and are located in the perisinusoidal-Disse space. These cells act as antigen-presenting cells and are also involved in liver fibrosis by secreting collagen.

The histopathological features of spongiosis hepatic includes multi-loculated cyst(s) lined with fine septa containing eosinophilic material. The cysts are not lined by endothelial cells and do not compress the surrounding liver parenchyma. They may be observed within altered hepatic foci and liver tumours and affected cells may be markedly enlarged (Thoolen et al. 2010).

Substances that induce spongiosis hepatis

Several substances can induce spongiosis hepatis, for example 1,4-dioxane (Health Council of the Netherlands 2011), diethylnitrosamine (Mukherjee et al. 2005) methylethylketoxime (Newton et al. 2001), tocotrienol (Tasaki 2008), tetrafluoroethylene, pentachlorophenol, benzyl acetate (Maronpot 2000 as reported in CPSC 2010a), and the phthalates DINP, DIDP and DEHP.

Clinical case studies in humans

Bannasch and Zerban (1997; as referenced in ACC 2005) stated that "*Only in one report of human hepatic adenomas that appeared in users of oral contraceptives has a picture been published with features resembling spongiosis changes within an adenoma (Nime et al. 1979).*" The lesion reported by Nime et al. (1979) was peliosis hepatis.

Kaiserling and Müller (2005) reported a previously unknown tumour type in human liver observed in a 35-year-old woman under chronic medication with contraceptives. The tumour had unusual focal accumulations of spindle cells and areas of abnormal liver parenchyma. The main clinical finding was an increase in transaminases. The tumour had fusiform CD34 cells and was initially misconceived as a tumour of the liver sinusoids. However, it was found to involve accumulation of cells that appeared to be hepatic stellate cells (Ito cells) and also contained hepatic cells arranged in plates, lobules, and capillarized sinuses. After the contraceptive medication was halted the patient recovered and did not show any symptoms. Liver enzymes also returned to normal levels. The authors came to the conclusion *"that the tumor has to be classified as an Ito cell tumor as previously described in experimental animals, called spongiotic pericytoma"*.

With the exception of these cases, no human case of spongiosis hepatis has been described (according to a PubMed search on 19th of July 2011).

Karbe and Kerlin (2002) wrote: "*A very few reports suggest that this lesion may occur rarely in other mammals, such as mice or humans. However, there is no doubt that it occurs in teleost fish, especially after treatment with the hepatocarcinogen diethylnitrosamine*." and "*The induction of cystic degeneration of the liver in laboratory rats by a test compound has no direct implication for the risk in humans, because such a lesion does not appear to exist in man: Human Ito cells do not seem capable of forming the lesion "cystic degeneration" as observed in rats. However, an indirect implication cannot be excluded, because a compound that adversely affected rat Ito cells might lead to corresponding cellular alterations in different human cells or tissues. To include such a hypothetical possibility in the risk assessment, it should be considered whether the alteration induced in rats is nonneoplastic, preneoplastic, or neoplastic in nature.".*

Bannash (2003) considered the lesion of concern to humans, whether or not the lesion would be classified as neoplastic.

In the absence of information that clearly indicates a species-specific mode of action for development of spongiosis hepatis, US EPA (2005b) assumed the occurrence of this lesion in rats to be relevant to humans. US EPA (2005b) used the effect for NOAEL selection for DINP "*based on indications of serious liver damage (i.e. statistically significant increased incidence of spongiosis hepatis and increased liver weight and liver enzyme activities) in male rats chronically exposed to DINP for two years*".

CHAP (2001) considered spongiosis hepatis the critical endpoint to determine the ADI. Furthermore, US CPSC (2010a) pointed out that cystic degeneration has been observed in human pancreas, frequently in association with squamous carcinoma or adenocarcinoma, and in human kidney.

ACC (2005) considered that "*the evidence does not support a conclusion that DINP can reasonably be anticipated to cause serious or irreversible chronic liver toxicity in humans*", and used as part of its argumentation publications by Su et al. (1997) and Su et al. (1998) that had not reported spongiosis hepatis in an investigation of almost 200 diseased livers. It should be noted that Su et al. (1997) selected 163 livers from which 144 showed advanced primary liver disease (mostly cirrhosis, alcoholic and posthepatitic), 12 with space occupying lesions other than hepatocellular carcinoma[27]. Seven (healthy) donor livers were included in the study. Su et al. (1998) selected only hepatitis B infected livers (n=39). If humans would develop spongiosis hepatis, the selection bias makes it highly unlikely that spongiosis hepatis could have been detected in these studies. Moreover, detailed studies of human liver are not common (Berry 2012).

On behalf of the European Council for Plasticisers and Intermediates (ECPI) the significance of spongiosis hepatis for humans has been evaluated by Berry (2012), who concluded the following:
"In my experience, there is no comparable human lesion, a view shared by expert human pathologists in this field. In Professor Sir Roderick MacSween's book the authors state "to the best of our knowledge no human counterpart of spongiosis hepatis has ever been described". The authors use the term spongiocytic pericytoma but are considering the lesion we discuss here and Bannasch is a contributor to the volume. Further, there was no evidence of a lesion resembling spongiosis hepatis in a review of 163 human livers con-ducted by members of the Bannasch laboratory (Su et al., 1997) nor in my autopsy

[27] With the following break-down: 3 polycistic, 1 adenocarcinoma of bile duct, 1 with malignant epithelioid haemangioendothelioma and 1 with a metastatic carcinoid, 2 with focal nodular hyperplasia, 2 with metastatic colon adenocarcinomas, 1 with a metastatic breast adenocarcinoma and 1 with echinococcosis.

study of 1500 livers at autopsy.

The broad consensus of pathologists appears to support the view that spongiosis hepatis is a degenerative change. From NTP studies, spongiosis hepatis is a lesion that appears to be confined to rats, particularly male rats, and teleost fish.

In human terms, Peliosis hepatis (PH) is a histopathological entity which might be compared with rat spongiosis.

PH has been defined in Man as a change in hepatic vasculature characterised by randomly distributed multiple blood-filled cavities throughout the organ. The size of the cavities usually ranges between a few millimeters to 3 cm in diameter and the change is commonly used as a macroscopic descriptive term. It has been reported in Man after exposure to drugs (including anabolic steroids, oral contraceptives, corticosteroids, tamoxifen, and diethylstilbestrol) and to some toxicants including arsenic and thorium oxide. […] I do not believe this lesion has a relationship to rat spongiosis hepatis as defined in the documents here. […] Although cell division may take place these are not proliferative lesions as Karbe and Kerlin (2002) point out in their consideration of the lesion in the rat."

On behalf of Exxon Mobil Company, Cullen (2012) updated his 2005 review and comments on the "Revised Technical Review of Diisononyl Phthalate (DINP). The author stated the following regarding the significance of spongiosis hepatis to humans: *"The relationship to human health is, at most, unclear, and based on the scientific evidence discussed here, it is highly doubtful that there is any relationship."*

RAC noted in ECHA (2013b) that the relevance of spongiosis hepatis for humans has been questioned by some, while others have indicated that treatment-related lesions similar to spongiosis hepatic are described in human pathology (sinusoidal dilations or sinusoidal ectasia), but that the terminology differs.

Spongiosis hepatis and association with cancer

Spongiosis hepatis was originally found to be associated with exposure to hepatocarcinogens (Bannash 1981 in Karbe and Kerlin 2002), but it was later associated also with exposure to non-hepatocarcinogens (Karbe and Kerlin, 2002). Although spongiosis hepatis may be considered a proliferative change usually associated with hepatocellular neoplasms, Karbe and Kerlin (2002) do not consider it as a neoplasm.

In their review they indicated that the morphology of spongiosis hepatis does not show the characteristics of a normal neoplastic lesion although it may be composed of cells with an increased mitotic index. This statement was based on a review of the NTP database and other literature in which they identified 12 studies showing spongiosis hepatis induced by hepatocarcinogens, by noncarcinogens, by genotoxins, and by non-genotoxins in addition to its spontaneous occurrence in aging rats. It was also concluded that the lesion sometimes causes adaptive changes such as hyperplasia and hypertrophy in the liver. Karbe and Kerlin (2002) interpreted spongiosis hepatis as a secondary change associated with damage to the parenchyma due to growth of a hepatocellular neoplasm or other liver changes. They argued that the evidence of increased proliferation in the Ito cells alone is not sufficient to call spongiosis hepatis a neoplastic lesion, and that even if spongiosis hepatis is a lesion that can be associated with hepatocarcinogens it is also seen in animals without liver tumours. The latter conclusion was also supported by Aristech (1994 as cited in CHAP 2010).

Bannasch and coworkers on the other hand regard spongiosis hepatis as a preneoplastic lesion or even as a benign neoplasm, based on its proliferative properties and persistent increased cell turnover rate in studies with liver carcinogens (e.g., Stroebel et al. 1995; Bannasch and Zerban 1997; Bannasch and Schroder 2002; Bannasch 2003 in US EPA 2005). In his comments to Karbe and Kerlin (2002), Bannasch (2003) also concluded that irrespective of the classification of spongiosis hepatis as a benign neoplastic or a preneoplastic lesion, there is compelling evidence for its reliability as a sensitive marker for (hepato)carcinogenic effects in rats and fish and saw the data collected by Karbe and Kerlin (2002) rather as supportive for this view.

It can be concluded that spongiosis hepatis can be considered a proliferative change, but it is unclear whether it may itself be regarded as a pre-neoplastic or even benign neoplastic lesion.

Proliferation of hepatocytes

Foci of cellular alteration

Other common findings in DINP- or DIDP-treated animals are altered hepatic foci or foci of cellular alteration. These foci represent a localized proliferation of hepatocytes phenotypically different from the

surrounding hepatocyte parenchyma. As is the case with spongiosis hepatis, foci of cellular alteration can occur spontaneously in aged rats and other rodents but they can also be induced by chemical treatment. The incidence, size, and/or multiplicity of foci are usually increased and time to development usually decreased after administration of hepatocarcinogens. They may show fat deposition and characteristic features of cystic degeneration and angiectasis. Foci of cellular alteration are not necessarily preneoplastic (Karbe and Kerlin 2002).

Hepatocellular hyperplasia and hypertrophy

Diffuse or focal *hepatocellular hyperplasia* (non-regenerative) is another spontaneous or treatment-associated proliferative lesion consisting of a collection of hepatocytes which may span over several liver lobules (Thoolen et al. 2010). There are two types of non-regenerative hepatocellular hyperplasia. One is relatively smaller and is accompanied by angiectasis and/or spongiosis hepatis (occurs in both sexes) and the other tends to be larger than several lobules and without spongiosis hepatis (predominantly in untreated female control F344 rats but occasionally reported in treated rats) (Thoolen et al. 2010).

Hepatocellular hypertrophy is found secondary to increase in microsomal enzymes. It often occurs with a zonal or specific lobular pattern and commonly occurs following exposure to enzyme inducing xenobiotics. There is enlargement of the hepatocyte cytoplasm secondary to increase in the cytosolic protein or number of organelles (e.g., smooth endoplasmic reticulum, peroxisomes, mitochondria) (Thoolen et al. 2010).

Both hepatocellular hyperplasia and hypertrophy, depending on the presence of other lesions and the etiopathogeny of the liver effects are sometimes considered as pre-neoplastic lesion. They are commonly induced by compounds which show tumour promoting activity in rodents such as phenobarbital (acting on CAR) and PPARα activators (Casarett et al. 2008). Nevertheless, hepatocellular hyperplasia and hypertrophy may also be considered as adaptive mechanisms for metabolizing high doses of xenobiotics (Maronpot et al. 2010).

DINP and DIDP induces both focal hepatocellular hyperplasia and hepatocellular hypertrophy. It is not known whether these liver effects may exacerbate development of spongiosis hepatis as a secondary change.

Role of peroxisome proliferation in the pathology of DINP and DIDP

Many chemicals, including certain herbicides, drugs and plasticizers, have the ability to induce peroxisome proliferation in the liver and in other tissues of rodents (Klaunig et al. 2003). Several studies are available addressing the peroxisome proliferation potential of DINP and DIDP. Some of these studies are cited below to illustrate the link between biochemical measurements and pathological/histopathological outcomes.

In a dietary study to investigate the carcinogenicity of DIDP in F344 rats using a 2-year bioassay Cho et al. (2008) noted significant decreases in the overall survival and body weights, and increases in the relative weights of kidneys and liver in both sexes of the highest dose groups (8000 ppm). Oval cell hyperplasia, hypertrophy, necrosis and peliosis of the liver were increased in males exposed to 0.8% (corresponding to 479 mg/kg bw/day in males). Measurement of increased H_2O_2-generating oxidases and H_2O_2-degrading enzyme catalase contained within the peroxisomes can be used as a marker of peroxisome proliferating activity (Cheung et al. 2004 as referenced by Cho et al. 2008). Immunohistochemical staining for H_2O_2-degrading enzyme catalase showed significant elevated levels after 12 weeks at 0.8%. However, at weeks 32 and 104 no changes in expression of catalase were observed from western blotting, immunohistochemistry and enzyme activity measurements, and no treatment-related neoplastic lesions were observed in the internal organs, including the liver. The authors concluded that the non-carcinogenicity of DIDP was due to its limited potential for peroxisomal proliferating activity.

Increases in palmitoyl-CoA oxidase, a monitor of the level of peroxisome proliferation, were observed at all four time points (1, 2, 13 and 104 weeks) for the high dose (1.2%) in the Aristech (1994) study with DINP. In the Exxon (1986) study with DINP, ultrastructural examination of liver specimens from two rats of each sex from three treatment groups (low, mid, and high dose) did not show peroxisome proliferation and/or proliferation of smooth endoplasmic reticulum. This might indicate that peroxisome proliferation does not play a significant role at the lower doses (below 1.2% or 733 mg/kg bw/day in males) with DINP.

A 21d feeding study to 0, 0.3, 1.2, and 2.5% DIDP to F344 rats (n = 5/sex/dose) by BIBRA (1986 as cited in EC 2003b) showed a significant increase in liver palmitoyl-CoA oxidation activity and variable increases in the number and size of hepatocyte peroxisomes at 2.5% (corresponding to 2100 mg/kg bw/day in males). Absolute and relative liver weights were increased in the 1.2% and 2.5% dose levels in both sexes. A study with DINP using the same dose levels BIBRA (1985 as cited in EC 2003a) showed significant increased palmitoyl-CoA oxidation in both sexes at 1.2% and 1.2%. At the high dose level (corresponding to 2195 mg/kg bw/day in males) a marked increase in peroxisomes was observed. Absolute and relative liver weights were increased from the lowest dose in both sexes (corresponding to 639 mg/kg bw/day in males).

Another 28d feeding study (Lake et al. 1991 as cited in EC 2003b) in male F344 rats (n = 5/dose) with doses 0, 0.02, 0.05, 0.1, 0.3 and 1% DIDP showed increased liver weights at 0.1% (corresponding to 116 mg/kg bw/day in males) and above. Increased liver palmitoyl-CoA oxidation activity was observed at 0.1% and above.

In sub-acute studies there is evidence of peroxisome proliferation at very high doses of DINP and DIDP (>2000 mg/kg bw/day), with some evidence at lower dose levels. Chronic studies however indicate that at lower dose levels (below 733 mg/kg bw/day in males) peroxisome proliferation might not play a significant role.

Role of peroxisome proliferation in liver carcinogenesis

Klaunig et al. (2003) published an extensive in-depth assessment of peroxisome proliferators and the role of PPARα in the tumorigenesis. According to the review by Klaunig et al. (2003), activation of PPARα is causally related to carcinogenesis in rodent liver. One of the arguments is that PPARα-null mice are refractory to the potent agonist WY 14643. This finding lead to the decision by IARC in 2000 to review the classification of DEHP from 'possibly carcinogenic to humans' to 'not classifiable as to its carcinogenicity to humans'. As routine screening showed that even compounds with a very high specificity to PPARα or PPARγ produced significant peroxisome proliferation, it was pointed out that the hypothesis of PPARα mediated carcinogenesis would be strengthened with further bioassays using other known peroxisome proliferators (Klaunig et al. 2003). This suggestion materialised with the publications by Ito et al. (2007) and Ito and Nakajima (2008). The authors studied DEHP in PPARα null mice and unexpectedly found that the incidence of liver tumors was higher (26%) in PPARα-null mice than in wild-type mice (10%) exposed dietary to 0.05% DEHP over two years (corresponding intake in mg/kg bw/day was not reported). The authors suggested the involvement of a non-PPARα pathway in rodent carcinogenesis. They hypothesised that oxidative stress and subsequent induction of inflammation might result in tumorigenesis in PPARα-null mice, and/or expression of protooncogenes. Their hypothesis was supported by dose dependant increased 8-OhdG and NFκB levels in both knock-out and wild type. Furthermore the protooncogene c-jun-mRNA was induced.

Rusyn and Corton (2012) reviewed the evidence that gathered the decade following the publication of Klaunig et al. (2003). The authors considered that activation of PPARα remains an important mode of action for DEHP carcinogenicity, but were of the opinion that the data suggest that multiple pathways in several cell types contribute to cancer in rats and mice. These were summed up as follows: *"(i) rapid metabolism of the parental compound to primary and secondary bioactive metabolites that are readily absorbed and distributed throughout the body; (ii) receptor-independent activation of hepatic macrophages and production of oxidants; (iii) activation of PPARa in hepatocytes and sustained increases in expression of peroxisomal and non-peroxisomal metabolism-related genes; (iv) enlargement of many hepatocellular organelles (peroxisomes, mitochondria, etc.); (v) rapid, but transient increases in cell proliferation and decreases in apoptosis; (vi) sustained hepatomegaly; (vii) chronic low-level oxidative stress and accumulation of DNA damage; (viii) selective clonal expansion of initiated cells; (ix) appearance of pre-neoplastic nodules; (x) development of adenomas and carcinomas."*. The authors furthermore concluded that the overall body of evidence on human cancer hazard of DEHP remains inconclusive.

In the light of the new evidence IARC has in 2011 reviewed the classification of DEHP back to 'possibly carcinogenic to humans (Group 2B)' (Grosse et al. 2011; IARC 2012). The recent literature regarding DEHP and the conclusions of IARC might also be relevant in the interpretation of carcinogenicity observed in rodent studies with DINP (and to some extent with DIDP), see section 4.4.8.

Relationship of peroxisome proliferation with spongiosis hepatis
As stated above DINP and DIDP induces focal hepatocellular hyperplasia and hepatocellular hypertrophy. These effects are typically mediated by the peroxisome proliferator activated receptor and are common for phthalates. In contrast, spongiosis hepatis seems to occur independently of peroxisome proliferation as it seems only to be induced in male rats (CHAP 2001). According to US CPSC (2010a), of the 10 substances where spongiosis hepatis was seen in NTP studies with F344 rats, none were reported to be PPARa agonist. CHAP (2001) comes to this conclusion based on the finding of the Aristech (1994) study in rats and the Aristech (1995c) study in mice: "*The relationship of spongiosis hepatis to peroxisome proliferation in the livers of rats exposed to DINP is not clear. While DINP induced peroxisome proliferation in both sexes of rats and mice, spongiosis hepatis was increased in only the male rats. Moreover, spongiosis hepatis occurred in control rats and in treated rats at dosages which did not apparently induce peroxisome proliferation (Bird et al., 1987). All of this would suggest that spongiosis hepatis is unrelated to peroxisome proliferation.*"

Conclusion

The most sensitive effect in repeated dose toxicity studies with DINP and DIDP is spongiosis hepatis (also referred to as cystic degeneration and spongiotic pericytoma). Spongiosis hepatis can occur spontaneously in aging rats and can be induced by hepatocarcinogens (both genotoxic and non-genotoxic substances) and by non-carcinogenic substances. Although spongiosis hepatis can be considered a proliferative change, it is unclear whether it may itself be regarded as a pre-neoplastic or even benign neoplastic lesion. The mechanisms for induction of spongiosis hepatis are not clear, but they do not seem to be related to peroxisome proliferation. In addition to the rat, spongiosis hepatis has been found in medaka fish and is less commonly found in mice. One recent case report and an earlier report described lesions in human liver that were similar to spongiosis hepatis in rats, but it has not been described in dogs and non-human primates. It is concluded that there is evidence that spongiosis hepatis is a rather mild effect commonly seen in aging male rats. Even if the precise relevance for humans remains under debate it can nevertheless be used as an endpoint for hazard and risk assessment.

The recent literature regarding DEHP and the conclusions of IARC to classify DEHP as 'possibly carcinogenic to humans (Group 2B)' might also be relevant in the interpretation of liver carcinogenicity observed in rodent studies with DINP (and to some extent with DIDP).

4.4.7 Mutagenicity

4.4.7.1 DINP

The following cites the 'Summary of mutagenicity' from the EU Risk Assessment:
"*DINP is not mutagenic in vitro in bacterial mutation assays or mammalian gene mutation assay (with and without metabolic activation) and is not clastogenic in one cytogenetic assay in vitro on CHO cells and in one in vivo assay on bone marrow cell of Fisher 344 rats. This suggests that DINP is not genotoxic in vivo or in vitro.*" (EC 2003a)

The information and conclusions from the EU Risk Assessment (EC 2003a) are considered valid for this endpoint. It was not considered necessary to actively gather new information for this endpoint.

4.4.7.2 DIDP

The following cites the 'Summary of mutagenicity' from the EU Risk Assessment:
"*DIDP is not mutagenic in vitro in bacterial mutation assays (with and without metabolic activation) and is negative in a mouse lymphoma assay. It is not clastogenic in a mouse micronucleus assay in vivo. This indicates that DIDP is a non-genotoxic agent.*" (EC 2003b)

The information and conclusions from the EU Risk Assessment (EC 2003b) are considered valid for this endpoint. It was not considered necessary to actively gather new information for this endpoint.

4.4.8 Carcinogenicity

Introductory remark
When ECHA initiated its in-depth re-evaluation of DINP and DIDP in 2010 there was a general consensus that the liver carcinogenicity seen in rodents in response to exposure to DEHP, DINP and DIDP was of little or unclear relevance to humans. Therefore, ECHA's draft review report of 7 May 2012 (ECHA 2012) did not review carcinogenicity in detail. During the preparation of the draft review report, ECHA was not aware of the decision of IARC in 2011 to review the classification of DEHP from 'not classifiable as to its carcinogenicity to humans (Group 3)'[28] to 'possibly carcinogenic to humans (Group 2B)'[29] (Grosse et al. 2011, IARC 2012). Only during the finalisation of the current report, and after RAC had issued its opinion (ECHA 2013a,b), ECHA became aware of the IARC decision and the importance of several studies that were published after the EU Risk Assessment reports had been completed (EC 2003a,b and EC 2008). The review by IARC might indicate further need for caution when interpreting the relevance of rodent carcinogenicity with DINP and DIDP to humans.

4.4.8.1 DINP

4.4.8.1.1 EU Risk Assessment conclusion

The following cites the 'Summary of carcinogenicity' from the EU Risk Assessment:
"DINP Cell transformation studies give various results: 3/7 tests are negative, 3/7 tests are doubtful (slight increases of transforming activity without statistical significance) and 1/7 test is clearly positive. The experimental conditions are not quite identical and those results are not inconsistent and such positive results are in accordance with those of well-known peroxisome proliferators. Interestingly the three negative studies were done at higher doses.

In Chronic / Carcinogenicity studies DINP was found to induce significant excess of liver neoplasia in rat and mouse after oral administration: in the Fisher strain, incidence of hepatocellular neoplasic changes was significantly increased in both sexes at dietary levels of 12,000 ppm DINP. In mice, liver neoplasia were seen in males and females from dietary levels of 4,000 and 1,500 ppm, respectively and lead to a NOAEL of 500 ppm (112 mg/kg/d in females).

It was demonstrated that DINP induced peroxisome proliferation in rodents (as evidenced by histological and biochemical analysis). It should be noted that hepatic peroxisomal ß oxidation was not affected in monkeys after 14 days DINP administration (Pugh et al. 1999; 2000) neither in a 13-week study in which no changes related to peroxisome proliferation were reported (Huntington Life Sciences, 1998).

From the literature data, it is known that all peroxisome proliferators which were investigated are able to induced increased cell proliferation which was sustained for several months for some compounds. In the Covance's studies, aimed to assess cell proliferation, the mean labelling index was increased during the first week in both sexes of rats and mice at the 12,000 ppm dietary dose level and DNA synthesis was

[28] IARC (2012) gives the following rationale for classification of a substance in 'Group 3' (not classifiable as to its carcinogenicity to humans): *"This category is used most commonly for agents for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals. Exceptionally, agents for which the evidence of carcinogenicity is inadequate in humans but sufficient in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans. Agents that do not fall into any other group are also placed in this category. An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations."*

[29] IARC (2012) gives the following rationale for classification of a substance in 'Group 2B' (possibly carcinogenic to humans): *"This category is used for agents for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals. It may also be used when there is inadequate evidence of carcinogenicity in humans but there is sufficient evidence of carcinogenicity in experimental animals. In some instances, an agent for which there is inadequate evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data."*

also increased in rats and mice after 2-4 weeks of treatment by dietary dose of 12,000 and 6,000 ppm, respectively. Interestingly, replicative DNA synthesis is not affected in the monkey-14 day-study.

From recent studies (Vanden Heuvel, 1999) it is assumed that PPARα was involved in hepatic tumour promotion as demonstrated in PPARα -knockout mouse (Valles et al. 1999) and that it was also implicated in apoptosis repression. In term of extrapolation, (or relevance) to human it is discussed as follow: "Based on the studies showing the importance of PPAR in cancer, the question may become: do humans possess PPARα in liver and cloned human PPARα functions in a manner similar to its rodent counterpart. However, it has been known for quite some time that human cells are refractory to peroxisome proliferation and induction of PPAR-responsive genes is less than that of rat or mouse cells; a partial explanation for decreased PP-responsiveness may be that PPAR expression is lower in human cells". Most of the epidemiological data supports the fact that humans exposed to fibrate hypolipidemic drugs are not at increased cancer risk. The most recent information (Woodyatt, 1999) provides a possible explanation at the genomic level to the lack of response of human to hepatocarcinogenic effects of PPs. The data presented in this paper suggest that the human ACO (acyl coA oxidase) gene promoter, one of the -responsive genes, is inactive in most of the individuals.

It should be noted that recently, IARC gave a ruling on the carcinogenicity of DEHP and concluded that the mechanism (peroxisome proliferation and PPARα activation) by which DEHP increased the incidence of liver tumours in rodents was not relevant to humans.

Regarding MNCL, a clear increase incidence is observed in the two studies conducted with Fisher rats (outside the historical range of spontaneous leukemia), along with shortening of the onset of MNCL. However, MNCL is a common neoplasm in the Fischer 344 rats and the increased incidence after chronic exposure to some substances is likely a strain specific effect with little relevance for humans. Of interest, the IARC categorised MNCL as "an unclassified leukemia with no known human counterpart" and substances which increase MNCL frequency as "not classifiable as to carcinogenicity in humans" (IARC, 1990).

Pertaining to kidney tumours, the species and sex-specific alpha 2u globulin mechanism likely responsible for kidney tumours seen in male rats is not regarded as relevant to humans." (EC 2003a)

4.4.8.1.2 Risk assessments from other international organizations and bodies

There has been a general agreement that the carcinogenic responses (liver tumors, MNCL and renal carcinomas) in rodents are of little or unclear relevance to humans (CSTEE 2001a; EFSA 2005a,c; SCENIHR 2008; CHAP 2001; US CPSC 2010; ECPI 2009; ACC 2005). However, the views of the US EPA and US CHAP/CPSPS indicated also a need for some caution when interpreting the relevance of rodent carcinogenicity to humans.

US EPA 2005

US EPA (2005) concluded as follows on the issue of MNCL: *"the Agency notes that there are several sources of uncertainty in the interpretation of the MNCL data. These include high and variable background rate, possible strain-specificity, and lack of information on the mode of action for induction of MNCL. As a result of this scientific uncertainty, EPA reserves judgement on the human significance of MNCL and whether DINP can reasonably be anticipated to cause cancer in humans."*. Regarding liver carcinogenicity US EPA (2005) concluded: *"The mode of action for the induction of DINP-mediated liver tumors may involve peroxisome proliferation. Peroxisome proliferation is a process that induces predictable physiological responses which may include the development of liver tumors in rats and mice. EPA is currently addressing this science issue through a technical panel formed by the Risk Assessment Forum of the Science Policy Council. The technical panel is studying peroxisome proliferator activated receptors (PPAR) alpha-agonist activation as the mode of action for some chemical carcinogens applying this information to assess human relevance. EPA therefore reserves judgement on whether DINP can reasonably be anticipated to cause cancer in humans, pending the results of this Agency-wide formal process."*

CHAP 2001 and US CPSC 2010a
CHAP (2001) concluded that "While the evidence is overwhelming that events downstream of PPARα activation lead to liver cancer in rodents, the relative roles of the possible, nonexclusive, downstream mechanisms – oxidative stress, apoptosis, cell proliferation, with or without Kupffer cell involvement – are unclear.". The overall conclusion was that based on information reviewed, *"DINP causes liver cancer in rodents by a PPARα-mediated mechanism, that is pronounced in rodents and believed not readily induced in humans, especially at doses resulting from current use of consumer products. The findings of mononuclear cell leukemia and renal tubular carcinoma in the rodent bioassays for DINP are of questionable relevance to humans."* Therefore, CHAP did not use the carcinogenicity findings for risk assessment. CHAP (2001) also pointed out that DINP had not been tested for carcinogenicity in young rodents, an considered this an important limitation given that infants and toddlers are the ones most exposed to DINP.

The US CPSC (2010a) considered DINP to be a 'possibly carcinogenic'. US CPSC (2010) concluded that *"It is unknown whether the non-PPARα MOA can occur in humans. However, since humans express functional PPARα, this might suppress the non-PPARα MOA as it seems to do in mice. Additional research is needed to assess the significance of the study by Ito et al. (Ito and Nakajima 2008)."* Overall, the US CPSC (2010) did not consider the evidence sufficient to consider carcinogenicity in evaluating the potential risks of DINP exposure to humans.

4.4.8.1.3 New studies/description of key studies

Liver
A small but significant increase of liver carcinoma was observed in male F344 rats in the Exxon (1986) study at the high dose level (307 mg/kg bw/day). A NOAEL of 152 mg/kg bw/day may be derived from this study. Lington et al. (1997) however concluded that DINP did not produce treatment-related preneoplastic or neoplastic lesions in the liver based on these data. See also Table 4.27.

In Aristech (1994) a clear increase was observed in neoplastic nodules or carcinoma in male and female F344 rats at the highest dose level (733 and 885 mg/kg bw/day for males and females respectively). A NOAEL of 359 mg/kg bw/day may be derived from this study. See also Table 4.28.

In Aristech (1995c as cited in EC 2003a) reported significantly elevated incidences in hepatocellular carcinoma in the mid-high and high dose male and female B6C3F1 mice (corresponding to 742 and 1560 mg/kg bw/day in males and 910 and 1888 mg/kg bw/day in females). Furthermore the total liver neoplasms was significantly elevated in mid-low dose females (336 mg/kg bw/day). A NOAEL of 112 may be derived for this study based on liver carcinogenicity in female mice. See also Table 4.29.

Table 4.27 Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in Fischer 344 by Exxon (1986) (Table 8-2 from CPSC 2010a)

	Percent DINP in feed			
	0	0.03	0.3	0.6
Males				
Carcinoma[a]	0/81	0/80	0/80	3/80[b]
Neoplastic nodules + carcinoma[c]	3/81	1/80	1/80	4/80
Females				
Carcinoma	1/81	0/81	0/80	1/80
Neoplastic nodules + carcinoma	1/81	2/81	0/80	2/80

a p=0.015 for Fisher's exact trend test.
b p=0.12 for Fisher's exact test for pairwise comparison with control.
c "Neoplastic nodules" are regarded as adenomas. The distinction is not clear.

Table 4.28 Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in Fischer 344 rats by Aristech (1994) (Table 8-3 adapted from CPSC 2010a)

	Percent DINP in feed					
	0	0.05	0.15	0.6	1.2	1.2 R[a]
Males						
Carcinoma	1/65	0/50	0/50	1/65	12/65	2/50
Carcinoma + adenoma	5/65	3/50	2/50	7/65	18/65	-
Females						
Carcinoma	1/65	0/49	0/50	1/65	5/65	2/55
Carcinoma + adenoma	1/65	1/49	0/50	2/65	8/65	-

a Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.

Table 4.29 Incidence of hepatocellular neoplasia in a 2-year dietary study of DINP-1 in B6C3F1 mice by Aristech (1995c) (Table 8-5 adapted from CPSC 2010a)

	Percent DINP in feed					
	0	0.05	0.15	0.4	0.8	0.8 R[a]
Males						
Carcinoma	10/70	8/67	10/66	17/65	20/70	12/50
Carcinoma + adenoma	16/70	13/67	18/66	28/65	31/70	-
Females						
Carcinoma	1/70	2/68	5/68	7/67	19/70	8/50[b]
Carcinoma + adenoma	3/70	5/68	10/68	11/67	33/70	-

a Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.
b The EU Risk Assessment reported a different incidence: 13/50

Mononuclear cell leukemia

In the key study by Exxon (1986), MNCL was statistically significant at the high dose levels in both sexes as well as in males at the 0.3% level, with a quite clear dose-response (see Table 4.30). A NOAEL of 15 mg/kg bw/day (0.03%) for MNCL could be derived from this study.

In the Aristech (1994) study MNCL was statistically significant at 0.6 and 1.2 % dose levels, although with a less apparent dose response (see Table 4.31). A NOAEL of 88 mg/kg bw/day (0.15%) for MNCL could be derived from this study.

Table 4.30 Incidence of mononuclear cell leukemia in a 2-year dietary study of DINP-1 in Fischer 344 by Exxon (1986) (Table 8-10 from CPSC 2010a)

	Percent DINP in feed			
	0	0.03	0.3	0.6
Males	33/81	28/80	48/80	51/80
Females	22/81	20/81	30/80	43/80

Table 4.31 Incidence of mononuclear cell leukemia in a 2-year dietary study of DINP-1 in Fischer 344 rats by Aristech (1994) (Table 8-11 from CPSC 2010a)

	Percent DINP in feed					
	0	0.05	0.15	0.6	1.2	1.2 R[a]
Males	22/65	23/55	21/55	32/65	30/65	31/50
Females	17/65	16/49	9/50	30/65	29/65	24/50

a Recovery group. Animals were exposed for 78 weeks, followed by a 26-week recovery period.

4.4.8.1.4 Discussion

Liver
Several publications were published after the latest literature search (2001) of the EU Risk Assessment (EC 2003a) discussing the different preneoplastic and neoplastic changes in the liver related to exposure to DINP in rodents and their relevance to humans. It is believed that peroxisome proliferation is the underlying mode of action for development of liver tumors with DINP. However, the literature indicates that the mechanisms of carcinogenicity in rodents with peroxisome proliferators have not entirely been elucidated and that multiple pathways might exist. Some of those pathways might be PPARα-independent. It could be noted in this context that IARC has reviewed the classification of DEHP to 'possibly carcinogenic to humans (Group 2B)'. This conclusion was reached considering new evidence that activation of PPARα might not be the only pathway for cancer with DEHP in rats and mice (Grosse et al. 2011; IARC 2012).

See Box 1 for more details.

Mononuclear cell leukemia
The observed significant dose-related increase in incidences of MNCL are seen in both key studies and in both sexes in F344 rat strain. A dose response is relatively clear, but however within (the very large) historical control range.

MNCL was not observed in Sprague-Dawley CD rats treated with "Santicizer 900" (also known as DINP-A)(Bio/dynamics 1986, see also section 4.4.6) nor in long-term DINP exposed mice (Aristech 1995c as cited in EC 2003a). Spontaneous incidences of MNCL are rare in other than F344 rat strains. In Sprague-Dawley the background incidence was reported to be 0.6% (Frith 1988 in Thomas et al. 2007). MNCL does not occur naturally in mice (Thomas et al. 2007; US EPA 2005).

Increased incidences of MNCL are a common finding in treated F344 rats in chronic studies (Lington et al. 1997; Thomas et al. 2007; US CPSC 2010). MNCL has a high background rate, which has been increasing over time, with in the more recent studies a mean incidence of 52 and 59% in males (NTP-2000 and NIH-07 diets respectively) and 24 and 32% in females (NTP-2000 and NIH-07 diets respectively) (as reviewed by Haseman et al. 2003 for 2 year NCI/NTP studies). The background incidences for the laboratories conducting the Exxon (1986) and Aristech (1994) studies have not been reported.

The incidences in control animals for the studies with DINP were 34 and 41% in males and 26 and 27% in females. High background rates hamper statistical analysis and are therefore likely to contribute to false (not treatment related) positive findings in long term studies with F344 rats (Lington et al. 1997; US CPSC 2010). However, in the case of DINP, increased dose-related incidences of MNCL are seen in both of the key studies and in both sexes, and as a consequence the probability that the observed statistical significance would be the result of chance findings seems low.

The cause of MNCL is unknown, although the evidence points to an age related genetic basis (Thomas et al. 2007). According to Caldwell (1999), the database of studies with alkyl phthalates shows that MNCL occurs with a threshold, which is further supported by the non genotoxic properties of phthalates. Some factors are known to influence the incidence, in particular the vehicle used for administration (Thomas et al. 2007). The cellular origin of MNCL in F344 rats is not fully proven, but is likely of the NK cell type (Thomas et al. 2007). IARC has categorised MNCL as "an unclassified leukemia with no known human counterpart" (IARC 1990). This was however questioned by Thomas et al. (2007) who concluded that MNCL (or LGLL) is similar to a rare human counterpart known as NK-LGLL. The authors acknowledge however that despite the similarities, mechanisms may be very different.

4.4.8.1.5 Conclusion

The available new information on the carcinogenicity of DINP further supports the conclusions of the EU Risk Assessment concerning renal tumors (EC 2003a). These neoplasms are assumed to have modes of actions which are not considered to be relevant for humans (alpha-2u-globulin).

It is believed that peroxisome proliferation is the underlying mode of action for development of liver tumors with DINP. However, the literature indicates that the mechanisms of liver carcinogenicity in rodents with peroxisome proliferators have not entirely been elucidated and that multiple pathways might exist. Some of those pathways might be PPARα-independent, which might indicate a need for some caution when interpreting the relevance of rodent carcinogenicity with DINP to humans.

With regard to MNCL, the review by Thomas et al. (2007) suggests that unlike previously thought there might be a human counterpart to MNCL in rats. The probability that the MNCL seen in the Exxon and Aristech studies would be a result of chance findings seems low. Nevertheless, the increased incidences of MNCL remain difficult to interpret in the light of the high and variable background incidences and the unclear relevance to humans. DINP is not genotoxic and it is argued (Caldwell 1999) that MNCL follows a threshold mode of action. The available information does not allow to draw definite conclusions on the matter. However, as a reasonable approach it would be possible to conclude that the MNCL findings further strengthen the selected NOAELs for repeated dose toxicity (15 and 88 mg/kg bw/day). Since such conclusion would not influence the outcome of the current risk assessment, the endpoint is not taken further to the risk characterisation step.

4.4.8.2 DIDP

4.4.8.2.1 EU Risk Assessment conclusion

The following cites the section 'Carcinogenicity long-term study' from the EU Risk Assessment:
"No carcinogenicity long-term study is available for DIDP but an increase in incidence of hepatocellular tumours in rats related to peroxisome proliferation might be anticipated, in regard with the increased incidence in tumour liver cells observed with DEHP and DINP in carcinogenicity studies. Indeed, DINP and DIDP show comparable responses for peroxisome proliferation parameters at comparable dose levels (BIBRA, 1986). However, in response to peroxisome proliferators a marked species difference could be foreseen. The current literature reported that only rats and mice are responsive to the carcinogenic effects of peroxisome proliferators, while dogs, non-human primates and humans are essentially non-responsive or refractory (IARC, 1995; Doull, 1999).

Thus, there is no concern in regard with carcinogenicity. Indeed it is now well-accepted that peroxisome proliferation is specific to rodents. It has been established that peroxisome proliferators exhibit their pleitropic effects due to activation of PPARα and that PPARα is expressed only at low level in humans, explaining the absence of significant response in humans to the action of peroxisome proliferators." (EC 2003b)

<u>Commentary to the EU Risk Assessment</u>
At the time of the finalisation of the EU Risk Assessment (EC 2003b) no carcinogenicity studies on DIDP were available to be assessed. The risk assessment concluded however that the available information from certain repeated dose toxicity studies (BIBRA 1986) showed that DIDP induced peroxisome proliferation responses similar to DEHP and DINP and that an increased incidence of liver tumours could be anticipated in rodent livers after exposure to DIDP.

4.4.8.2.2 Risk assessments from other international organizations and bodies

There is no known disagreement with the conclusions of the EU Risk Assessment (EC 2003b) that there is no concern for carcinogenicity of DIDP in humans.

4.4.8.2.3 New studies/description of key studies

Cho et al. 2008, 2010
In a 2-year rodent carcinogenicity study with DIDP by Cho et al. (2008, 2010) Fischer 344 rats were fed diets containing 0.04, 0.2 or 0.8% DIDP (average daily doses for male rats were 21.86, 110.25 and 479.20 mg/kg bw/day and for females 22.92, 128.18 and 619.59 mg/kg bw/day).

Significantly increased incidences of MNCL were observed in males and females at the highest dietary concentration of 0.8% (see Table 4.32). The incidences were within the historical range of controls in the NTP database (as reported by Haseman et al. 1998 in Cho et al. 2008) during the same time period but laboratory/breeder specific ranges were not reported. The incidences of MNCL in the Cho et al. study with DIDP were lower in males and females than the reported incidences in the Exxon (1986) study with DINP, and lower in males compare to the Aristech (1994) study with DINP. A NOAEL of 110 mg/kg bw/day (0.2%) for MNCL could be derived from this study.

Table 4.32 Incidence of MNCL lesions in rats exposed to DIDP for 2 years (Cho et al. 2008).

	Control	0.04%	0.2%	0.8%	NTP
Number examined	50	50	50	50	1354
Males	10 (20%)	16 (32%)	14 (28%)	23** (46%)	32-74%
Females	11 (23%)	7 (14%)	11 (22.4%)	22* (44.9%)	14-52%

* significantly different ($p<0.05$) from control by the poly-3 test
** significantly different ($p<0.01$) from control by the poly-3 test

Cho et al. 2011
In a recent 26-week carcinogenicity study, DIDP was administered to CB6F1 –Tg rasH2 mice at dietary levels of 0, 0.1, 0.33, or 1% and to wild-type mice at 0 and 1% for 26 weeks (Cho et al, 2011). See also section 4.4.6.2.

A statistically significant number of hepatocellular adenomas was observed in the male transgenic mice receiving 1% DIDP (n = 5/15), but not in the wild-type mice receiving 1% DIDP. The corresponding intakes in mg/kg bw/day were not reported, but might be in the order of 1500 mg/kg bw/day for the males receiving 1% (LOAEL) and 500 mg/kg bw/day for the 0.33% dose group (NOAEL)[30].

No hepatocellular adenomas were observed in female mice. No MNCL was observed in the study.

4.4.8.2.4 Discussion

Transgenic rasH2 mice are reported to have a relatively low susceptibility for liver carcinogenicity (Mitsumori et al. 1998 as cited in Cho et al. 2011). Importantly, Cohen et al. (2001) indicated that the rasH2 model did not respond to most classes of non-genotoxic rodent carcinogens.[31] As DIDP is assumed to be non-genotoxic, and the study covered only

[30] Assuming an adult mouse weighs 0.02 kg and eats 3 g per day this would yield 150 g/kg bw/day. With 1% of DIDP in the feed, this would lead to a dose level of 1500 mg/kg bw/day and with 0.33% the dose can be estimated to be 500 mg/kg bw/day.
[31] The rasH2 mouse is said to be sensitive to genotoxic and non-genotoxic carcinogens and is considered to be a promising model for short-term carcinogenicity studies (Kanno 2003). However, Cohen et al. (2001) concluded that despite the fact that the model was designed to increase sensitivity to carcinogens, it was actually less sensitive than the traditional 2 year mice or rat studies. Nevertheless, Cohen et al. considered the model appeared to have greater specificity. Cohen et al. observed that in studies with models such as the rasH2 frequently only responses were observed in the high dose groups as a consequence of the low number of animals used in those studies (usually 15). In addition, it has been suggested that rasH2 mice have low susceptibility for hepatocarcinogenesis (Mitsumori et al. 1998).

1/4th of the life-time assay in mice, the results from the Cho et al. 2011 need to be interpreted with care (with respect to lack of tumors in the lower dose levels and lack of tumors in all dose levels in female transgenic mice). Interestingly however, no liver adenomas were observed in the 2-year carcinogenicity study with rats (Cho et al. 2008, 2010).

It is believed that peroxisome proliferation is the underlying mode of action for development of liver tumors with DIDP (Cho et al. 2011). However, regarding the relevance to humans the same considerations need to be made as with DINP (see above and Box 1).

Similarly to DINP, increased incidences of MNCL were observed in the 2-year carcinogenicity study with rats (Cho et al. 2008, 2010). The incidences in males were lower than observed in the key studies with DINP, however, and were only significant in the high dose group.

4.4.8.2.5 Conclusion

Although no treatment-related tumours were observed in a 2-year carcinogenicity study with rats, DIDP has been shown to induce liver adenomas in a 26-week study in rasH2 mice. It is assumed that the increased incidence of liver adenomas in mice is related to peroxisome proliferation. However, the literature indicates that the mechanisms of liver carcinogenicity in rodents with peroxisome proliferators have not entirely been elucidated and that multiple pathways might exist. Some of those pathways might be PPARα-independent, which might indicate a need for some caution when interpreting the relevance of rodent carcinogenicity with DINP to humans.

As discussed in the previous section 4.4.8.1 for DINP, the increased incidences of MNCL are difficult to interpret in the light of the high and variable background incidences and the unclear relevance to humans. DIDP is not genotoxic and it is argued (Caldwell 1999) that MNCL follows a threshold mode of action. The available information does not allow to draw definite conclusions on the matter. If the MNCL findings were to be considered relevant to humans, a reasonable approach would be to assume that MNCL follows a threshold mode of action. Following this argumentation, the conclusion would not influence the outcome of the current risk assessment (NOAEL of 110 mg/kg bw/day), and the endpoint is not taken further to the risk characterisation step.

4.4.9 Toxicity for reproduction

4.4.9.1 DINP

4.4.9.1.1 EU Risk Assessment conclusion

The following cites the 'Summary of toxicity for reproduction' from the EU Risk Assessment:
"Fertility assessment may be inferred from effects on reproductive organs and the two-generation study. In adult rats, some minor effects were observed not histologically confirmed in any of the studies mentioned: in the one-generation study, a statistically significant increase in the mean absolute and relative right testis, left testis and right epididymis weights and the mean relative left epididymis and seminal vesicle weights in the high-dose males were observed; in a few subacute and/or subchronic studies, slight increases (statistically significant) of relative testes weights were also noted at high doses. Taken as a whole, no overt toxicity was observed on reproductive organs in rats.

In mice, very high dose (5,770 mg/kg/d) leads to decrease in testicular weight with abnormal/immature sperm forms and uterus/ovaries atrophy in the 13-week study. In the 104-week chronic study, a NOAEL of 1,500 ppm (276 mg/kg/d) can be assumed for testicular effects, based on decrease in testicular weight (relative and absolute) observed from 742 mg/kg/d. The NOAEL for systemic toxicity in male is 1,500 ppm as well.

In the two-generation study no changes in reproductive indices are observed.

From those assays, no adverse effects on fertility may be anticipated.

In regard with offspring survival in rats, at 1.5% (corresponding to a range of 966-2,246 mg/kg/d), a decrease of life birth and survival indices was observed in the one-generation range-finding study but not observed in the two-generation study, conducted up to 0.8%. For decrease in life birth and survival indices a NOAEL of 622 mg/kg/d (the lowest dose of the estimated range) is determined and is taken into account in the risk characterisation.

In the developmental studies, visceral (dilated renal pelvis and hydroureter) and skeletal (rudimentary cervical and accessory 14th ribs) variations were significantly increased at 1,000 mg/kg/d this lead to a NOAEL of 500 mg/kg/d. Slight (1,000 mg/kg/d) or no (500 mg/kg/d) maternal toxicity was observed in those studies.

A decrease of mean offspring body weight was observed following parental administration of DINP in the one and two-generation studies from the lowest dose tested (0.2% in the two-generation study), leading to a estimated LOAEL of 159 mg/kg/d, the lowest value of the maternal dose range during post-partum. In the two-generation study parental toxicity was limited to lower mean body weight and hepatic changes from 0.2% (eosinophilia and rarely enlargement of the hepatocytes), thus a LOAEL of 114 mg/kg/d (the lowest level of the 0.2% dose) may be derived.

The NOAEL and LOAEL quoted above will be considered in risk characterisation for developmental effects.

Regarding fertility and development, the effects observed in the available studies, do not justify classification according to the EU classification criteria." (EC 2003a)

<u>Summary of the examination of endocrine activity in the EU Risk Assessment</u>
EU Risk Assessment did not conclude on endocrine activity, it rather summarizes the results of studies available under a chapter of "Additional studies" and suggests to revisit and update the Risk Assessment report of DINP, if necessary, in light of a review of new studies proposed by US National Toxicology Program (NTP). The following summary is based on the text provided in summary in the EU Risk Assessment.

According to the EU Risk Assessment (EC 2003a), DEHP, DINP and DIDP showed no activity in the different in vitro assays conducted to test the ability of binding to rodent or human oestrogen receptors or to induce oestrogen receptor-mediated gene expression (Harris et al. 1997, Zacharewski et al. 1998). DINP showed ability to stimulate proliferation of human breast cancer cells in one in vitro assay (Harris et al. 1997). In an uterotrophic assay/vaginal cell cornification assay with orally dosed rats, the response for uterine wet weight and vaginal

cornification were both considered negative for the phthalates tested (DEHP, DINP and DIDP) – although the value of the test was questioned in relation to the uterine response (Zacharewski et al. 1998). Vaginal haemorrhages observed in some reproductive toxicity studies (developmental toxicity studies) might be indicative of a perturbation of endocrine homeostasis. Possible mechanisms of endocrine disruption for androgenic function were ongoing at the time of EU Risk Assessment. One study indicated that DINP might have anti-androgenic potency in neonatal rats (Gray et al. 2000).

Table 4.33 Summary of reproductive toxicity studies of DINP (modified from EC 2003a).

Species	Protocol/ doses	Results NOAEL/LOAEL	Test substance	References
One-generation studies (oral)				
Rat Crl: CDBR	0.5-1-1.5% (0; 301-591; 622-1157; 966-1676 mg/kg bw/day for males and 0; 363-923; 734-1731; 1087-2246 mg/kg bw/day for females)	LOAEL Parents, offspring 0.5% (301 mg/kg bw/day)	CAS 68515-48-0 MRD 92-455	Exxon (1996a) Waterman et al. (2000)
Two-generation studies (oral)				
Rat Crl: CDBR	diet 0-0.2-0.4-0.8% (0; 118-215; 236-426; 477-852 mg/kg bw/day for P0 premating period, 0; 114-264; 235-523; 467-1090 mg/kg bw/day for P1 premating period, 0; 133-153; 271-307; 543-577 mg/kg bw/day for gestation period (P0 and P1), 0; 159-395; 347-758; 673-1541 mg/kg bw/day for postpartum period (P0 and P1)	LOAEL parents, offspring 0.2% (159 mg/kg bw/day)	CAS 68515-48-0 MRD 92-455	Exxon (1996b) Waterman et al. (2000) Nikiforov et al. (1995)[a]
Developmental toxicity studies				
Rat Sprague Dawley	gavage 0-100-500-1,000 mg/kg bw/day	NOAEL (F, dams) 500 mg/kg bw/day[b]	CAS 68515-48-0 MRD 92-455	Exxon (1994) Waterman et al. (1999)[b)]
Rat Crl: CDBR	range finding study by gavage 0-40-200-500-1,000 mg/kg bw/day	NOAEL (F, dams) 1,000 mg/kg bw/day	CAS 68515-48-0	Nikiforov and Koehler (1994)
Rat Wistar	screening study 0-40-200-1,000 mg/kg bw/day	NOAEL (F, dams) 200 mg/kg bw/day	DINP1 CAS 68515-48-0	Hellwig et al. (1997)
Rat Wistar	screening study 0-40-200-1,000 mg/kg bw/day	NOAEL (F, dams) 200 mg/kg bw/day	CAS 28553-12-0 DINP 2, Palatinol N (91/26), purity: 99.8%	BASF (1995b) Hellwig et al. (1997)

Rat Wistar	screening study 0-40-200-1,000 mg/kg bw/day	NOAEL (F, dams) 200 mg/kg bw/day	CAS 28553-12-0 DINP 3, Palatinol DN (92/64) purity: >99.9%	BASF (1995a) Hellwig et al. (1997)
Rat Sprague Dawley	gavage 0-10-500-1,000 mg/kg bw/day	NOAEL (F, dams) 1,000 mg/kg bw/day	DINP, not further specified	Hazleton (1981b)

[a] Abstract only available
[b] The lower NOAEL of 100 mg/kg bw/day was agreed by NTP-CERHR (2003a), US EPA (2005b) and US CPCS (2010a)

Commentary to the EU Risk Assessment
In this commentary, further details and clarifications to the summary of the EU Risk Assessment are given.

High dietary concentrations of DINP affected reproductive organ weights in rats and mice. Dietary concentrations corresponding to 966 – 1676 mg/kg bw/day increased absolute and/or relative weights of testis, epididymis, and seminal vesicles in a one-generation reproductive toxicity study in rats (Waterman et al. 2000 [Exxon 1996a]). The same dietary concentration (corresponding to 1087 – 2246 mg/kg bw/day in females) caused a decrease in absolute and relative right ovarian and mean absolute of left ovarian weights in females. No histopathological examinations were conducted. In a few of the subacute/subchronic studies reviewed, relative and/or absolute testis/epididymis weights were increased at a high dietary level in rats (at and above ~690 mg/kg bw/day: Bio/dynamics 1982b, c; Hazleton 1991a) and decreased in mice (at and above ~1,377 mg/kg bw/day: Hazleton 1991b; Hazleton 1992). At even higher dose levels, abnormal/immature sperm in the epididymis and reduced weight with hypoplasia in the uterus and absence of corpora lutea in the ovaries were found after an exposure of 13-week in mice (Hazleton 1982). After chronic exposure, increase in relative testis weight was reported at 307 mg/kg bw/day in rats (Exxon 1986) and relative and absolute testis weight at and above 742 mg/kg bw/day in mice (Aristech 1995c). The NOAEL for testicular effects was determined to be 276 mg/kg bw/day based on reduced absolute and relative testis weight at 742 mg/kg bw/day in a 2-year study (Aristech, 1995c). However, the liver and kidney effects were observed at a lower dose levels than the effect on testis weight.

Life birth and survival indices were decreased at 966-2246 mg/kg bw/day in a one-generation study in rats and a NOAEL of 622 mg/kg bw/day was determined based on these findings (Waterman et al. 2000 [Exxon 1996a]). A decrease in mean offspring body weights in one- and two-generation studies were observed at the lowest dose examined (159 mg/kg bw/day; the lowest postpartum dose) and was considered as a developmental LOAEL (Waterman et al. 2000 [Exxon 1996b]). The parental toxicity at this dose level was limited to lower body weights and minimal to moderate increased cytoplasmic eosinophilia with a rarely enlargement of the affected hepatocytes with a LOAEL of 114 mg/kg bw/day in both generations (the lowest premating dose level for F1 adults). A NOAEL of 500 mg/kg bw/day was derived based on visceral and skeletal variations and slight maternal toxicity at 1000 mg/kg bw/day in prenatal developmental toxicity studies (Waterman et al. 1999 [Exxon 1994]). A NOAEL of 200 mg/kg bw/day, also referred to in some of the international evaluations summarized below, was identified based on screening developmental toxicity studies using three related forms of DINP with developmental LOAELs of 1000 mg/kg bw/day (Hellwig et al. 1997). However, the developmental NOAEL of 500 mg/kg bw/day was not supported by the evaluation of NTP-CERHR (2003a) and US CPSC (2010a) who proposed a NOAEL of 100 mg/kg bw/day based on skeletal findings.

In the EU Risk Assessment, the following NOAELs/LOAELs were selected for risk characterisation: 276 and 622 mg/kg bw/day for reproductive toxicity, and 159 and 500 mg/kg bw/day for developmental toxicity (Table 4.34).

Table 4.34 Studies showing the critical endpoints of DINP (Modified from EC 2003a).

Endpoint	Study	LOAEL (mg/kg bw/day) and critical effects	NOAEL (mg/kg bw/day)	Reference
Reproductive toxicity	One-generation reproductive toxicity study, dietary, rat	966 (1.5%), decreased live birth and survival indices	622 (1%)	Exxon (1996a) Waterman et al. (2000)
	104-week dietary study, mouse	742 (4,000 ppm), decreased testicular weight	276 (1,500 ppm)	Aristech (1995c)
Developmental toxicity	Prenatal developmental toxicity study, dietary, rat	1,000 skeletal and visceral variations	500[a]	Exxon (1994) Waterman et al. (1999[a])
	Two-generation reproductive toxicity study, dietary, rat	159 (0.2%), decreased body weight in offspring	No NOAEL	Exxon (1996b) Waterman et al. (2000)

[a] A lower NOAEL of 100 mg/kg bw/day was agreed with the sponsor by NTP-CERHR (2003a) and supported/ reported by US EPA (2005b) and US CPCS (2010a)

4.4.9.1.2 Risk assessments from other international organizations and bodies

EU bodies

CSTEE 2001a
The Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE 2001a) reviewed the EU Risk Assessment (EC 2003a) and acknowledged in its opinion the identified NOAELs and LOAELs for reproductive toxicity: the LOAEL of 0.2% (159 mg/kg bw/day) for parents and offspring established from the oral two-generation reproductive toxicity study in rats (Waterman et al. 2000 [Exxon 1996a]), the NOAEL of 500 mg/kg bw/day for maternal toxicity and developmental toxicity based on a developmental toxicity study (Waterman et al. 1999 [Exxon 1994]), and the lower NOAEL of 200 mg/kg bw/day for skeletal variations and maternal toxicity (Hellwig et al. 1997).

Regarding possible endocrine disrupting properties of DINP, the EU Risk Assessment (EC 2003a) pointed out that investigations on possible mechanism of endocrine disruption for androgenic function were ongoing at the time of the review by CSTEE (2001a), investigating in vitro androgen receptor binding for a number of phthalates including DINP. A study by Gray et al. (2000) investigating the effects of several phthalates on neonatal rats indicated that DINP might have anti-androgenic potency. However, the reported changes (occurrence of female-like areolas/nipples in infant males) were slight and were only seen at a very high dose (750 mg/kg from gestational day (GD) 14 to postnatal day (PND) 3). In this respect DINP was about an order of magnitude less active than DEHP and BBP as stated by CSTEE.

The CSTEE agreed with the conclusions of the EU Risk Assessment that the effects observed in the available studies did not justify classification for effects on fertility and development according to the EU classification criteria.

EFSA 2005a
The European Food Safety Authority (EFSA) did not carry out a new extensive risk assessment, but instead took cognisance of the previous evaluations by the Scientific Committee for Food (SCF 1999) and in particular of the EU Risk Assessment (based on the 2001 text versions), as

well as the comments made by the CSTEE on the EU Risk Assessment (CSTEE 2001a). EFSA concluded in its opinion that "*No overt toxicity was observed on reproductive organs in rats. NOAELs of 500 mg/kg bw/day and 622 mg/kg bw/day were established for minor developmental effects and decreases in live birth and survival indices, respectively. Maternal toxicity was limited to lower mean body weight and hepatic changes with a LOAEL of 114 mg/kg bw/day".*

EFSA referred in their conclusion to the NOAELs of 500 mg/kg bw/day for minor developmental effects and maternal toxicity from a prenatal developmental toxicity study (Waterman et al. 1999 [Exxon 1994]) and 622 mg/kg bw/day for decreases in live birth and survival indices from one-generation reproductive toxicity study in rats (Waterman et al. 2000 [Exxon 1996a]). In two rat developmental toxicity studies considered, visceral variations and/or skeletal variations were observed with a NOAEL of 500 mg/kg bw/day (Waterman et al. 1999 [Exxon 1994]) and 200 mg/kg bw/day (Hellwig et al. 1997 [BASF 1995a]), respectively. They seemed not to consider in their conclusion the NOAEL of 276 mg/kg bw/day based on reduced testis weight in the 2-year mice study (Aristech 1995c) as a critical finding for fertility possibly due to the rather high LOAEL of 742 mg/kg bw/day. Some subacute, subchronic and chronic rat studies indicated increase in relative testis weights with or without increase in absolute testis weights in high doses (Biodynamics 1982a, b, c; Hazleton 1991a; Exxon 1986). The LOAEL of 159 mg/kg bw/day based on decreased body weight in one-and two-generation reproductive toxicity studies (Waterman et al. 2000 [Exxon 1996a and b]) was not considered in the conclusions by EFSA, perhaps due to the LOAEL of 114 mg/kg bw/day for parental toxicity identified for decreased body weight and hepatic changes.

EFSA presents the CSTEE (2001a) opinion regarding possible endocrine disrupting properties of DINP "*Regarding possible endocrine disrupting properties of DINP the report points out that investigations on possible mechanism of endocrine disruption for androgenic function are currently being conducted by investigating in vitro androgen receptor binding for a number of phthalates and an adipate including DBP, DEHP, DIDP, DINP, DEHA and DNOP. Furthermore, a recent study by Gray et al. (2000) investigating the effects of several phthalates on neonatal rats indicated that DINP might have anti-androgenic potency. However, the reported changes (occurrence of female-like areolas/nipples in infant males) were slight and ~~was~~ were [sic] only seen at a very high dose (750 mg/kg from gestational day 14 to postnatal day 3). In this respect DINP was about an order of magnitude less active than DEHP and BBP. There has been a proposal by the US National Toxicology Program that further testing be carried out in this area."*

SCENIHR 2008

Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) adopted an opinion on the safety of medical devices containing several plasticizers, such as DINP, on neonates and other groups possibly at risk. SCENIHR referred in their evaluation of reproductive toxicity of DINP to EU Risk Assessment (EC 2003a) and opinion of CSTEE (2001a).

For reproductive effects SCENIHR referred to the decreased testis weights with abnormal/immature sperm forms and uterus/ovary atrophies in mice at high doses after a 13-week exposure (Hazleton 1992) and identified a NOAEL of 276 mg/kg bw/day for testicular effects based on reduced testis weight at 742 mg/kg bw/day in a 104-week rat study [in fact a mouse study; Aristech 1995c]. A developmental NOAEL of 500 mg/kg bw/day was referred based on visceral and skeletal variations on litter basis at 1000 mg/kg bw/day (Waterman et al. 1999 [Exxon 1994]) and an offspring LOAEL of 159 mg/kg bw/day for decreased body weight from one-and two-generation studies were communicated (Waterman et al. 2000 [Exxon 1996a and b].

SCENIHR acknowledged that DINP is not oestrogenic in vitro but referred to studies indicating areolas/nipple retention in males after perinatal exposure. The incidence of reproductive

malformation was found to be slightly but significantly increased (7.7% with DNIP versus 91% with DEHP based on the study of Gray et al. (2000). SCENIHR concluded that the profile of the reproductive effect of DINP is similar to that of DEHP but DINP is only half or less as potent as DEHP. The mechanism of action seems to be via effects on steroidogenesis in the foetal male rat (as shown for DEHP).

SCHER 2008
Scientific Committee on Health and Environmental Risks (SCHER) was requested to provide an opinion on the information contained in the report of Phthalates in School Supplies report (Danish Environmental Protection Agency, Danish EPA).

SCHER acknowledged the NOAELs of 500 and 622 mg/kg bw/day for minor developmental effects and decreases in live birth and survival indices, respectively (Waterman et al. 1999; 2000 [Exxon 1994; 1996a]). The latter NOAEL is from one-generation range-finding study (Waterman et al. 2000 [Exxon 1996a]) and not from a two-generation study as erroneously stated in the SCHER opinion.

The LOAEL of 159 for offspring from a two-generation reproduction toxicity study (Waterman et al. 2000 [Exxon 1996b]) or the NOAEL of 276 mg/kg bw/day for testicular effect (Aristech 1995c) is not considered by SCHER. It is to be noted that the LOAEL for the testicular effects was 742 mg/kg bw/day (Aristech 1995c) and the one-generation study allows setting the NOAEL of fertility to 622 mg/kg bw/day (Waterman et al. 2000 [Exxon 1996a]) meaning that the highest NOAEL below the lowest LOAEL for fertility is 622 mg/kg bw/day.

The United States

NTP-CERHR 2003a
In the Monograph on the Potential Human Reproductive and Developmental Effects of Di-isononyl Phthalate (DINP), NTP-CERHR[32] concluded that there is minimal concern for adverse effects on human development or reproduction. Studies of reproductive and developmental toxicity in rats indicated that relatively high doses of DINP can affect development of the kidneys and skeletal system of the foetus and result in reduced birth weight. The reproductive toxicity studies reviewed did not indicated adverse effects on the reproductive system of rats. The study of Gray et al. (2000) provided some evidence that DINP, like other phthalates such as DEHP and DBP, affects the male rat reproductive system at a single high dose according to the NTP assessment.

The NTP Expert Panel did not agree with the NOAEL of 500 mg/kg bw/day for developmental toxicity based on calculations presented in the report by Waterman et al. (1999; based on the study by Exxon 1994). The re-analysis performed by the sponsor of the study confirmed the Panel's interpretation of skeletal variations and the Panel was confident that 500 mg/kg bw/day was an effect level, and 100 mg/kg bw/day was a NOAEL (Table 4.35). The Panel considered cervical ribs as of greater toxicological concern than lumbar ribs although the effect on lumbar ribs was more pronounced. Cervical ribs are rare in control animals and their presence may indicate a disruption of gene expression. Cervical ribs may interfere with normal nerve function and blood flow. The sponsor of the study calculated the benchmark dose for the rudimentary lumbar rib variant. At the 5% excess risk level, the BMD05 was 193 mg/kg bw/day with a 95% lower confidence interval value of 162 mg/kg bw/day.

[32] National Toxicology Program – Centre for the Evaluation of Risks to Human Reproduction

Table 4.35 Mean percent of pups in litter with skeletal variations after in utero exposure to DINP (according to NTP-CERHR report after a re-evaluation of data using the generalized estimating equation approach to linearized model). The original values reported by Waterman et al. (1999) are indicated in parenthesis as foetal/litter incidences (values attaining statistical significance in original analysis are in bold and in new analysis with asterisk(s)).

Parameter	Oral dose levels of DIDP (mg/kg bw/day)			
	0	100	500	1000
Skeletal variations	16.4	15.0	28.3*	43.4**
Visceral variations	0.5	3.3	3.7	5.8*
Rudimentary lumbar ribs	3.5 (3.7/25.0)	4.7 (5.4/20.2)	18.1* (**18.6**/54.2)	34.2** (**34.5**/**78.3**)
Supernumerary cervical ribs	1.6 (1.6/12.5)	1.5 (1.6/12.0)	1.0 (1.0/8.3)	5.5* (**5.7**/**30.4**)
Dilated renal pelvis[a]	0 (0/0)	3.3 (3.7/12.0)	3.7 (4.0/16.7)	5.3* (4.5/**26.1**)

*p≤0.05, ** p≤0.01
[a] Two different statistical methods were used with show reasonable agreement.

Based on the other prenatal developmental toxicity study, NTP-CERHR agreed with the NOAEL of 200 mg/kg bw/day for maternal and developmental effects at 1000 mg/kg bw/day (Hellwig et al. 1997).

The LOAEL for the developmental effects derived from a two-generation reproductive toxicity study (Waterman et al. 2000 [Exxon 1996a]) was 0.2% (143-285 mg/kg bw/day during gestation through lactation) based on adverse effect on weight gain in pups. The Panel notes that neither of the prenatal studies extended dosing into the late gestation period to include the critical window of developmental effects reported for other phthalates. The study designs neither allowed assessing sexual maturation and endpoints sensitive to other phthalates.

US EPA 2005b

US Environmental Protection Agency (US EPA) performed a hazard assessment of DINP in order to prepare a proposed for inclusion in the EPCRA Section 313 list. This is a list of chemicals under the so-called Section 313 of the Emergency Planning and Community Right-to-Know Act, supported by a Technical Review (US EPA 2005a). The assessment and conclusions are reported in the "Revised Technical Review of DINP" (US EPA 2005b).

US EPA concludes that there are no data on the reproductive toxicity of DINP in humans. In one- and two-generation reproductive toxicity studies conducted in rats there was no reproductive toxicity at oral doses up to 1000 mg/kg bw/day Waterman et al. 2000 [Exxon 1996a and b]. Minimal to slight histopathological changes were observed in the ovaries and testes of rats exposed perinatally (in utero and during lactation) at dietary maternally toxic doses of 1164-2656 mg/kg bw/day (Masutomi et al. 2003).

US EPA concludes on developmental toxicity as follows: "*EPA believes that the weight of evidence from available reproductive and developmental toxicity studies suggest that DINP causes adverse developmental effects in animals that the Agency considers to be serious. The adverse effects include decreased body weight of pups during lactation in rat one- and two-generation reproductive toxicity studies and a perinatal exposure study; adverse renal and skeletal effects observed in two rat developmental toxicity studies; and altered sexual differentiation observed in a study of perinatally-exposed male rats. These effects, taken either individually or in combination, showed a clear toxicological continuum of severity and/or marked progression of response with increasing dose.*"

The available data for developmental toxicity showed a consistent pattern of effects. DINP affected postnatal growth in one- and two-generation reproductive toxicity studies, as evident from significantly reduced pup growth at doses of 143-285 mg/kg bw/day (during gestation and lactation; Waterman et al. 2000 [Exxon 1996a and b]). The results of two developmental toxicity studies on DINP (Waterman et al. 1999 [Exxon 1994]) and Hellwig et al. 1997 [a screening study]) are also consistent showing increased incidences of skeletal variations (rudimentary lumbar and/or supernumerary cervical ribs) and adverse renal effects in foetuses. EPA identified NOAEL and LOAEL values of 100 and 500 mg/kg bw/day, for skeletal variations, and 200 and 1000 mg/kg bw/day for the kidney effect, respectively (Waterman et al. 1999; Hellwig et al. 1997). In addition, DINP increased incidences of reproductive malformations in male offspring and alterations in foetal testicular testosterone production and content after exposing pregnant rats during gestation/perinatally to 750 mg/kg bw/day (Gray et al. 2000; Borch et al. 2004).

EPA was of the opinion that a reduction in the mean body weight of pups exposed to DINP is a sensitive indicator of developmental toxicity. The pup weight decrements were considered serious "*because (1) they were statistically significant, (2) dose-related, (3) ranged from 9-22% below control values, (4) tended to increase with DINP exposure over time via milk, (5) were consistently observed in both sexes, and in both F1 and F2 generations of the two-generation study, (6) were noted in both one- and two-generation studies and (7) may have long term consequences*."

EPA also considered that the kidney and skeletal variations observed in rats treated with DINP are to be regarded as serious as they are structural effects that indicate that development has been disrupted. EPA pointed out that the occurrence of extra cervical ribs may also lead to serious health consequence and they refer also to NTP-CERHR (2003a) who concluded: *"…..supernumerary cervical ribs are an uncommon finding and their presence may indicate a disruption of gene expression leading to this structural anomaly. In addition, there is concern that cervical ribs may interfere with normal nerve function and blood flow"*. EPA further concluded that the effects observed in male rats by Gray et al. (2000) are to be regarded as serious as they represent gross morphological malformations which are not normally seen. The observed effects in the study by Gary et al. (2000) indicate that DINP has the potential for anti-androgenic effects in neonatal male rats when tested at 750 mg/kg/day. The absence of similar effects in the two-generation reproductive toxicity study conducted by Waterman et al. (2000) than those reported by Gray et al. (2000) may be explained, in part, by the differences in dose and protocols used. There were differences in exposure levels during gestation (approximately 560 mg/kg bw/day vs. 750 mg/kg bw/day, respectively), parameters measured, number of animals examined (statistical power) and routes (gavage vs. diet).

The findings of a recent study by Borch et al. (2004) showed decreased T production and content in foetal testis after maternal exposure to DINP and increase the weight of evidence together with observation from other structurally related phthalate esters for disruption of testosterone synthesis as a potential mode of action for the observed effects on the male reproductive system. EPA concludes that "*although information is currently lacking on 1) the precise mechanism(s) responsible for DINP-induced malformations and its relevance to humans and 2) the critical window of susceptibility for these effects during reproductive development, the Agency believes that it is premature to conclude that humans would not be effected if exposed to sufficient concentrations of DINP or its metabolites at critical stages of reproductive development*."

EPA also reviewed the new information on DINP exposure and concluded that there are not sufficient data to conclude that DINP does not cause adverse effects on humans at critical stages of reproductive development.

US CPSC 2010a

The United States Consumer Product Safety Commission's (US CPSC) Health Sciences' staff published their assessment of the potential toxicity associated with DINP (US CPSC 2010a). CPSC staff assesses product's potential health effects to consumers under the Federal Hazardous Substance Act (FHSA). The main purposes were the hazard identification and dose response assessment but it also briefly summarizes information relating to exposure. The information in this report is provided to the Chronic Hazard Advisory Panel of Phthalates which assesses the potential health effects of cumulative exposure to phthalates from all sources. The conclusions by NTP-CERHR (2003a) were included into the report.

CPSC staff concludes for reproductive effects that "*Only one study of the reproductive effects of DINP in mammals has been reported (Waterman et al. 2000). The CERHR (2003) concluded that male and female rat reproductive function and structure of reproductive organs are unaffected by exposure to DINP at maternal doses of 555/1,129 mg/kg-d during gestation and lactation, respectively, and adult doses as high as 1,676 mg/kg-d in males and 1,694 mg/kg-d in females…"* and further *"Overall, the CPSC staff concludes that there is inadequate evidence for reproductive toxicity of DINP in animals. No studies on the reproductive effects of DINP in humans are available."*

For developmental toxicity CPSC staff concludes that *"there is sufficient evidence for developmental effects of DINP in animals based on the observation of malformations of the kidneys, male reproductive organs, and skeletons in multiple studies in rats. The lowest NOAEL for developmental malformations in animals is 100 mg/kg-d (Waterman et al. 1999). NOAEL's have not been established for reduced pup weight (LOAEL = 143-285 mg/kg-d)(Waterman et al. 2000) or decreased testosterone production (LOAEL = 750 mg/kg-d)(Borch et al. 2003, 2004). Furthermore, the staff concludes that there is inadequate evidence for developmental effects in humans. DINP is considered to be a probable developmental toxicant in humans, based on sufficient evidence in animals."*

US CPCS staff (2010a) referred in their evaluation to three studies with a prenatal exposure (Hellwig et al. 1997; Waterman et al. 1999; Hellwig and Jackh 1997 (isononyl alcohols)). The studies examining developmental toxicity after perinatal exposure and reviewed by US CPSC (2010a) are presented in Table 4.36. In addition to these, results from Adamsson et al. (2009) and a Herschberger assay (Lee and Koo 2007) are reported. For human data, the report refers to the studies from Main et al. (2006), Swan et al. (2005) and Zhang et al. (2009).

Table 4.36 Developmental studies of DINP in animals – perinatal exposure (from CPCS 2010a).

Study	Doses, species/strain	NOAEL mg/kg-d	LOAEL mg/kg-d	Effects
Waterman et al. (2000) DINP-1	0, 0.2, 0.4, 0.8% in feed (2 – generation study) SD rat	ND	143-285 (0.2%)	Decreased pup weight
Gray et al. (2000) DINP-1	0, 750 mg/kg-d GD 14 – PND 3 SD rat	ND	750	Areolas/nipples; testicular malformations; epididymal agenesis (males)
Ostby et al. (2001) DINP -1	0, 1000, 1500 mg/kg-d GD 14- PND 3 SD rat	ND	1000	In males only: areolas; reduced AGD

Hass et al. (2003)[b]	0, 300, 600, 750, 900 mg/kg GD 7- PND 17 Wistar rat	300 300	600 600	Nipple retention (males) [c] Maternal pup retrieval
Borch et al. (2003, 2004) DINP - 2	0, 750 mg/kg-d (PND 7-21) Wistar rat	ND	750	Decreased testosterone production & content (males)
Masutomi et al. (2003) DINP - 2	0, 400,4000, or 20,000 ppm in feed GD 15 – PND 10 SD rat	30-66 (0.04%) 307 – 657 (0.4%)	307 – 657 (0.4%) 1164 – 2657 (2.0%)	Decreased pup weight Testicular atrophy & histopathology
Masutomi et al. (2004) DINP - 2	0, 20,000 ppm in feed GD 15 – PND 10 CD (SD)IGS rat	20,000 ppm[d]	ND	No effect ER gene transcription in the SDN No other endpoints were studied.[c]
Takagi et al. (2005) DINP - 2	0,4000, 20, 0000 ppm in feed GD 15 – PND 10 CD (SD) IGS rat	20,000 ppm[d]	ND	No effect ER gene transcription in the SDN No other endpoints were studied. [c]
Lee et al. (2006b) DINP - 2	0,40, 400, 4000, 20,000 ppm in feed GD 15 – PND 21 Wistar - Imamichi rat	ND	40 ppm[d]	Reduced pup weight Reduced AGD (males)[c] Increased grn gene transcription (females) Increased p130 gene transcription (males) Reduced lordosis quotient (females)

a AGD, anogenital distance; ER, estrogen receptor; GD, gestational day; grn, granulin precursor; LOAEL, lowest observed adverse effect level; ND, not determined; NOAEL, no observed adverse effect level; PND, postnatal day; SDN, sexually dimorphic nucleus.
b DINP type or source unspecified.
c Other malformations, variations, and developmental delays were not studied or not reported.
d Food consumption or dose in mg/kg-d were not reported.

It should be noted that US CPSC takes over the developmental NOAEL determined by NTP-CERHR Panel (2003a) from the study of Waterman et al. (1999; [Exxon 1994]). The NTP-CERHR Panel identified an effect level of 500 mg/kg bw/day and a NOAEL of 100 mg/kg bw/day which is lower than the NOAEL of 500 mg/kg bw/day in EU Risk Assessment for that study. The report of US CPSC states that "*There was a statistically significant increase in the percentage of fetuses with dilated pelves at 1,000 mg/kg –d. There was also a significant positive trend in dilated renal pelves. Rudimentary lumbar ribs were also significantly increased at the high dose. No other fetal effects were reported.*

The authors concluded that the LOAEL for maternal and developmental toxicity was 1,000 mg/kg-d, with a NOAEL of 500 mg/kg-d. However, the Expert Panel concluded that developmental effects (skeletal variations and rudimentary lumbar ribs) were present at 500 mg/kg-d (CERHR 2003). At the request of the CERHR panel, the study sponsor reanalyzed the data, the results of which supported the Expert Panel's conclusion. Thus, the Expert Panel determined that the NOAEL for the study was 100 mg/kg-d. The Expert Panel also calculated a benchmark dose (BMD_{05})(dose at which 5% of the animals are affected) of 193 mg/kg-d and a lower confidence limit ($BMDL_{05}$ of 162 mg/kg-d, based on the incidence of rudimentary ribs.

This study is limited because the dams were not exposed during the optimum window for antiandrogenic effects (GD 16-19)."

US CPSC as well as NTP-CERHR Panel (2003a) identified a LOAEL of 143-285 mg/kg bw/day (during gestation and lactation, respectively) for developmental effects (reduced weight gain in pups during perinatal and preweaning period) based on two-generation reproductive toxicity study of Waterman et al. (2000). In EU Risk Assessment (EC 2003a), LOAEL of 159 mg/kg bw/day is referred for this effect. The US CPSC report also notes that effects on male reproductive tract development, such as those associated with phthalate exposure, were not examined and that the study design may have lacked sufficient power to detect such effects.

For the endocrine effects (see Table 4.37), US CPSC concludes the following: *"Certain of the developmental effects in animals, specifically effects on male sexual development, are believed to be largely to the inhibition of testosterone synthesis (Gray et al. 2000; Parks et al. 2000). … DINP exposure also led to reduced testicular weights following chronic or subchronic exposure in mice and rats. These antiandrogenic effects of DINP on testosterone synthesis are not due to direct interaction of DINP with the androgen receptor. There is no evidence that DINP binds significantly to the estrogen receptor (Harris et al. 1997; Zacharewski et al. 1998).*

One study in humans suggests that lactational exposure to DINP metabolites in male humans may be associated with increases in levels of luteinizing hormone and decreased testosterone (Main et al. 2006). This study is confounded by exposure to multiple phthalates. The CPSC staff concludes that there is inadequate evidence of endocrine effects in humans.

Apart from certain developmental effects, there are few studies on the possible endocrine effects of DINP. There were certain testicular effects in mice and rats, including reduce testicular weight and abnormal sperm (Bankston 1992; Moore 1998a, b; Moore 2000; Myers 1991). It is not clear whether the testicular effects are caused by, or are a cause of, endocrine effects in the animal. A Herschberger assay in rats suggests that DINP may cause antiandrogenic effects in developing males (Lee and Koo 2007). The CPSC staff concludes that there is limited evidence of endocrine effects in animals.

Overall, DINP is considered "possibly toxic" in humans with regard to endocrine effects, based on inadequate evidence in humans and limited evidence in animals. "Possibly toxic" means that the evidence does not satisfy the regulatory definition of "toxic" with regard to endocrine effects. The CPSC staff will not consider endocrine effects in risk assessment or risk reduction activities. However, the staff will continue to monitor new information as it becomes available."

Table 4.37 Endocrine effects of DINP and MiNP exposure in males[a] (from US CPSC 2010a)

Species/strain	Study design	Effect	NOEL[b]	LOEL[b]	Reference
Human	Lactational exposure to MiNP	Increased LH and LH: testosterone ratio	ND	ND	Main et al. (2006)
Rat, F344	13 weeks in feed	Reduced testicular weight	292	584	Myers (1991)
Rat, F344	2 years in feed	Reduced testicular weight	733	ND	Moore (1998a)
Mouse, B6C3F1	13 weeks in feed	Reduced testicular weigh; Abnormal sperm	904	2365	Bankston (1992); Moore (2000)
Mouse, B6C3F1	2 years in feed	Reduced testicular weight	276	742	Moore (1998b)
Rat, SD	Hershberger assay	Decreased seminal vesicle weight	ND	20	Lee and Koo (2007)

		Decreased levator ani/bulbocavemous weight; increased LH; decreased testosterone	100	500	

[a] LH, luteinzing hormone; LOEL, lowest observed affect level; MiNP, monoisononyl phthalate;ND, not determined; NOEL, no observed effect level
[b] Doses in mg/kg-d

Industry

ECPI 2009, 2011a
European Council of Plasticizers and Intermediates (ECPI) reviewed recent scientific data on DINP and carried out a risk characterisation for its use in toys and childcare articles (ECPI 2009). Their main conclusions are in line with the conclusions of the EU Risk Assessment (2003a).

The one-generation dose range finding study (reported by Waterman et al. 2000) was said to be sufficient to indicate that exposure to DINP is not associated with detectable effects on reproductive function. Increase in cervical and lumbar ribs observed in Waterman et al. (1999) and Hellwig et al. (1997) at 1,000 mg/kg bw/day are considered to be evidence of developmental delays and reversible changes in rodents and lead to a NOAEL of 500 mg/kg-bw/day. The two-generation reproductive toxicity study (reported by Waterman et al. 2000) suggest a reduction in weight gain in pups during the perinatal and pre-weaning period of life. The LOAEL based on this effect was said – similarly to the EU Risk Assessment - to remain approximate since pups switched diet from milk to solid food between postnatal day (PND) 14 and 21 but may be estimated to be 159 mg/kg/d, the lowest dose of the estimated maternal range of dose (159 – 395 mg/kg bw/day) during post-partum.

ECPI has made available a report on the evaluation of endocrine data for selected phthalates (ECPI 2011a). Additional endpoint studies have been conducted since the EU Risk Assessment and ECPI's report provides a review of all endocrine data relevant to human health for selected high molecular weight (HMW) phthalates, among those DINP, using the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals ("OECD Conceptual Framework"). Based on this information ECPI concluded there are sufficient data to conclude that DINP is not an endocrine disrupting substance for mammals. The key conclusions by level of the OECD Conceptual Framework for DINP are presented below:

Level 1 Sorting & prioritization based upon existing information
DINP has a rich safety dataset available. These data have previously been collated and evaluated by international regulatory authorities and assessed for their potential risk to human health and the environment. The EU Risk Assessment concluded that DINP should not be classified as hazardous under EU regulations, and that risk reduction is not required for any current use. SCTEE concluded that the available data indicates that the potency of DINP for anti-androgenic effects is very low.

Level 2 In vitro assays providing mechanistic data
No significant responses were observed with DINP in any of the in vitro assays. Taken as a whole, the available data indicate that DINP does not have significant interactions with the oestrogenic or androgenic receptors. It is noteworthy that in vitro data need to be evaluated very carefully as the tests may have involved either substances which are not relevant for in vivo conditions or may have employed non-physiological conditions.

Level 3 In vivo assays providing data about single endocrine mechanisms and effects
Taken as a whole, these data support the conclusion that DINP does not cause adverse endocrine effects in in vivo screening studies. DINP shows no significant adverse effects in the Uterotrophic Assay (for oestrogenic effects), and no consistent significant adverse effects in the Hershberger Assay (for anti-androgenic effects). In non-validated research studies for anti-androgenic effects DINP showed no, minor or inconsistent effects at high doses, and with no or limited evidence of a dose response. While one animal study shows no effects on foetal testicular T, one study shows variable effects with no dose response, and one study shows reduced foetal testicular T at a single high dose, this was not associated with any adverse health effects in the animals. If there is an effect on T this would appear to be occurring at high doses only and without adverse health effects being seen in animals. Given this potential effect it is appropriate to assess Level 4 and Level 5 studies and confirm whether or not adverse health effects are being seen.

Level 4 In vivo assays providing data about multiple endocrine mechanisms and effects
Taken as a whole these data support the conclusion that DINP does not induce endocrine mediated chronic toxicity in rodents or non-human primates.

Level 5 In vivo assays providing data on effects from endocrine & other mechanisms
Based on the comprehensive one-generation, two-generation reproductive studies and the developmental studies it can be concluded that DINP is not an endocrine disruptor in OECD guideline in vivo studies. The adverse health effects mediated via an endocrine mechanism of cryptorchidism, hypospadias, and significant testicular pathology which are seen with DBP in laboratory animals are not seen with DINP.

ACC Panel 2005
The Phthalate Ester Panel of American Chemistry Council (ACC) submitted comments on US EPA's Revised Technical Review of DINP (2005) in support of EPA's proposal to add DINP into the list of chemicals under section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA). The ACC Panel argued that "*DINP cannot reasonably be anticipated to cause serious or irreversible developmental toxicity in humans. EPA had focused on the few marginal effects observed in developmental studies of rats to conclude that DINP causes developmental toxicity in humans."* Developmental effects referred include decrease in pup body weight gain, renal effects (dilated renal pelvis), skeletal variations (cervical and lumbar ribs), "reproductive malformations cited by EPA" (presence of areolas and retained nipples in males, small testes and testicular atrophy in two animals), and histological lesion in the gonads of male and female rats). In ACC's opinion none of these effects nor the occurrence of minimal to slight histological lesions in the ovaries and testes of rats were indicative of serious or irreversible chronic effects in humans. Their conclusions were supported by the opinion of an expert (an owner of an independent consulting firm specializing in developmental, reproductive and general toxicology) in developmental and reproductive toxicology.

ACC were of the opinion that there is no evidence that decreased body weight gains observed in rat pups are irreversible, that they impact health or reproductive success and were likely the result of postnatal ingestion of DINP rather than due to in utero exposure. They were considered to be due to peroxisomal proliferation, not relevant to humans. In ACC's opinion, the pup weight effects were within historical control limits and had no long-term effects, suggesting that they are not serious.

ACC further argues that dilated renal pelvis observed in rat development toxicity studies were transient findings and of doubtful significance occurring only at maternally toxic dose levels and within historical control ranges. EPA concluded that dilated renal pelvis might lead to kidney damage levels which does not meet the standards leading to a conclusion that DINP can reasonably be anticipated to cause developmental effects in humans according to ACC.

Regarding cervical and lumbar rib variants ACC concluded they are common developmental variants in developmental toxicity studies and are usually reversible and generally not considered as toxicologically significant. The lumbar ribs were more frequent only at high, maternally toxic doses when presented per litter basis, and within the historical control range. The cervical ribs were more frequent but without statistical significance at maternally toxic doses. In addition, these findings were not reported together with other signs of embryotoxicity and were therefore regarded as of little biological significance.

ACC concluded that the reproductive malformations in male rats reported by EPA were marginal, transient and of questionable statistical significance and occurring only at a high dose level. They pointed out that the retained nipples and areolae in males did not differ in adult animals. The testicular findings in two animals (small testes in one animal and testicular atrophy in another animal) without a reduction in reproductive organ weights in adult animals were corroborative of results from a two generation reproductive toxicity study showing no effect on reproduction. The histological lesions in the gonads of male and female rats were judged as minimal to slight in severity by the study authors according to ACC. The effects were slight, observed only at high, maternally toxic dose levels and not accompanied by changes in reproductive organs weights or other developmental parameters.

With regard to the endocrine effects of DINP, EPA cites the results of a study by Borch et al. (2004), in which in utero exposure to 750 mg/kg/day of DINP significantly reduced testosterone levels in male foetuses. ACC refers EPA's statement: *"These results indicate that in utero exposure to DINP disrupts steroidogenesis in male offspring and suggest a possible mode of action (MOA) for the antiandrogenic effects of DINP on male fetal reproductive tract development observed by Gray et al. (2000)."* ACC Panel agrees that the changes in testosterone reported in Borch et al. (2004) are merely a biomarker that may suggest a MOA for the effects in Gray et al. (2000) and they are not themselves toxic effects. Therefore, ACC Panel keeps the opinion that the effects reported in Gray et al. (2000) were marginal, of questionable statistical significance, and/or transient.

ACC concluded that the data reported by EPA do not support the conclusion that DINP can cause serious or irreversible developmental toxicity in humans.

ExxonMobil 2011a

In their comments to the CHAP-CPSC, ExxonMobil (2011a) concluded that extensive developmental and reproductive toxicity data are available for DINP indicating that the findings are different from those reported for other phthalates. ExxonMobil refers to studies on both DINP and DIDP, but only information related to DINP is presented in the following paragraphs. DINP has not been shown to cause cryptorchidism, hypospadias, or gross reproductive tract malformations and there is no strong evidence for adverse sperm effects. Multigeneration studies do not indicate effects on fertility (Watermann et al. 2000). Reported high dose findings in AGD, nipple retention and foetal T levels are of questionable significance in light of other studies and no evidence of adverse male reproductive tract development and reproductive performance.

A small increase in sperm count on PND 90 was observed after a gestational and lactational high dose exposure considered not indicative of an effect in testicular sperm production (Boberg et al. 2011). Kwack et al. (2009) reported a reduction in sperm count (~25%) in adult males after juvenile rats were exposed to 500 mg/kg bw/day for four weeks. Sperm motion/quality parameters, such as straight-line velocity, curvilinear velocity, straightness, and linearity, were decreased. Contradictory, there were no effects on fertility in two-generation reproductive toxicity studies indicating a questionable relevance of the findings. A significant reduction in sperm count is needed to affect the fertility in cases of good sperm quality (Parker 2006). Reproductive performance is critical to the interpretation of the findings of Kwack et al. (2007).

Male reproductive tract malformations, such as cryptorchidism or hypospadias, have not been reported for DINP (Waterman et al. 2000; Adamsson et al. 2009; Boberg et al. 2011; Borch et al. 2004; Gray et al. 2000; Hellwig et al. 1997; Kwack et al. 2009; Lee and Koo 2007; Lee et al. 2006a and b; Masutomi et al. 2003; 2004; Waterman et al. 1999). Four of 52 adult males (from three litters) exposed perinatally to DINP exhibited a malformation: one displayed a fluid-filled testis, a second displayed paired testicular and epididymal atrophy, the third displayed bilateral testicular atrophy and the fourth displayed unilateral epididymal agenesis with hypospermatogenesis and scrotal fluid-filled testis devoid of spermatids (Gray et al. 2000). The pooled incidence of effects was 7.7% (compared to 82% with DEHP treated animals). Based on historical control data and pooling of data to achieve significance, the significance of the reported findings is questionable.

Unaltered AGD was reported in two studies (Gray et al. 2000; Masutomi et al. 2003). Boberg et al. (2011) reported a small (6%) but statistically significant decrease in AGD at 900 mg/kg/day on PND 13 but not on PND 90. Reduced AGD at all dose levels was reported by Lee et al. (2006b) but the change was very small and potent anti-androgens had no effect in this study suggesting a reporting error.

DINP does not induce permanent nipple retention as indicated by Gray et al. (2000) and Boberg et al. (2011). On PND 13, an incidence of 22% males with areolae was observed, but this reduced to 2/52 cases at age of 5 month (Gray et al. 2000). In this study the incidence in the control group was 0 but in another study from the same laboratory it was 14% which confounds the interpretation (Ostby et al. 2001). An increase in nipples in males at 750 and 900 mg/kg bw/day (average of 3 nipples in each dose group) as compared to controls (average of 2 nipples) on PND 13 but not on PND 90 was reported by Boberg et al. (2011). The biological and/or toxicological significance of nipple retention observed in early postnatal male rats is questionable due to their temporary nature and the difference between humans and rats – human males do not lose their nipples.

DINP present a very different toxicity profile compared to other phthalates and not support inclusion of DINP in a cumulative (combined) risk assessment based on the vague and imprecise "rat phthalate syndrome." A reduction of foetal T and/or a reduction in insulin-like 3 peptide hormone biosynthesis (Insl3) during the critical window of male reproductive tract development have been hypothesized to lead this syndrome. Definitive two-generation and developmental toxicity studies indicate that cryptorchidism is not induced by DINP suggesting that DINP does not likely affect Insl3 as also supported by measurements of Insl3 mRNA levels (Lambright et al. 2011). An increase in Insl3 mRNA expression observed 2 days after the last dose may results from a "rebound effect" from a low T production at the time of dosing (Adamsson et al. 2009).

Humans differ from rats in aspect of testicular steroidogenesis and the relevance of reduced T in rats to humans is not supported. Limited data using human tissue does not indicate any effects of examined phthalates on the Leydig cell or suppression of T levels (Lambrot et al. 2009; Hallmark et al. 2007; Heger et al. 2010; 2011). DINP induces only a transient reduction in foetal plasma/testicular T levels at high doses. Four studies reported no effects (Adamsson et al. 2009; Boberg et al. 2011; Gray et al. 2000, Lee et al. 2006a;b), at a single high dose level (Borch et al. 2004) or without a clear dose-response (Boberg et al. 2011) and not post-gestation days (GD) 21 indicating that the effect is transient.

DINP does not induce decreased weights in androgen sensitive tissues (Adamsson et al. 2009; Boberg et al. 2011; Gray et al. 2000). The weights of the sex accessory tissues showed no consistent or dose-related significant changes (Lee and Koo 2007), and results do not meet the Organisation for Economic Co-operation and Development (OECD) or Environmental Protection Agency (EPA) criteria for being classified as having positive results since not all tissues were effected and no dose-response was observed.

DINP has no effects on the age of preputial separation (Gray et al. 2000; Masutomi et al. 2003) or male mating behaviour as measured as frequency of copulatory behaviours (Lee et al. 2006a and b). These observations support the findings of the definitive two-generation reproductive and developmental toxicity study (Waterman et al. 2000). A direct citation from ExxonMobil: *"Impaired fertility would be considered the decisive concern and ultimate result of the collective effects described for the male reproductive tract and termed "rat phthalate syndrome". As previously described, there were no effects on male fertility parameters or reproductive performance in either the parental (P) or first filial (F1) generation. These studies demonstrate that adult males (P) exposed to DINP prior to mating are successfully able to reproduce. More importantly, the reproductive capacity of the F1 generation males, which were exposed to DINP throughout their lifetime, is unaltered. Therefore, it is clear that DINP does not impair fertility. Conclusion: DINP and DIDP Do Not Induce "Rat Phthalate Syndrome"".*

ExxonMobil concluded that DINP does not modulate the endocrine system leading to adverse effects. According to OECD Conceptual Framework and using commonly recognized definitions, DINP is not an endocrine disrupting substance.

DINP didn't show significant responses in vitro studies examining oestrogen/ anti-oestrogen, androgen/ anti-androgen activity, modulating thyroidal active iodide uptake or other examined hormonal activities (Harris et al. 1997; Koch and Angerer 2007; McKee et al. 2002; Zacharewski et al. 1998; Akahori et al. 2005, 2008; Takeuchi et al. 2005; Kruger et al. 2008; Mlynarcikova et al. 2007; Breous et al. 2005; Wenzel et al. 2005; Ghisari and Bonefeld-Jorgensen 2009); this is supported from vivo studies. Based on the results from two-generation reproductive, sub-chronic, and chronic studies, DINP does not meet the criteria of an endocrine disrupter, neither under the Weybridge, IPCS or REACH guidance definitions. ExxonMobil Chemical Company refer to following points: 1) DINP or MiNP does not bind to androgen receptors in vitro, 2) DINP does not meet the OECD criteria for an androgen antagonist, and 3) no effects on androgen sensitive endpoints after high dietary exposure have been observed and only minor effects on male reproductive tract after high gavage dosing with high Cmax values. DINP is not anti-androgenic.

Commentary to ExxonMobil 2011a

ExxonMobil considers that without effects in functional fertility (reproductive performance), effects in sperm counts or sperm motility should not be seen as indications of impairment in fertility. In addition, the high dose findings in AGD, nipple retention and foetal T are argued to be of questionable significance in light of other studies and not evidences of adverse male reproductive tract development and reproductive performance.

It should be highlighted in this context that rodents have an excess of sperm in their ejaculates: as much as 90% reduction in sperm count may be needed to affect the fertility index in rodents. Human males on the contrary have highly variable sperm counts, generally lower than in rodents, and many men have sperm concentrations near or below WHO reference values for fertility (OECD 2008). Thus, in a case of human subfertility even a small change in sperm count or sperm motility may lead to infertility. For this reason, a statistically significant change in sperm count in a rodent study is considered to be indicative of a potential effect on fertility in humans (OECD 2008).

The findings in AGD, nipple retention and foetal T, suggest an anti-androgenic mode of action (androgen deficiency) and may be considered as relevant findings and predictors of potential adverse effect during human development. Decreased AGD in male offspring and nipple or areola retention has been shown to be predictive of other effects such as hypospadias and undescended testes (OECD 2008). Phthalates readily cross the placenta leading to foetal exposure levels reflecting the maternal exposure and epidemiological evidence on similar male reproductive tract malformations support the anti-androgenic mode of action.

ExxonMobil reports that humans differ from rats in aspect of testicular steroidogenesis and refer to limited data using human tissue. However, no detailed mechanistic hypothesis of the differences is provided. Many experts believe that steroidogenesis in the rat and human testes are rather comparable (for more details, see the Section 4.4.10). Issues related to species differences are discussed in the chapter of "Considerations on combined risk assessment of DINP and DIDP (and other phthalates)". ExxonMobil also questions the relevancy of the nipple retention as an adverse finding.

4.4.9.1.3 New studies

The studies conducted after the EU Risk Assessment (EC 2003a) are presented as divided according to the updated OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals (OECD Conceptual Framework) as presented in draft OECD guidance Document (GD) 150 (OECD 2011). This approach aims to make a clear presentation of the different type of studies and their results. The OECD Conceptual Framework should only be regarded as a system for categorising information and study types. The Levels should not be regarded as a study strategy that should be followed.

Briefly, the different assays are divided into the following levels:
Level 1: Existing data and non-test information
Level 2: In vitro assays providing data about selected endocrine mechanism(s) /pathways
Level 3: In vivo assays providing data about selected endocrine mechanism(s) /pathways
Level 4: In vivo assays providing data on adverse effects on endocrine relevant end-points
Level 5: In vivo assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organisms

For the purpose of this assessment, the information from the new studies is presented in two sets; information from Level 4 or 5 assays and information from Level 2 or 3 assays.

A) Information on reproductive toxicity and integrity of endocrine systems from Level 4 and 5 assays according to OECD Conceptual Framework
There are no new Level 4 or 5 guideline compliant studies following standard test guidelines and good laboratory practices (GLP) conducted with DINP such as two-generation reproductive toxicity studies or prenatal developmental toxicity studies. New studies concentrate on evaluation of effects after exposure during a critical window of masculinisation and investigates especially potentially adverse effects on male sexual development. The studies can be considered as developmental toxicity studies but they do not follow any standard test guidelines and are not done under GLP. Most of the studies are published but industry has also recently conducted two not (yet) published studies which were made available for review. There are also some information from investigations in humans comparing exposure and malformations.

Published in vivo animal studies

Masutomi et al. 2003, 2004
The effects of DINP (CAS No 28553-12-0, purity > 98%) to brain sexual differentiation and endocrine reproductive system were examined at dietary concentrations of 0, 400, 4000 or 20,000 ppm after perinatal exposure from GD 15 to PND 10 in SD rats. The calculated gestational and lactational maternal intakes were 0, 31, 307 or 1165 mg/kg bw/day during gestation and 0, 66, 657 or 2656 mg/kg bw/day during lactation corresponding to 0, 400, 4000 and 20,000 ppm, respectively.

Exposure to the highest concentration of DINP caused minimal/slight degeneration of meiotic spermatocytes (stage XIV) and Sertoli cells in the testis in 4 out of 5 males (none in control), and minimal/slight decrease of corpora luteae in the ovary in 4 out of 5 females as compared to one control female on PND 77 (week 11). At 20,000 ppm, a slight increase in scattered cell

debris in the epididymal ducts was also noted in 4 out of 5 males. There were no changes in the volumes of sexually dimorphic nucleus of the preoptic area (SDN-POA; PND 27), anogenital distance (AGD; PND 2), onset of puberty (cleavage of the balano-preputial skinfold PPS, vaginal opening VO), oestrous cyclicity (PND 56-77), organ weights or histopathology of endocrine organs at adult stage (PND 77).

Changes in the immunoreactive cell populations of luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin (PRL) were examined on PND 21 or PND 77. DINP had no effect on pituitary cell populations examined whereas oestrogenic methoxyclor and ethinylestradiol exhibited responses but different from each other. In addition to DINP, genistein, 4-nonylphenol and bisphenol A were also negative in this study.

There was a slight decrease in the mean number of live offspring at the highest dose level of DINP (11.2, 12.6, 13.0 and 9.2 at 0, 400, 4000 and 20,000 ppm, respectively). Pup body weight was also slightly decreased at the highest dose during PND 2-10. After the exposure period there was no change except on PND 27 body weights of female and male pups were reduced at the highest dose group and also in males at 400 ppm. Body weight gain was reduced in males during PND 21-42 at 20,000 ppm but was similar to that in control males from PND 42 to PND 77. In females, there was no change in body weight gain after PND 21. Both absolute and relative brain weights were reduced at 20,000 ppm in prepubertal males and females. Absolute and relative testes weights were also reduced at 20,000 ppm as well as absolute ovary and uterine weights and relative adrenal weights in females on PND 21.

The highest dietary concentrations (1165 mg/kg bw/day during gestation and 2657 mg/kg bw/day during lactation) were maternally toxic doses (decrease in body weight gain and reduced food intake) and caused also fetotoxicity (decrease in mean number of live offspring), toxicity during postnatal development (reduced body weights, body weight gain and some organ weights). These effects were not permanent after the cessation of the exposure. In spite of the systemic toxicity at the highest exposure level, there was no change in sexual development (AGD, VO or PPS) or brain sexual differentiation (volume of SDN-POA) when measured before the adulthood. The changes still seen in adults included degeneration of meiotic spermatocytes at stage XIV, vacuolar degeneration of Sertoli cells, scattered cell debris in ducts in epididymis and decrease in number of corpora luteae. All these changes were reported only at the highest exposure level and of minimal or slight changes but occurring in most of the exposed animals.

It may be concluded that perinatal exposure at dose levels causing systemic toxicity in the dams induce minimal or slight but permanent changes in testes and ovaries of the offspring. The NOAEL for the permanent (as well as nonpermanent) changes observed in this study is 307-657 mg/kg bw/day (4000 ppm).

Borch et al. 2004
Testicular T production ex vivo and T levels in testes and plasma of male foetuses at GD 21 were reduced after exposure to DINP during gestation (GD 7- GD 21) at 750 mg/kg bw/day. The reduction in T production and testicular T content was around 3-fold but the plasma levels were reduced only by approximately 25%, which was similarly to those seen after exposure to 300 mg/kg bw/day of DEHP. There were no other dose groups and, thus, no NOAEL can be derived. Co-exposure with 300 mg/kg bw/day of DEHP further decreased both the testicular T production and T levels. In addition, plasma T level was statistically significantly decreased after the co-exposure and in line with this the plasma LH level was increased on GD 21.

Takagi et al. 2005
Brain sexual differentiation was examined on PND 10 after exposure to DINP from GD 15 to PND 10 at dietary concentrations of 0, 4000 or 20,000 ppm (CAS No 28553-12-0). Expression levels of oestrogen receptor (ER) α and β, progesterone receptor (PR), steroid receptor coactivators (SRC-1 and SRC-2), gonadotropin releasing hormone (GnRH), and calbindin-D

(CALB) mRNAs in hypothalamic medical preoptic area (MPOA) were measured using real-time PCR. DINP did not change the expression levels of ERα, ERβ, PR or SRC-1 in males. The expression level of PR was reduced in females after normalization for glyseraldehyde-3-phosphate dehydrogenase (GAPDH) expression and a non-significant decrease after normalization for hypoxanthine-guanine phosphoribosyl transferase (HPRT) value was observed. After correction for total RNA there was no change. The rat PR gene contains oestrogen-responsive regions in the promoter region. It has been hypothesized that maternal progesterone plays a role in masculinisation of the male brain. Female rats exposed to oestrogen analogues during the perinatal period exhibit up-regulation of PR in the hypothalamic brain regions that include MPOA. In male rats, androgen receptors may have a role in suppression of PR gene expression.

Lee et al. 2006a,b
Lee and coworkers (2006a,b) examined the effects of perinatal exposure to DINP (CAS No 28553-12-0, purity >98%) on hypothalamic gene expression, hormonal levels and sexual behaviour. In addition to DINP, effects of DBP and di-(ethylhexyl)adipate (DEHA) were examined. Pregnant female Wistar-Imamichi rats were exposed to DINP in dietary concentrations of 0, 40, 400, 4000 and 20,000 ppm from GD 15 to the weaning on PND 21. The following measurements were done on the offspring: AGD on PND 1; serum sex-steroid hormone levels and hypothalamic gene expression of granulin (grn) and p130 on PND 7; and sexual behaviour, gonadotropin, T and oestradiol levels and oestrous cyclicity after maturation (postnatal weeks PNWs 8-9 and/or PNW 19-21).

The adjusted (normalised) AGD (AGD per cube root of body weight ratio) was decreased in males at all tested dose levels (40, 400, 4000 and 20,000 ppm) showing some dose-dependency. The adjusted AGD in females was increased at the dose level of 20,000 ppm.

Female rats showed a significant decrease in the lordosis quotient (number of lordosis reflexes/10 mounts by males x 100%), a measure of sexual responsiveness, at all tested dose levels. The lordosis quotient of female rats was approximately 75, 50, 45 and 25% at 0, 40, 400 and 4000 ppm, respectively, which indicates a clear dose-depend effect. The reduced copulatory behaviour in the low dose group males, without dose dependence, was discussed by the authors in light of the similar non-dose-depend findings reported also by others for another phthalate (DBP) (Masutomi et al. 2003). The sexual behaviour may be dependent on combined diverging effects on separate gene expression levels in hypothalamus. No effects were seen in male or female serum levels of LH, FSH and T or oestradiol on PND 7 or PNW 20. The oestrous cyclicity and gonadotropin surge on the day of proestrous were also normal in females.

Effects on sex-steroid regulated gene expressions in the neonatal female rat hypothalamus were seen such as increased grn mRNA levels at all doses (40, 400, 4000 and 20,000 ppm) but without a dose response, and increased levels of p130 mRNA levels in males at all dose levels, again without a dose response. The mRNA expression of grn and p130 in hypothalamus were measured because these sex steroid-regulated genes are believed to be involved in the sexual differentiation of the rat brain and are considered as relevant parameters for assessing the sex steroid properties of endocrine disrupting chemicals.

The results suggest that DINP has anti-androgenic properties based on the decrease in male AGD (AR antagonist) at all dietary concentrations but also weak androgenic properties based on increased AGD in females at the highest dietary concentration tested. There was no substantial change in serum T or oestradiol levels on PND 7, indicating that that inhibitory effect of the chemicals on foetal testicular T production, if any, may be transient. The effect of phthalates on gene expression in hypothalamus may be direct because they did not affect the serum sex steroid levels on PND 7. The authors concluded that inappropriate hypothalamic grn and/or gene expressions in neonatal rats after gestational and lactational exposure to DINP may suppress sexual behaviour both in adult males and females without affecting hormone levels of the hypothalamus-pituitary-gonad (HPG) axis. The increase in grn gene expression in

the female hypothalamus may be due to the oestrogenic properties of the phthalates. The anti-androgenic properties may account for the increase in p130 gene expression in male neonates. Grn and p130 may be involved in the processes of not only masculinisation (increase in male-type sexual behaviour) but also defeminisation (decrease in female-type sexual behaviour). The brain regions responsible for inducing preovulatory gonadotropin surge and responsible for inducing lordosis are thought to be different and, thus, the modulation of the gene expression during development in the brain area responsible for inducing lordosis may have affected the female sexual behaviour without any effects on the hormone levels.

Commentary to Lee et al. 2006a,b
In their first article, Lee et al. (2006a) indicate that they used 6-12 rats and four litters per dose group for measurements of male and female sexual behaviour, adult LH, FSH, T and oestradiol measurements. For AGD, there were 16-47 pups per group but no information on their litter distribution. It may, however, be anticipated that there were pups from at least 4 litters per dose group because the litter size was reduced to 8 pups (usually 4 males and 4 females) and theoretically 4 litters would be needed to have 16 pups which was the minimum number of pups in a dose group (Lee et al. 2006b). For the measurements on PND 7 (T, oestradiol, grn and p130 expression), the results are from 5-7 animals and it is not clear from the article if they are from different litters. The article does not provide information on how the possible "litter effect" (i.e., the effect of maternal environment and genetics) was controlled. This may play a role and reduce the validity of the statistical analysis which seems not to take this into account. In addition, a parametric analysis was used for non-parametric results (lordosis quotient). However, the lordosis quotient (number of lordosis reflexes/10 mounts by males x 100%) of female rats was approximately 75, 50, 45 and 25% at 0, 40, 400 and 4000 ppm, respectively, which indicates a clear dose-depend effect and seem to be a relevant finding irrespective of appropriateness of the statistical analysis. The methodology is not adequately described in the article and it is not absolutely clear how many mounts were recorded for the lordosis effect but assuming the time recording was the same as for the males, 30 min, it suggests that around 60 mounting was used in calculations per animal, which should be an adequate amount for recording lordosis reflexes. The rather low lordosis quotient of control females (75%) may indicate that the timing of the measurement may not have been optimal for females (proestrous). Positive control substances, oestradiol and T, inhibited the lordosis quotient (Lee et al. 2006a). It was clarified in the first article (Lee et al. 2006a) that the animals were from 4 different litters but this information was not given in the second article which also provided data on lordosis reflexes.

Female sexual behaviour has not been measured in other studies with DINP and the results from Lee et al. have consequently not been repeated yet by other researchers. Male copulatory behaviour was affected only at the lowest dose level (decreased frequency of mounts, intromissions and ejaculations, no effect in post-ejaculation interval) and, thus, even if it may display non-dose-dependency due to a complex net effect of differently expressed relevant genes in hypothalamus, in contradiction to the conclusions drawn by the authors, we do not consider the changes in male copulatory behaviour to be treatment-related.

There was no calculation of corresponding doses in mg/kg bw/day in the studies. Assuming that for an adult rat 1 ppm would correspond to 0.05 mg/kg bw/day, this would lead to a LOAEL of 2 mg/kg bw/day based on decreased sexual responsiveness in females (and also based on changed gene expression in hypothalamus and a limited finding of reduced AGD in males). A slightly higher LOAEL can be estimated using a food intake of 20 g/day for a pregnant animal. This would lead to a LOAEL of 3.2 mg/kg bw/day.

In conclusion, there is limited evidence that perinatal exposure to DINP (as well as to DBP and DEHA) change the expression of grn and/or p130 genes in the hypothalamus in neonatal animals might lead to decreased sexual behaviour after maturation, without affecting the endocrine system of the HPG axis. DINP seems to have anti-androgenic activity causing reduced AGD in males but also weak androgenic activity increasing the AGD in females. For

several reasons, however, the results of this study are not considered the primary driving force for the NOAEL/LOAEL setting: 1) there are critical limitations in reporting of the methodology and statistical analysis relating to the findings, such as reduced AGD in males and lordosis quotient; 2) the low LOAEL for AGD is not supported by other studies (Boberg et al. 2011; Clewell et al. 2011b) and the change in AGD is minor; 3) the gene expression findings show no clear dose response and would need to be confirmed; 4) the reduced lordosis reflex warrants replication due to limitations in the methodology and statistical analysis; 5) The rather low lordosis quotient of control females (75%) may indicate that the timing of the measurement may not have been optimal for females (proestrous); 6) measurement of lordosis reflex is not included in internationally accepted standard test methods; and 7) the results from one- and two-generation reproductive toxicity studies do not indicate affected fertility it is however acknowledged that fertility parameters measured in the available one- and two-generation reproductive toxicity studies may not adequately reflect lordosis quotient. Mating and pregnancy may be successful inspite of reduced lordosis reflex.

Overall, the drastically reduced female sexual behaviour observed in this study need to be followed up before any firm conclusions can be drawn.

Adamsson et al. 2009
The foetal testicular T content was not changed on embryonic day (ED) 19.5 after exposure to 250 or 750 mg/kg bw/day of DINP from ED 13.5 to 17.5. The finding suggests that DINP does not have an effect on foetal testicular steroidogenesis when measured two days after the exposure period. Plasma corticosterone concentration showed an increasing tendency at 250 mg/kg bw/day without affecting steroid acute regulator (StAR), side chain cleavage enzyme (P450scc) or 3ß-hydroxysteroid dehydrogenase (3ßHSD) expression levels in foetal adrenals at ED 19.5. At 750 mg/kg bw/day, DINP increased testicular mRNA levels of P450scc, transscriptor factor GATA-4 and (Insl3) on ED 19.5 although protein levels of testicular StAR, P450scc or 3ßHSD or T content were not changed. The authors suspected that the detected increased expression level is a "rebound effect" on steroidogenesis at the time of dosing a couple of days earlier. The different outcome of this study compared to results of Borch et al. (2004) may be due to the shorter exposure time used, according to the authors.

<u>Commentary to Adamsson et al. 2009</u>
It is considered plausible that testicular T content, if reduced due to exposure, has recovered in two days between the last dosing and the point in time when T levels were measured, and that the gene expressions associated with steroidogenesis were increased for several days after cessation the exposure and after the exposure-induced decrease.

Kwack et al. 2009
Kwack and coworkers (2009) reported a reduction in sperm count (~25%) in adult male SD rats after 4 weeks of exposure of juvenile rats to DINP at 500 mg/kg bw/day. DINP lowered the sperm counts and sperm motility of epididymal sperm. There was also a statistically significant decrease in sperm motion/quality parameters, such as straight-line velocity and curvilinear velocity. Liver weights were significantly increased at this dose level but testis weights were unchanged. There were no changes in haematological parameters, but glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP) and triglycerides were increased.

For comparison, DEHP decreased sperm count (~ 70%), motility, average path velocity and straight-line velocity at the same dose level. In addition, DEHP increased liver and thymus weights and decreased testis weights and increased serum levels of glucose and calcium. Based on the results from the phthalate diesters and monoesters examined, the adverse effects on sperm parameters were greater with phthalate diesters than monoesters according to the authors.

Boberg et al. 2011 (Hass et al. 2003(abstract))
In another study, effects on reproduction and sexually dimorphic behaviour were studied (Boberg et al. 2011). Pregnant Wistar rats, 16 animals per group, were administered DINP by gavage (CAS No 28553-12-0, purity 99%) at doses of 0, 300, 600, 750 or 900 mg/kg bw/day from GD 7 to PND 17. Foetuses from four dams per dose group were investigated for foetal T production (ex vivo) and testicular histopathology on GD 21. Maternal pup retrieval (interaction between mother and pups) was assessed on PND 1. After birth dams and live pups were weighed, the AGD of the pups was measured on PND 1 and the pups were examined for presence of nipples/aerolas on PND 13. Sexual maturation of the offspring was investigated by recording the day of vaginal opening (VO) in the females and cleavage of the balano-preputial skinfold (PPS) in the males. On PND 90, males were examined for presence of nipples, penile malformations and testicular descent and blood samples were collected from 1–7 males per litter for hormone analysis. In addition, the following organs were removed and weighed: liver, kidney, adrenal, thyroid, right and left testis, left epididymis, seminal vesicle, ventral prostate, levator ani/bulbocavernosus muscle (LABC), and bulbouretral gland. From all males one testis was fixed for histopathology and the contralateral testis was frozen for hormone analysis. Semen quality (motility and sperm count) was analysed on 1–3 males per litter and from further 1 to 3 males per litter, the epididymides were fixed for histopathology. On PND 90, females were killed on the day of oestrus and their uteri and ovaries were weighed. On PND 21, one or two male and female pups from each litter were weaned and used later for behavioural testing including assessment of motor activity and habituation capability, various learning and memory tests and sweet preference. Approximately on PND 200, the males were examined for malformations of external genitals and nipple retention.

Testicular histology on GD 21 was affected in all dose groups. All examined animals were affected in the two highest dose groups (750 and 900 mg/kg bw/day). MNGs were seen in some animals in the lowest dose group with dose dependent increase at higher doses (number of affected foetuses per examined foetuses: 0/7, 2/8, 3/5*, 6/7*, 6/6* at 0, 300, 600, 750 and 900 mg/kg bw/day, respectively (* = $p \leq 0.05$, one-sided Fisher's exact test); number of affected litters per examined litters: 0/3, 2/4, 3/3*, 3/3*, 3/3*). In addition, most testes at the two highest dose levels had an increased number of gonocytes with a central location in seminiferous chords, and chord diameters were significantly increased. No changes in testicular histopathology were noted at PND 90.

A dose-dependent reduction in the percentage of motile sperm was seen at and above 600 mg/kg bw/day on PND 90. The percentage of progressive sperm was decreased at 750 mg/kg bw/day where one animal had very low sperm motility (also small testes and epididymides). There was no effect on Inhibin B levels (indicator of Sertoli cell number and function). Mean testicular T content was 63% of control levels at the highest dose level on PND 90, but this was not statistically significant. Sperm count was significantly higher in the highest dose group. This 17% increase in sperm count per gram cauda epididymis reflected a 9% increase in sperm counts per sample and a 7% decrease in cauda epididymis weight in the highest dose group compared to controls.

There was a tendency towards reduction in ex vivo testicular T production in all exposed groups. Testicular T content was significantly reduced in the group exposed to 600 mg/kg bw/day alone. Plasma T of LH levels did not change due to the exposure. Uncorrected AGD in males were significantly lower at ≥ 600 mg/kg bw/day on PND 1, but when using birth weight as a covariate the reduction was statistically significant only at 900 mg/kg bw/day reflecting the slight decrease in pup body weights at higher doses. On PND 13, a statistically significant and dose-dependent increase in nipple retention in male pups was seen at 750 and 900 mg/kg bw/day with a borderline change at 600 mg/kg bw/day. At PND 90, two control males had one nipple each and three males per group from the three highest dose groups had one to six nipples each. Of these, two animals at 600 and 750 mg/kg bw/day, respectively, had 4 and 6 thoracic nipples. There was no statistically significant difference between groups in the number of nipples or AGD on PND 90. There were no changes in liver, thyroid or reproductive organs

weights. One male at 600 mg/kg bw/day and one at 750 mg/kg bw/day had small testes and epididymides.

In behavioural test pattern, investigating spatial learning and memory abilities in the Morris water maze, DINP exposed females showed a dose-dependent improvement in the highest dose group on the first day of memory testing. The improved performance of the females made their performance similar to the male performance, and may indicate masculinization of the brain in the high dose females. Effects of DINP on female behaviour were not observed on the other test days in the Morris water maze, nor in the other behavioural tests performed. Hormonal imbalance during the critical periods of brain development seems to be sufficient to alter the default female outcome in a more masculine direction. According to the authors this is supported by the fact that both androgens (T) and anti-androgens (such as cyproteronacetat) have been previously reported to improve female maze learning. On the other hand, anti-androgens such as DEHP, have not been shown to have similar effects.

There were transient changes in AGD, nipple retention (partly permanent, i.e. nipple/areolae retention was still observed at a later time point, but less nipples/areolae per animal were observed or less animals were affected than at the earlier time point) and testicular effects after exposure to DINP. The current study by Boberg and coworkers suggests that alterations in semen quality and sexual dimorphic behaviour may result after perinatal exposure to DINP. The authors conclude that the effects seen are similar to the observed effects, e.g., in foetal testicular histopathology, with other phthalates (DEHP and DBP), but that the effects of DINP are less potent. DINP also shows a similar mechanism of action as that of DEHP and DBP, namely reduction of factors involved in steroidogenesis leading to reduced foetal T levels. This is indicated by reduced staining intensity for the steroidogenic factors StAR, P450scc and Cyp17 based on the results of a combination study on DEHP and DINP and other studies (as referred by Boberg et al. 2011). Furthermore, the anti-androgenic effects (partly permanent) from Boberg et al. (2011) support the previously reported findings of low incidence of permanent malformations and lower semen quality in DINP exposed males (Kwack et al. 2009; Gray et al. 2000). The study also provides additional knowledge on effects on sexually dimorphic behaviours indicating masculinization of behaviour in DINP exposed females.

A NOAEL of 300 mg/kg bw/day (LOAEL of 600 mg/kg bw/day) was set for reproductive toxicity and anti-androgenic effects [seemingly mainly based on decrease in the percentage of motile sperm; a permanent change, although reversible changes such as nipple retention on PND 13 were also seen at and above 600 mg/kg bw/day, with a low incidence of permanent nipples (four animals) and 2 animals with very small testes and epididymides].

In summary, the authors concluded that the findings in this study of similar dose-related effects of DINP as previously seen with DEHP and DBP (nipple retention, reduction of AGD, disruption of semen quality) clearly support that DINP is an anti-androgen and a reproductive toxicant, but also that it is less potent than DEHP and DBP.

Commentary to Boberg et al. 2011

In spite of the low number of litters examined for testicular histology on GD 21, the result is convincing as MNGs were seen in animals from all dose groups dose-dependently. The NOAEL/LOAEL of 300 mg/kg bw/day is based on this effect (no NOEL). Most testes at the two highest dose levels (750 and 900 mg/kg bw/day) showed an increased number of gonocytes with a central location in seminiferous chords, and chord diameters were significantly increased.

There was a tendency towards reduction in ex vivo testicular T production in all exposed groups studied with a NOAEL for anti-androgenic effect at 900 mg/kg bw/day illustrated by significantly decreased measures of AGD on PND 1 using birth weight as a covariate in the reduction. However, there was no change in AGD on PND 90, indicating a reversible or transitional effect only although it should be noted that the same animals were not studied at

both instances. The number of males with nipples on PND 13 was increased at 750 and 900 mg/kg bw/day with a borderline effect at 600 mg/kg bw/day. The same animals were not examined on PND 90, but there seem to be at least a slight increase in males with nipples and number of nipples in one male, but there was no statistical significance.

The two treated males having small testes and epididymides (one at 600 and the other at 750 mg/kg bw/day) are considered as suggestions of an adverse effect only because there is no dose-response. On the PND 90, the histopathology of male reproductive organs was not affected. It is important to note that there was a slight decrease in pup body weights at higher doses indicating some general toxicity. The suggested masculinisation of female behaviour needs further clarification.

Hannas et al. 2011a,b
Foetal T production and gene expression levels in foetal testes were examined after oral gavage dosing of pregnant SD rats with corn oil (control), 500, 750, 1000 or 1500 mg DINP /kg/day on GDs 14-18. Two separate formulations of DINP were tested: CAS# 28553-12-0 and CAS 68033-90-2. At necropsy on GD 18 (1-3 hours after the last dose), there were a total of 37 pregnant dams with 6, 3, 4, 6, and 6 litters treated with CAS 28553-12-0 and 3, 2, 1, 3, and 3 litters treated with CAS 68033-90-2).

Both DINP formulations reduced foetal testicular T production similarly in a dose responsive manner at and above doses of 500 mg DINP/kg (no NO(A)EL; ED50 852 mg/kg bw/day) and reduced testis StAR and Cyp11a gene expression (NO(A)ELs of 750 mg/kg bw/day; ED50 901 and 1357 mg/kg bw/day, respectively). DINP was 2.3 fold less potent than DIBP DIHP, and DEHP in reducing foetal testicular T production (studied with a similar test set up) and 18-fold less potent that DPeP. Based on individual ED50 values for each phthalate, T production was the most sensitive foetal testicular endpoint from the 5-day in utero exposure to DEHP, DINP and DPeP, whereas Cyp11a expression was the most sensitive to DIBP. The order of potency for decreasing testicular StAR expression was DPeP >DIBP =DEHP >DINP and that for Cyp11a DPeP =DIBP >DEHP >DINP.

Commentary to Hannas et al. 2011a,b
DINP reduced foetal testicular T production at a lower dose than StAR and Cyp11a gene expression. There was no NO(A)EL but the lowest dose was already quite high; 500 mg/kg bw/day. The measurements were done right after the exposure which explains the difference from other studies where the measurements were done a couple of day after the last dosing (Adamsson et al. 2009).

Lambright et al. 2011 (abstract)
Foetal testes T production and gene expression were studied by exposing SD rats via oral gavage using a single dose of 750 mg/kg bw/day during GD 14-18. The T levels were measured after a 3 hours culture on GD 18 and pooled remaining testes were used for gene expression measurements of Insl3, StAR and Cyp11a (termination 1-3 hours after the last dose). DINP exposure significantly reduced foetal T production compared to control, as did several other phthalates (BBP, DBP, DIBP, DiHP, DHeP(diheptyl-), DHP(dihexyl-), DCHP (dicyclo-)). DEP, DOTP or DiNCH did not have any effect on T production. Some of the phthalates that reduced T production also significantly reduced gene expression of Insl3, StAR and Cyp11a. Results with some phthalates reducing both foetal T production and gene expression indicated that the reduced T production was the most robust response. Some phthalates with straight chains shorter than C4 or longer than C6 also disrupted foetal T synthesis.

DINP (CAS No 282553-12-0) reduced the foetal testosterone production by 50% at a dose level of 750 mg/kg bw/day administered during GD 14-18 (a poster presented in the 51st Annal Meeting of Society of Toxicology by Gray et al. "A fetal rat testes endocrine and genomic "signature" accurately predicts the phthalate syndrome of malformations"). For other DINP

mixture (68515-48-0) the testosterone production was around 60 and 77 % of the control value. For comparison, DEHP and DBP reduced T production down to around 10% of that of the controls at the same dose level.

Hannas et al. 2012
To define the relative potency of several phthalates to genomic biomarkers of male developmental effects, DINP was administered by gavage at dose levels of 0, 500, 750, 1000 or 1500 mg/kg bw/day on GDs 14-18. Phthalates positive for anti-androgenic activity as measured by ex vivo foetal testicular T production (1-3 hours after the last dose) were measured for effects on gene expression levels in foetal testes (e.g., Cyp11b1, Scarb1, StAR, Cyp11a1, Cyp17a1, Insl3 and Hsd3b). DPeP was the most potent for reducing each gene expression and DINP was the least potent. The ED50-values (mg/kg bw/day) for gene expression of DINP were: 326 for Cyp11b1, 597 for StAR, 602 for Scarb1, 797 for Cyp17a1, 852 for T production, 1148 for Cyp11a1, 963 for Hsd3b, 1488 for Insl3 and 2239 for Cyp11b2. The overall sensitivity of each gene endpoint and T production was Cyp11b1 >StAR =Scarb1 >Cyp17a1 =T production >Cyp11a1 =Hsd3b =Insl3 >Cyp11b2. The overall potency of the individual phthalates was DPeP >DHP >DIBP ≥DHeP >DINP.

DINP mixtures differing in the content of different ester side chain structures were used. DINP 1 included more isodecanol than DINP 2 (15-25% vs. 0%, respectively) and less methyl octanols (50-20% vs. 35-40%, respectively). DINP 1 (CAS 68515-48-0) and DINP 2 (CAS 28553-12-0) did not differ significantly in their ability to reduce foetal testicular T production or testis gene expression. It was clear from the results that phthalates did not affected PPAR-related genes in foetal testes. DINP down regulated acyl-CoA oxidase (Acox1) expression only at the highest dose level of 1500 mg/kg bw/day.

It was concluded that the anti-androgenic phthalates act through a similar mode of action but the proximate molecular target is still unclear.

New studies provided by Industry

ExxonMobil (2011d) has provided summaries of two new studies (Study #1 and Study #2, Clewell et al. 2011a and b) conducted in the Hamner Institutes in 2011. The studies are not guideline compliant GLP studies for regulatory purposes but targeted studies to examine the mode of action and permanency of effects after exposure during the critical time window of male reproductive tract development. The quality control of the studies follows the Research Quality Standards of The Hamner Institutes for Health Sciences. Based on the quality statement, the protocol and the study report have been checked by the quality assurance but there was no quality checks during the in life phase of the studies. In addition, there are no GLP statements in the reports from the pathology laboratories examining the slides, but one of the laboratories provides a quality assurance certification.

In both of these studies animals were exposed during gestational and/or the postnatal period (GD 12-19 or GD 12 – PND 14). The first study is a gavage study whereas the second one used dietary administration.

Study #1, ExxonMobil Biomedical Sciences, The Hamner Institutes for Health Sciences 2011 (Clewell et al. 2011a)
Male offspring of pregnant rats administered 0, 50, 250, or 750 mg/kg/day DINP from gestation day (GD) 12-19 (Clewell et al. 2011a) were examined for foetal development of the male reproductive tract, kinetics in maternal and foetal compartments. In addition, the physiologically based pharmacokinetic (PBPK) model developed for DEHP was evaluated using maternal and foetal kinetic data of DINP. DINP treated animals were euthanized at 0.5, 1, 2, 6, 12, and 24 hrs after the final (GD 19) dose and blood and liver were collected from dams, and in addition, 3 placentas per litter, pooled amniotic fluid and pooled foetal blood were sampled per litter and the testes pair from each foetuses were collected. AGD was measured in all pups

from the 24 hr time-point group (from all dose and control groups). Testes from 1-2 pups from each litter in the 2 and 24 hr time points (all dose and control groups) were stored for total T concentration measurements. Testes from one foetus from each litter of the 24 h group were processed for histopathology.

Cumulative maternal urine was collected at 7 and 24 hrs after the final dose for metabolite analysis. Distribution of the major metabolites of DINP was examined in maternal and foetal serum. Metabolite concentrations were also measured over time in maternal liver, placenta, urine, and foetal plasma and testes in order to characterize the absorption, distribution, metabolism and elimination of DINP during gestation, and to provide the data needed to extend a previously developed physiologically based pharmacokinetic (PBPK) model for DEHP to DINP.

In this study, absolute and scaled AGD (AGD/body weight$^{1/3}$) was not altered by exposure to DINP at up to the highest dose tested (750 mg/kg/day) on GD 20 (24 hours after the last dose). T concentration in testis was significantly reduced at 2 hr post-dosing, but no longer 24-hrs post-dosing at and above 250 mg/kg bw/day (Table 4.39). The decrease was approximately 50% at 250 mg/kg bw/day and approximately 60% at 750 mg/kg bw/day 2 hr post-dosing. There was no statistically significant change 24 hours after the final dose; the T concentration was increased more at 250 mg/kg bw/day than at 750 mg/kg bw/day [Evaluator's comment: this may suggest an over-compensation which may occur later at 750 mg/kg bw/day than at 250 mg/kg bw/day].

Table 4.38 Mean concentration of T (ng/mL) in foetal testis. Mean of the litters (percentage from control, range). Mean is calculated from 4-5 litters, 2 males per litter. Statistically significant decrease was indicated at 2 hours after dosing at 250 mg/kg bw/day (p<0.01) and 750 mg/kg bw/day (p<0.001)(1-way ANOVA with Dunnett's post test). (Based on Clewell et al. 2011a)

Hours after the last dose (and group)	Dose level (mg/kg bw/day)			
	0	50	250	750
2 (LD-1)	0.947 (0.520-1.1539	0.929 (98.1%; 0.586-1.689)		
2 (LD-3)	0.462 (0.292-0.705)	0.504 (109.1%; 0.301-0.866)		
24 (LD-1)	0.622 (0.155-1.597)	0.455 (73.2%; 0.165-0.794)		
24 (LD-3)	0.528 (0.281-0.843)	0.566 (107.2%; 0.260-0.763)		
2 (MD-1)	1.187 (0.817-1.747)		0.525 (**44.2%**; 0.375-0.748)	
2 (MD-3)	0.642 (0.264-0-870)		0.356 (**55.5%**; 0.072-0.676)	
24 (MD-1)	0.418 (0.264-0.633)		0.619 (148.1%; 0.212-1.560)	
24 (MD-3)	0.725 (0.185-2.005)		0.936 (129.1%; 0.572-1.332)	
2 (HD-3)	0.589 (0.067-1.180)			0.215 (**36.5%**; 0.082-0.420)
2 (HD-7)	0.451 (0.184-0.807)			0.184 (**40.8%**; 0.150-0.229)
24 (HD-5)	0.984 (0.733-1.609)			1.028 (104.5%; 0.602-1.706)
24 (HD-6)	1.560 (0.624-2.398)			1.584 (101.5%; 0.258-3.066)

There was no change in seminiferous tubule diameter. However, the number of multinucleated gonocytes was increased at ≥ 250 mg/kg bw/day 24 hours after the last dose (Table 4.39). The average number of MNGs was approximately 0.75 and 1.25 per testis section at 250 and 750 mg/kg bw/day, respectively. An increased incidence of large Leydig cell aggregates was observed in the highest dose group.

Table 4.39 Histopathology findings in testes on GD 20 (Huntingdon Life Sciences) (adopted from Clewell et al. 2011a)

	Control	DINP 50 mg/kg bw/d	DINP 250 mg/kg bw/day	DINP 750 mg/kg bw/day
Number of animals examined	27	8	8	8
Animals with MNGs	0	0	2	6*
Animals with increased number of gonocytes	0	0	0	2
Animals with large Leydig cell aggregates	2	3	1	7*

* p<0.001, 1-way ANOVA
MNGs=Multinucleated gonocytes

Absolute and relative maternal liver weights were increased at ≥ 250 mg/kg bw/day in both at 2 and 24 hr time points. Maternal weight gain or maternal and foetal body weights were not altered.

All the measured metabolites monoisononyl phthalate (MiNP), monocarboxyisooctyl phthalate (MCiOP), monohydroxyisononyl phthalate (MHiNP), monooxoisononyl phthalate (MOiNP) and monoisononyl phthalate glucuronide conjugate (MiNP-G) were present in the maternal and foetal plasma and tissues after DINP administration (Table 4.42). MCiOP was the major metabolite in plasma and tissues, followed in decreasing order by MiNP, MHiNP, MOiNP, and MiNP-G. The calculated half-live for MiNP in the maternal plasma was 4 hrs at all doses. Foetal half-live was a slightly longer, 4.5-4.7 hrs. The peak foetal plasma level (Cmax) of MiNP was 21 µM and that for dams 56 µM at 50 mg/kg bw/day (2.7-fold difference). The difference remained rather similar at higher doses. The area under the curve (AUC) for MiNP were almost similar in foetuses and dams at 50 mg/kg bw/day; 180 µM*hr for foetuses and 211 µM*hr for dams. At higher doses the maternal AUC was clearly larger than foetal AUC. MCiOP was present at comparable concentrations in foetal and maternal plasma as MiNP at 50 mg/kg bw/day. However, the AUC-values were higher for MCiOP than for MiNP at 50 mg/kg bw/day. At higher dose levels, the maternal peak concentrations as well the AUC-values were higher for MCiOP than MiNP. On the other hand, only the AUC-values of MCiOP were higher than those of MiNP and the Cmax-values of both metabolites were at similar range for the foetuses.

Table 4.40 Pharmacokinetic parameters for DINP metabolites in foetal (and maternal) plasma after the final dose of 50, 250 or 750 mg/kg bw/day administered from GD 12 to GD 19. (based on Clewell et al. 2011a)

Metabolite	Dose (mg/kg bw/day)	Foetal (maternal) plasma			
		Cmax (µM)	Tmax (hr)	T½	AUCinf (µM*hr)
MiNP	50	**21 (56)*[1]**	**1 (1)**	**4.5 (3.9)**	**180 (211)**
	250	85 (264)	1 (1)	4.7 (4.0)	535 (1241)
	750	87 (189)	2 (0.5)	4.7 (4.0)	865 (1393)
MCiOP	50	**20 (52)**	**6 (1)**	**13.8 (5.8)**	**485 (628)**
	250	61 (465)	6 (2)	17.8 (4.9)	1836 (5071)
	750	100 (601)	6 (2)	15.3 (5.7)	2838 (6080)
MHiNP	50	4 (10)	6 (1)	6.1 (5.5)	45 (79)
	250	13 (47)	2 (1)	7.1 (5.5)	155 (479)

	750	22 (60)	2 (2)	6.4 (5.8)	300 (636)
MOiNP	50	1 (2)	6 (6)	9.4 (10.5)	13 (26)
	250	4 (14)	2 (2)	11.6 (3.4)	53 (209)
	750	7 (26)	2 (2)	5.7 (7.3)	110 (308)
MiNP-G	50	2 (3)	6 (1)	5.6 (1.6)	23 (8)
	250	10 (11)	6 (1)	3.6 (2.8)	130 (112)
	750	28 (11)	6 (0.5)	5.3 (5.0)	336 (102)

*[1] values between brackets represent maternal values.
Cmax = maximum concentration
Tmax = time point for maximum concentration
T½ = half live
AUCinf = area under curve
MiNP = monoisononyl phthalate
MCiOP = monocarboxyisononyl phthalate
MHiNP = monohydroxyisooctyl phthalate
MOiNP = monooxyiosnonyl phthalate
MiNP-G = monoisononyl phthalate glucuronide conjugate

The highest concentration of MCiOP in foetal testes was 6 hours after the last dose at all dose levels reaching 25 µM concentration at the lowest dose of 50 mg/kg bw/day and 70.6 µM at the LOAEL of 250 mg/kg bw/day (Table 4.39). The highest concentration of MiNP was observed at 1 hour after the dosing and was 15.5 µM at 50 mg/kg bw/day and 92.9 µM at 250 mg/kg bw/day. Other metabolites occurred at lower concentrations in foetal testes.

Table 4.41 Concentrations of DINP metabolites in rat foetal testes after the final dose of 50, 250 or 750 mg/kg bw/day DINP from GD 12 to GD 19. (Based on Clewell et al. 2011a)

Metabolite	Dose (mg/kg bw/day)	Mean concentration, µM, (range) at different time point after final dose (h)			
		0.5	1	6	12
MiNP	50	7.7 (5.0-9.9)	**15.5** (5.8-22.9)	11.0 (8.8-12.2)	3.4 (2.7-4.4)
	250	17.3 (9.6-26.4)	92.9 (55.8-162.1)	35.0 (24.7-45.3)	16.5 (13.0-19.0)
	750	56.4 (35.1-71.1)	84.0 (52.5-129.5)	85.6 (63.1-111.7)	40.4 (24.6-70.0)
MCiOP	50	8.6 (6.8-10.7)	10.3 (7.7-12.4)	**25.0** (19.1-31.6)	13.1 (11.4-15.8)
	250	24.1 (19.4-29.3)	55.5 (38.3-89.5)	70.6 (52.6-83.7)	62.0 (45.7-71.2)
	750	52.3 (34.2-59.5)	88.4 (79.5-94.9)	160.4 (95.3-233.2)	133.3 (87.1-218.2)
MHiNP	50	0.6 (0.3-1.1)	1.3 (0.3-1.9)	3.1 (2.6-3.8)	1.1 (0.9-1.2)
	250	1.7 (1.4-1.9)	9.9 (5.8-17.1)	9.2 (6.6-12.6)	5.0 (4.4-5.7)
	750	6.0 (5.4-7.1)	12.2 (8.6-14.9)	25.1 (12.7-37.6)	16.0 (8.1-29.2)
MOiNP	50	0.2 (0.1-0.2)	0.3 (0.1-0.5)	0.9 (0.7-1.1)	0.3 (0.2-0.3)
	250	0.6 (0.4-0.7)	2.8 (1.2-5.2)	2.9 (2.2-4.4)	1.6 (1.3-1.9)
	750	1.7 (1.3-2.3)	3.6 (2.3-4.6)	9.3 (4.5-15.0)	6.0 (2.9-12.6)
MiNP-G	50	1.5 (1.3-1.6)	1.6 (1.2-1.9)	3.8 (2.9-4.8)	2.0 (1.8-2.4)
	250	3.6 (2.9-4.4)	8.4 (5.8-13.6)	10.7 (8.0-12.7)	9.4 (6.9-11.5)
	750	7.9 (5.2-9.0)	13.4 (12-14.4)	24.2 (14.4-35.3)	20.2 (13.2-33.1)

MiNP = monoisononyl phthalate
MCiOP = monocarboxyisooctyl phthalate
MHiNP = monohydroxyisononlyl phthalate
MOiNP = monooxoisononyl phthalate
MiNP-G = monoisononylphthalate glucoronide conjugate

Urinary excretion within 24 hours after the last dose was 54, 47 and 22% of the administered dose of 50, 250 and 750 mg/kg bw/day, respectively. MCiOP was the major urinary metabolite accounting for 76-81% of the metabolites, followed by MHiNP (15-20%) and MOiNP (4%). MiNP and MiNP-G were minor metabolites (<1% of the urine metabolites). The pharmacokinetic model suggests similarities in the absorption, distribution, metabolism, and excretion of DEHP and DINP. The differences in vivo effects are likely due to pharmacodynamic differences.

Study #2, ExxonMobil Biomedical Sciences, Inc., The Hamner Institutes for Health Sciences, 2011 (Clewell et al. 2011)
The second study was designed to determine a NOEL for effects on the developing male rat reproductive tract for DINP (Jayflex™, CAS 68515-48-0) (Clewell et al. 2011b, conducted under the Research quality standards of the Hamner Institutes for Health Sciences). Male offspring of dams administered 0, 760, 3800, and 11400 ppm DINP and 7600 ppm DBP in the diet from GD 12 to PND 14 (target daily doses were 0, 50, 250, and 750 mg/kg bw/day DINP and 500 mg/kg bw/day DBP) were studied. The mean maternal doses calculated from body weight and food consumption data were 56, 288, and 720 mg DINP/kg bw/day or 642 mg DBP/kg bw/day during gestation and 109, 555, and 1513 mg DINP/kg bw/day or 1138 mg DBP/kg bw/day during lactation.

Male pups were examined for a number of effects including reduced AGD (PNDs 2, 14 and 49), nipple/areola retention (PNDs 14 and 49), testes T concentration (PND 2), alterations of the urogenital tract (PND 49), testes and epididymides weight and histopathology (PND 49), and preputial separation (PPS; PND 49). Urogenital tract examinations included examinations for hypospadias, cleft phallus, undescendent testes, epididymal agenesis and measurements of the right and left gubernacular cords. In addition, the following organs were weighed: seminal vesicles, glans penis, ventral prostate, levator ani plus bulbocavernosus muscles (LABC), Couper's glands, kidney, liver and adrenal glands. Plasma from pups on PND 2 were pooled and analysed for major metabolites of DINP and DBP.

There was maternal toxicity at the highest exposure level as indicated by decreased food consumption and maternal body weights. Food palatability may have led to decreased maternal body weights. Offspring body weights were reduced on PND 2 at the highest dietary concentration and on PND 14 at ≥ 250 mg/kg bw/day. Body weights were comparable on PND 49 at all exposure levels.

Spot measurements of metabolite plasma levels in pups were lower compared with Cmax concentrations measured in foetuses previously by Clewell et al. (2011a). After DINP administration at 50 mg/kg bw/day via oral gavage to pregnant animals resulted in peak foetal plasma levels of 20 µM MINP (Clewell et al. 2011a), which is approximately 1000-fold higher than the levels achieved in the pups from lactation dams given a similar dietary dose (0.02 µM; Clewell et al 2011b, Table 4.42). In addition, measured concentrations of MCiOP were more than 10-fold higher than MiNP in neonate plasma in study #2 whereas peak levels of MCiOP and MiNP levels were similar in maternal and foetal plasma in Study #1 (Clewell et al. 2011a). For DBP, the foetal/pup plasma levels of MBP were 15 times lower (3 µM vs. 45 µM) during lactation than during pregnancy (PND 2 vs. GD19-20) as reported by Clewell et al. 2011b. Monoester metabolites do not transfer well into milk and, thus, pup exposure is reduced after parturition.

Table 4.42 Plasma concentrations (µM) of metabolites of DINP or DBP in pups on PND 2 (dietary administration of DINP, Clewell et al. 2011b)

	Control	DINP 56/109[a] mg/kg bw/d	DINP 288/555[a] mg/kg bw/day	DINP 720/1513[a] mg/kg bw/day	DBP 642/1138[a] mg/kg bw/day
MiNP	nd	0.02	0.13	0.49	-
MCiOP	nd	1.70	7.80	14.5	-
MHiNP	nd	0.10	0.27	0.45	-
MOiNP	nd	0	0.07	0.15	-
MBP	nd	-	-	-	2.81

[a] Calculated substance intake during pregnancy/lactation
nd = not detected
-= not measured
MiNP = monoester of DINP
MCiOP = monocarboxyisooctyl phthalate
MHiNP = monohydroxyisononyl phthalate
MOiNP = monooxoisononyl phthalate
MBP = monoester of DBP

Treatment with DINP or DBP did not reduce the testicular T concentration on PND 2. The variation was very large ranging from 0.5 to 5 ng/ml in controls and the authors conclude that T measurements on PND 2 unlikely provide reliable information due to large variation. In the mid and high dose DINP group and DBP group the T levels were higher than in controls. On PND 49 there were no changes in concentrations of T in testes neither after DINP or DBP exposure.

There was no evidence for DINP-induced nipple retention, preputial separation, or sexual organ weights at doses up to 750 mg/kg/day. DINP reduced the scaled AGD (AGD/body weight$^{1/3}$) on PND 14 but not on PND 2 or PND 49 at the high dose level (scaled AGD was decreased on PNDs 2 and 14 but not on PND 49 for DBP). Males exposed to DINP perinatally did not display retained nipples at the two observed days (PND 14 and PND 49). Animals treated with DBP displayed a significant increase in nipple retention on PND 14 (but not on PND 49).

Increased incidence of animals with multinucleated gonocytes (MNGs) was noted at and above 250 mg/kg bw/day for DINP and at 500 mg/kg bw/day for DBP (Table 4.43). The incidences were 1/24, 2/20, 7/20* and 18/19* for DINP at 0, 50, 250 and 750 mg/kg bw/day, respectively. In the positive control group (DBP), all the animals had MNGs. There was also increased incidence and severity of large Leydig cell aggregates at and above 250 mg/kg bw/day. At the highest exposure level of DINP all 19 animals had large Leydig cell aggregates which were increased in severity compared to control. Most of the positive control animals (DBP) had also large Leydig cell aggregates. Increased number of Leydig cells and MNGs were also reported on PND 2 at the high exposure of DINP as well as DBP group by Experimental Pathology Laboratories, Inc. There were approximately 2.3 MNGs per one section of testis at 750 mg/kg bw/day and approximately 0.4 MNGs per one section of testis at 250 mg/kg bw/day. Testis sections from control animals and animals from the lowest exposure level (50 mg/kg bw/day) were without MNGs.

Table 4.43 Histopathology findings in testes on PND 2 (Huntingdon Life Sciences)(dietary administration of DINP, Clewell et al. 2011b)

	Control	DINP 56/109[a] mg/kg bw/d	DINP 288/555[a] mg/kg bw/day	DINP 720/1513[a] mg/kg bw/day	DBP 642/1138[a] mg/kg bw/day
Number of animals examined	24	20	20	19	21
Animals with MNGs	1	2	7*	18**	21**
Animals with increased number of gonocytes	0	0	0	0	5*
Animals with large Leydig cell aggregates	4	4	8	19*	18**

a Calculated substance intakes during pregnancy/lactation
* p<0.05, ** p<0.001, 1-way ANOVA
MNG=Multinucleated gonocytes

The toxicological significance of MNGs in PND 2 animals is not known. Rats may have a mechanism of eliminating polyploidy embryonic germ cells (Kleymenova et al. 2005). MNGs were observed in one high dose male (5%) on PND 49 and the positive control animals had also rather low incidence 12% (Table 4.44).

Table 4.44 Histopathology findings in testes on PND 49 (Huntingdon Life Sciences)(Dietary administration of DINP; Clewell et al. 2011b)

	Control	DINP 56/109[a] mg/kg bw/d	DINP 288/555[a] mg/kg bw/day	DINP 720/1513[a] mg/kg bw/day	DBP 642/1138[a] mg/kg bw/day
Number of animals examined	25	20	20	20	25
Multinucleated germ cells	0	0	0	1	3
Tubular/rete dilation	1	0	0	1	4
Occational atrophic tubules	2	1	0	1	6
Tubular dysplasia	0	0	0	0	1
Necrosis /hypopasia/ mineralization	0	0	0	0	1

[a] Calculated substance intakes during pregnancy/lactation

A high exposure of DINP and DBP may be associated with a decrease in the number of ductular profiles indicative of decreased coiling in epididymides on PND 2. Lack of serial sectioning hampered the evaluation and further analysis would be needed to confirm the presence or absence of the epididymal effect. Reproductive organ weights were not altered by DINP. The LABC, ventral prostate and seminal vesicles weights were decreased by DBP on PND 49. The

average score PPS was not altered by DINP but was reduced by DBP from nearly complete separation (score 2.77) in control animals to prepuce no longer attached to tip of glans, but not completely separated (score 1.99).

Three low dose and one middle dose males had incomplete (hypoplastic) epididymides on PND 49 (vs. none in control group; Table 4.45). The incidence and severity of segmental ductular dilatation was similar to the controls. One low dose animal had slight interstitial edema in epididymides. In addition, one low dose and one mid dose male had undescendent testis. Gubernacular cord length was slightly increased in the low dose DINP group but was not considered treatment related. Two high dose males had very slight hypospadias of similar severity than in one control male (historical control range not provided). Complete spermatogenesis was recorded for both DINP and DBP treated animals on PND 49. One high dose male had minimal numbers of MNGs in occasional tubules on PND 49. Minimal numbers of MNGs were found also in a few tubules in 3 DBP treated males. Leydig cells were normal in all DINP groups.

Table 4.45 Reproductive tract malformations in male rats on PND 49 exposed to DINP or DBP in the diet during gestational and lactational period of GD 12 – PND 14.

	Control	DINP 56/109[a] mg/kg bw/d	DINP 288/555[a] mg/kg bw/day	DINP 720/1513[a] mg/kg bw/day	DBP 642/1138[a] mg/kg bw/day
Number of animals/litters examined	111/24	87/20	83/20	84/20	84/21
Mild/slight hypospadias	1	0	0	2 (1)[b)]	9 (5)
Exposed os	0	0	0	0	3 (1)
Atropic testis/epididymis	0	0	0	0	1
Unilateral enlarged testis	0	0	1	0	5 (5)*
Undescendent testes	0	1	1	0	1
Epididymal agenesis	0	0	0	0	2 (2)
Incomplete Epididymis	0	3 (2)	1	0	9 (8)**
Flaccid epididymis	2 (2)	3 (2)	8 (4)	4 (3)	17 (7)*
Absent seminal vesicles	0	0	0	0	1
Total affected animals[c)]	3	7	11	6	48

a Calculated substance intakes during pregnancy/lactation
b = affected litters in parenthesis
c = not tested statistically, provided as a sum of the effects reported
*= $p<0.05$, ** =$p<0.01$, pairwise test using jackknife methodology

In positive control group (DBP), two animals had agenesis of epididymides and 9 had incomplete (hypopastic) epididymides, one had undescendent testes and another had an atrophic testis and associated epididymides. Histopathology of the atrophic testis/epididymis showed extensive necrosis, hypoplasia and mineralization of the tissues. There was also interstitial edema in epididymides of four rats. The incidence and severity of segmental ductal

dilation was slightly increased. DBP caused mild hypospadias in 9 rats, of which 3 cases with exposed os.

A NOEL of 760 ppm DINP (approximately 50 mg/kg bw/day) was established for alterations in male sexual development based on increase in MNGs in the testis on PND 2. The NOELs for Leydig cell aggregates and reduced AGD was 3800 ppm (~250 mg/kg bw/day). These effects were reversible by adulthood. According to the authors the biological significance of these observations is unknown and, thus, it is not clear whether these observations should be considered adverse.

Commentary (to both studies #1 and 2, Clewell et al. 2011a and b)
At 250 mg/kg bw/day a significant increase in MNGs was observed on GD 20/PND 2 after dosing from GD 12 until termination. Reduction in testicular T content on GD 19 is in line with earlier studies where dosing was done until termination during foetal phase. It is noted that foetal testicular T levels reduced by 50% at 250 mg/kg bw/day dose level and by ~60% at 750 mg/kg bw/day dose level indicating that at lower dose levels the reduction is larger than would be expected by linear extrapolation from higher dose levels. The highest measured intratesticular concentrations of MiNP and MCiOP related to decreased T levels were 92.9 vs 15.5 µM for MiNP at one hour after the final dose of 250 and 50 mg/kg bw/day, respectively, and 70.6 vs 25.0 µM for MCiOP at 6 hours after the final dose of 250 and 50 mg/kg bw/day, respectively.

Urinary excretion of DINP metabolites were 54% of the administered dose at 50 mg/kg bw/day within 24 hours (plasma half-time for MiNP was 4 hours in dams). The total balance, biliary excretion and fecal elimination were not reported. Based on the urinary data, absorption was at least 54% at the lowest dose level. The maternal and foetal AUC values for MiNP indicate that an approximation of 100% transfer to foetal compartment is justifiable.

AGD was reduced on PND 14 at the highest DINP dose. At the same dose level decreased body weight on PND 14 may indicate more general developmental toxicity during lactation (exposure through milk) but the pups start also to eat around PND 14. The dosing ended on PND 14 and on PND 49 there was only one animal with multinucleated germ cells and a few cases of very slight hypospadias, undescended testes, flaccid epididymis or incomplete epididymis. The incidences of malformations and histopathology findings in the positive control group (642/1138 mg/kg bw/day DBP, see Tables 4.43-4.45) in the dietary study by Clewell et al. were considerably lower than following gavage dosing of DBP at a lower dose of 500 mg/kg bw/day (see e.g., NICNAS 2007). This may support the notion that a peak concentration is more important in inducing these effects compared to the area under the curve. It is furthermore noted that the number of animals with MNGs on PND 49 was only 3/25 (12%) in the positive DBP control group (642/1138 mg/kg bw/day), and 1/20 (5%) in the highest DINP dose group (720/1513 mg/kg bw/day)– showing not a remarkable difference. In addition, it can be argued that PND 49 may not yet be considered adulthood.

A NO(A)EL for effects on the developing male rat reproductive tract was established for DINP of 760 ppm (50 mg/kg/day). A LO(A)EL of 3800 ppm DINP (250 mg/kg/day) was determined based on the significant increase in MNGs on GD 20/PND 2, T reduction on GD 19, and decreased pup body weight on PND 14.

Information in humans

There are several studies where association of phthalate exposure with impaired human reproductive health have been suggested and discussed. However, in the following only studies referring exposure to DINP are described.

Lottrup et al. 2006
In their review Lottrup and co-authors discuss the findings of two human studies (Main et al. 2005 [2006]; Swan et al. 2005) suggesting similar findings to those observed in rodent studies and indicating that prenatal phthalate exposure is associated with lower androgen levels and shorter AGD. In the first study, concentrations of the monoester phthalates MMP, MEP, and MBP in breast milk positively correlated with the ratio between infant serum LH level and free androgen index (FAI; nmol T/nmol AGD/body weight$^{1/3}$sex hormone binding globulin x 100) (Main et al. 2005 [2006]). In boys without cryptorchidism the correlation was further strengthened and the LH/FAI ratio to MEHP and MiNP levels in breast milk was significantly increased. The authors concluded that the findings in combination were considered as indicators of decreased androgen activity due to phthalate exposure. Increased SHBG levels led to a reduced free T. The increase of the ratio between LH and FAI with increasing concentrations of phthalate monoesters was suggested to reflect the gonadotrophin drive on the testis to increase T synthesis. Phthalates seemed to adversely affect Leydig cells, which in turn led to reduction of the T production and further increase of LH secretion from the pituitary.

In another study, women with the highest gestational urinary levels of MEP, MBP, MBzP and MIBP gave birth to boys with smaller than expected AGD (Swan et al. 2005). Boys with a short anogenital index (AGI) defined as a ratio between AGD and body weight, had a very high prevalence of concomitant cryptorchidism, a smaller and less rugated scrotum and smaller penis. In humans, both the hormonal surge at around 3 months of age and the measurement of AGD may reflect androgen activity and perinatal exposure.

Higher urinary metabolite levels of phthalates have been observed in men with lower semen quality (Murature et al. 1987; Duty et al. 2003) indicating that sensitive time periods when the adequate androgen levels are critical for normal development may also exist in humans.

Main et al. 2006
The study did not find any association between phthalate monoester levels and cryptorchidism. However, there was a positive correlation between MiNP and LH. For comparison, MEP and MBP correlated positively with SHBG, MMP, MEP and MBP with LH:free testosterone ratio and MBP correlated negatively with free T. Other phthalate monoesters showed similar but non-significant tendencies. The reproductive hormone levels and information on phthalate exposures in newborn boys suggest that human Leydig cell development and function may be vulnerable to perinatal exposure to some phthalates. Data support also other findings indicating incomplete virilization in infant boys exposed to phthalates perinatally. However, the study groups may have been too small to detect subtle effects and the postnatal exposure assessment during lactation may have missed the critical window.

B) New information on endocrine disruption potency from Level 2 and 3 assays according to OECD Conceptual Framework
In addition to the new in vivo studies already referred, there are several new studies (a Hershberger assay, an Uterotrophic assay, both in vivo assays, and several new in vitro experiments) measuring endocrine disruption potency of DINP. These are listed and shortly described in this chapter.

In vivo studies (Level 3 assays)

Lee and Koo 2007
In a Hershberger assay to examine the anti-androgenic properties, DINP was administered by oral gavage to castrated SD rats at dose levels of 20, 100 or 500 mg/kg bw/day for 10 days. A significant decrease in seminal vesicle weight was observed in all groups and a significant decrease in weight of levator ani/bulbocavernosus muscle (LABC) was observed in the high dose group males.

Commentary to Lee and Koo 2007
According to the test guideline, a substance should be considered as positive in the test if at least two of the five organs show an effect. Thus, the dose level of 500 mg/kg bw/day can be considered as anti-androgenic in this test. For comparison, DEHP decreased ventral prostate weights at and above 20 mg/kg bw/day, seminal vesicle weights at >100 mg/kg bw/day, and LABC weights at 500 mg/kg bw/day.

Akahori et al. 2008
DINP exhibited neither oestrogenic nor anti-oestrogenic responses in the uterotrophic assay according to OECD TG 440 (nor did DIDP or DEHP).

In vitro studies (Level 2 assays)

Akahori et al. 2005 (as cited in ExxonMobil 2011a)
Akahori et al. (2005) used combined quantitative structure-activity relationship (QSAR) models from discriminant and multi-linear regression analysis to predict the binding potency to human oestrogen receptor alpha (ERα) and compared these results to an in vitro human ERα binding assay. In the in vitro assay, DINP exhibited minimal human ERα binding; reported as the relative binding affinity (logRBA = -3.49). When examined in the computer models, weak binding was predicted for DINP.

Akahori et al. 2008
Akahori and co-workers (2008) examined a series of chemicals in a human ERα binding assay and compared the results to observations from an in vivo uterotrophic assay performed according to the OECD Test Guideline 440 and in compliance with good laboratory practices (GLP). DINP exhibited minimal human ERα binding in the in vitro assay; reported as the relative binding affinity (logRBA = -3.49). DINP exhibited neither oestrogenic nor anti-oestrogenic responses in the in vivo assay (nor did DIDP or DEHP). The discrepancies between in vitro and in vivo assays in phthalates (some ER-mediated activities in in vitro assays but no oestrogenic response in in vivo model) are probably caused by the deactivation of phthalates to mono alkyl phthalates (Harris et al. 1997; Picard et al. 2001; Zacharewski et al. 1998).

Krüger et al. 2008
DINP was tested for agonist/antagonist activity on the aryl hydrocarbon receptor (AhR) and the androgen receptor (AR) using luciferase reporter gene expression bioassay in recombinant mouse Hepa1.12cR cells (AhR-CALUX) and in transient transfected Chinese Hamster Ovary (CHO-K1) cells (AR-CALUX). No significant effect was observed neither on AhR activity or AR activity (concentration ranges 1 x 10^{-10} – 1 x 10^{-4} M). For comparison, DIDP exerted agonistic AhR activity by increasing the response compared to control value by 378% at the lowest tested non-cytotoxic concentration causing the maximum effect (at 0.1 µM). For DEHP, the corresponding value was 175% at 0.1 µM. In AR-Calux also DIDP and DEHP were negative. The result for AR is consistent with the results of studies by others (Roy et al. 2004; Takeuchi et al. 2005).

Takeuchi et al. 2005
Takeuchi et al. (2005) characterized the activities of the human ERα, human ERß and human androgen receptor (AR) using a reporter gene assay in Chinese Hamster Ovary (CHO) cells. Neither DINP nor DIDP showed any oestrogenic/anti-oestrogenic or androgenic/anti-androgenic activity at the tested concentrations (up to 10^{-5} M). For comparison, DEHP induced ERα mediated oestrogenic activity, antagonized ERß and was not active via AR (showing 20% of the agonistic activity of 10^{-9} M E2 at 5.5 x 10^{-6} M and 20% of the antagonistic activity of 10^{-10} M E2 via ERß).

Commentary to Takeuchi et al. 2005
Other tests reporting positive effects were carried out at higher concentrations (e.g., 1 mM)

Mlynarciková et al. 2007
DINP did not affect the basal progesterone production in porcine ovarian granulosa cell culture. However, DINP amplified FSH-stimulated progesterone release into the culture medium. Basal oestradiol production was not affected but FSH-stimulated oestradiol production was inhibited after the treatment with DINP.

Commentary to Mlynarciková et al. 2007
ExxonMobil (ExxonMobil 2011a) stated in its comments to CHAP-CPSC that DINP did not induce basal hFSH stimulated progesterone production. However, based on the original article, although the basal production was not affected, the FSH-stimulated progesterone release was increased.

DINP has a potential capacity to cause anti-oestrogenicity via antagonizing the stimulatory effects of FSH on ovary granulosa cells in vitro.

DeKeyser et al. 2011
Phthalates were shown to extensively interact with the human constitutive androstane receptor (CAR) and pregnane X receptor (PXR). CAR2 is especially sensitive to DINP (as well as to DEHP). Both Cyp2b6 and Cyp3a4 enzymes involving T metabolism are induced by CAR and PXR. Induced T metabolism via CAR and PXR activation may be one additional mechanism how DINP reduces foetal T levels. The estimated EC_{50} values for the activation were 0.34 µM and 0.1 µM via CAR2 and 3.6 µM and 3.8 µM via PXR for DINP and DEHP, respectively.

Wenzel et al. 2005; Breous et al. 2005
In two studies from the same group, the effects of DINP and other phthalates on the basal iodide uptake and the responsible mode of action were studied (Wenzel et al. 2005; Breous et al. 2005). DINP enhanced iodide uptake in a rat thyroid cell line (FRTL-5) at concentrations of 0.1 - 1 mM but not at lower concentrations (Wenzel et al. 2005). The effects of DINP (as well as other active phthalates) on iodine uptake were inhibited by perchlorate, a specific symporter inhibitor, at 30 µM concentration. This indicates that the enhancement of iodide uptake by DINP is mediated by sodium/iodide symporter (NIS). DINP, DIDP and DOP seem to be of approximately similar potency, DEHP a more potent and BBP less potent and DBP was not active at all.

Further examinations on mode of action revealed that DINP did not up-regulate the human NIS (hNIS) promoter construct. However, DINP slightly decreased the TSH induced activation of the promoter and enhancer construct (N3+NUE). The response of the hNIS promoter construct (N3) as well as the promoter and enhancer construct (N3+NUE) were investigated at 1 mM concentration of DINP in the presence of 1.5 mU/ml TSH using PC C13 rat thyroid cell line (Breous et al. 2005). Because DINP did not change the mRNA level of rat NIS (rNIS), the authors suggested that DINP modulate the activity of NIS at the post-transcriptional level. The slightly lowered TSH-induced transcriptional activities of N3+NUE may result of the interference with important accessory factors, e.g., adenylyl cyclase, according to the authors.

In addition to DINP also DIDP, BBP, DEHP and DOP decreased the TSH induced activation of N3+NUE. Expression levels of rNIS were also unaffected for DEHP and DBP, but were increased by DIDP, BBP and DOP. In addition to DINP, also DEHP and DBP may modulate the activity of NIS at the post-transcriptional level. In conclusion, DINP seem to enhance iodide uptake in thyroid by modulating the activity of NIS at post-translational level.

Commentary to Wenzel et al. 2005 and Breous et al. 2005
DINP enhanced the basal iodine uptake in the rat thyroid cell line at rather high concentrations which may not be biologically relevant.

Ghisari and Bonefeld-Jorgensen 2009
DINP inhibited the thyroid hormone (TH)-dependent rat pituitary GH3 cell proliferation (T-screen) without the thyroid hormone triiodothyronine (T3) (at concentrations of 10-100 nM), and at a higher concentration of 50 µM in the presence or absence of T3. DINP had no oestrogenic effect as measured in MVNL cells transfected with an oestrogen receptor (ER).

For comparison, other phthalates, such as BBP, DEHP, DBP, DIDP and DOP stimulated the proliferation of GH3 cells (but less than T3) in the absence of T3. In the presence of T3, only BBP and DIDP stimulated the cell proliferation and all the other examined phthalates (DEHP, DBP, DOP) inhibited cell proliferation. BBP and DBP enhanced weakly the ER transactivation but DEHP, DOP and DIDP were inactive without oestradiol (E2). In the presence of E2, BBP and DBP further enhanced the E2-mediated response. However, at concentrations above 10^{-5} M BBP, DBP and DEHP inhibited the E2-induced transactivation.

The findings indicated that in conditions mimicking the natural situation (with endogenous hormone), the effects of phthalates are far less potent than the endogenous hormones and that the potency varies depending on the phthalate. DINP seems to inhibit GH3 cell proliferation at rather low concentrations (10 and 100 nM) in the absence of T3 and at higher concentration (0.5 µM) in the presence of T3.

4.4.9.1.4 Discussion

In all of the new animal studies, animals were exposed in utero during gestation only or during gestation and lactation. Examinations on male reproductive tract malformations/alterations, behavioural changes, hormonal levels and gene expressions or protein levels were conducted to evaluate potency and mode of action of DINP. Measurements were done at different time points generally right after the exposure and/or later during development. In many studies the results were compared with other phthalates.

Foetal testicular testosterone levels and Leydig cell function
All tested DINP formulations reduced ex vivo foetal testicular T production in a dose responsive manner at and above of 500 mg/kg bw/day, the lowest dose tested, after exposure during GD 14-18 (Hannas et al. 2011b; Table 4.46). DINP also reduced ex vivo testicular T production and T levels in testes to one third after gestational exposure at 750 mg/kg bw/day (Borch et al. 2004), and foetal testicular T levels were reduced by 50% at 250 mg/kg bw/day after exposure during GDs 12-19 (Clewell et al. 2011a). The foetal T production was reduced to 50-75% of control value after exposure during GD 14-18 at 750 mg/kg bw/day (DINP 28553-12-0 was slightly more potent than DINP 68515-48-0) (a poster presented in the 51st Annal Meeting of Society of Toxicology by Gray et al. "A fetal rat testes endocrine and genomic "signature" accurately predicts the phthalate syndrome of malformations"). This is well in line with the reduction of the foetal testicular T levels by 70% at 750 mg/kg bw/day (Clewell et al. 2011a). However, if T content was measured one or a couple of days after ceasing the exposure during the pregnancy, only a tendency or no clear change in testicular T content or T production was found (Boberg et al. 2011; Adamsson et al. 2009; Clewell et al. 2011b). One study reported no change in plasma T level on during the exposure period on PND 7 (Lee et al. 2006b) and another no inhibition in testes T on PND 2 during dietary exposure (Clewell et al. 2011b). It should be taken into account that based on the results from Clewell and co-workers (2011b), the variation of T concentration in testis on PND 2 is very large and measurement during a critical window when T levels peak would give more relevant information.

A decrease in foetal T levels/production has been observed in several in vivo studies with DINP (Borch et al. 2004; Hannas et al. 2011b; 2012; Lambright et al. 2011; Clewell et al. 2011a). The foetal T level peaks during the masculinisation in utero and the level decreases towards birth and maintains then at a rather constant level until puberty (Welsh et al. 2008). Reduced (without statistical significance) testosterone T levels in testes in adults after gestational and

lactational DINP exposure (63% of control levels) have been reported in one study (Boberg et al. 2011). It appears that the NO(A)EL for decreased T content/production considering all available studies is 50 mg/kg/day. The LO(A)ELs from different studies were 250 mg/kg bw/day (NOAEL of 50 mg/kg bw/day; Clewell et al. 2011a), 500 mg/kg bw/day (no NOAEL; Hannas et al. 2011a and b), 750 mg/kg bw/day (no NOAEL; Borch et al. 2004; Lambright et al. 2011), and an ED50-value of approximately 850 mg/kg bw/day (Hannas et al. 2012).

Reduced T in testis may be interpreted as a marker for an anti-androgenic effect. The adversity of this effect depends on the impact it has on the sexual development, sexual behaviour, hormonal control, sperm parameters, functional fertility and structural abnormalities in later life. At present it is not known how much variability, in terms of magnitude, can occur in the testis T concentration during the critical window of masculinisation to assure normal sexual development. A small change in T concentration in foetal testis may not lead to malformations or changed sexual development. However, currently there is no information on whether or not there is a threshold. A reduction of 50% (as observed at 250 mg/kg bw/day in study by Clewell et al. 2011a) is considered harmful in terms of its potential to affect sexual development, function and behaviour later in life. For comparison, foetal testicular T production was reduced by 50% at 300 mg/kg bw/day with DEHP (Hannas et al. 2011b) indicating that there may be no large difference between these two phthalates regarding this effect in utero.

Furthermore, it is not known for how long the foetal testicular T levels can be reduced without impairing masculinisation process. A short temporal change may not lead to malformations or changes in sexual development. However, there is no information on the duration of the reduction in T levels that would be needed to affect sexual development. Measurements of the foetal testicular T levels at various time points after dosing are missing (dietary or bolus).

The testicular T levels peak for a couple of days in rats and for some weeks in humans during development in utero. Based on the results from the study by Clewell et al. (2011a), the testicular T levels were reduced at 2 hours but no longer at 24 hours following the gavage dosing of DINP at 250 mg/kg bw/day. This suggests that a more continuous exposure is needed in rats to keep the testicular T levels lowered for a whole day at this dose level. The measurements were done only at 2 hours and 24 hours time points and the duration or the level of T reduction at other time points is not known. However, it was noted that compared with the magnitude of the effect at the higher dose levels, the reduction in foetal testicular T levels at lower dose levels were higher than one would expect (50% and 60% at 250 and 750 mg/kg bw/day, respectively; Clewell et al. 2011a).

Information on the amount of reduction of the testicular T level or the duration of the reduction after dietary (exposure not constant – rather during the dark period because rats are active and eat mainly during the night) or other non-bolus exposure is not available in rats at the exposure level of 250 mg/kg bw/day. It is likely that high bolus doses magnify and prolong the duration of significant reduction in testicular T peak during the critical time window of masculinisation compared with low dose bolus. After 1-2 hours of the final gavage dosing of 750 mg/kg bw/day during the critical period the ex vivo testicular T production was reduced approximately by 23-50% (a poster presented in the 51st Annal Meeting of Society of Toxicology by Gray et al. "A fetal rat testes endocrine and genomic "signature" accurately predicts the phthalate syndrome of malformations") and by 3-fold after dosing (Borch et al. 2004). This dose level of DINP did produce reproductive tract malformations at a low frequency (Gray et al. 2000; Boberg et al. 2011). The exposure levels of human foetuses or the consistency/fluctuation of the levels of active phthalates in foetal testes during critical weeks are not known. It is also not known whether rats and humans are equally sensitive, or whether one of the species is more sensitive to the reduction of the foetal testicular T level due to the phthalate exposure and/or the consequences of that reduction on masculinisation/development. Without further knowledge (especially of the human situation), it is considered that the exposure to DINP at 250 mg/kg bw/day, which produces a significant

reduction (50%) in foetal testicular T concentration 2 hours after a bolus dose, is an adverse effect taking into account the essential role of androgens during the critical developmental window of foetal life. This is supported by the findings with other phthalates that 50-90% reductions in intratesticular levels of T is reflected in reduction of number of Sertoli cells (by 50%) at birth, AGD and reduced testis weight although with a complex relationship (discussed by Scott et al. 2008).

Increases in testicular P450scc (Cyp11a), GATA-4 and Insl3 mRNA expression on ED 19.5 support changes in steroidogenesis and Leydig cell function caused by gestational DINP exposure (Adamsson et al. 2009). DINP had an ED50-value of 326 mg/kg bw/day for the most sensitive gene (Cyp11b1) and almost identical ED50-values for StAR and Scarb1 (around 600 mg/kg bw/day; Hannas et al. 2012). The ED50-value for reduced T production was 852 mg/kg bw/day and almost 1500 mg/kg bw/day for Insl3. The most sensitive gene in the foetal testis (Cyp11b1) does not appear to be biologically linked to the postnatal outcomes of concern. The gene codes an enzyme converting 11-deoxycortisol to cortisol in the adrenal cortex and it is not expressed in adult testes. Recent studies indicate that a subpopulation of foetal Leydig cells express Cyp11b1 but no enzyme activity was detected (Val et al. 2006; Hu et al. 2007) indicating that translation of enzyme product is suppressed to prevent high levels of corticosteroid production in the foetal testes.

Proteins SR-B1, StAR and Cyp17a1 are critical for T synthesis. It has been shown that phthalates reduce T production by interfering with cholesterol regulation, and that anti-androgenic phthalates reduce Star, Scarb1 and Dhcr7 gene expression (Hannas et al. 2012). The Dhcr7 gene codes for an enzyme mediating the final step in cholesterol production (7-dehydrocholesterol). SR-B1 protein facilitates cholesterol uptake and StAR transports cholesterol across mitochondrial membranes. Cyp17a1 converts progesterone to androstenedione in rat (but not in human; Scott et al. 2009) which is further converted to testosterone. These gene products directly influence the production of T in foetal testis during the critical period of androgen-dependent tissue development (Hannas et al. 2012). PPARα, PPARβ and PPARγ pathways are not evidently activated in foetal testes due to phthalate exposure (Hannas et al. 2012). Insl3 is a foetal Leydig cell product; a hormone critical for transabdominal descent of testis. Reduced Insl3 gene expression may lead in altered gubernacular development and disrupted testicular descent.

Phthalates positive for anti-androgenic activity affect several gene expression levels in foetal testes (e.g., Cyp11b1, Scarb1, StAR, Cyp11a1, Cyp17a1, Insl3 and Hsd3b)(Hannas et al. 2012). DPeP was the most potent of tested phthalates for reducing each gene expression and DINP was the least potent (DPeP>DHP>DIBP≥DHeP>DINP; Hannas et al. 2012). The overall sensitivity of each gene endpoint and T production was Cyp11b1 >StAR =Scarb1 >Cyp17a1 =T production >Cyp11a1 =Hsd3b =Insl3 >Cyp11b2.

In conclusion, the new study provided by ExxonMobil shows a NO(A)EL value of 50 mg/kg bw/day based on e.g., significant reduction in T in testes at 250 mg/kg bw/day. Other studies recording decreased T production at higher dose levels with a LOAEL of 500 mg/kg bw/day are well in line with the new ExxonMobil study. The effect is not considered adverse by the authors because it was transient by nature. However, if associated with reproductive tract malformations or decreased fertility the main question would be how much and for how long foetal testicular T production/levels should be reduced to be considered as adverse. As discussed by Makris et al. (2010), although the inhibition of T synthesis is reversible, the biological effects resulting from reduced T levels during the critical developmental window are irreversible. Thus, the justification for considering foetal reduced testicular T concentration as adverse is that during the critical developmental window it has shown to induce male reproductive developmental effect. In addition to the male reproductive tract development it is not yet clear which all other aspects of male development and (sexual) brain differentiation are dependent on T production of foetal testes.

Testicular and ovary histopathology
Increased number of MNGs and increased central location of gonocytes was noted at and above 300 mg/kg bw/day (lowest dose) when measured on PND 13 (Boberg et al. 2011; Table 4.46) indicating no NOAEL in the study. A dose-dependent reduction in the percentage of motile sperm was seen from 600 mg/kg bw/day on PND 90 in the same study. The new study from ExxonMobil indicated a significant increase in MNGs at and above 250 mg/kg bw/day, leading to a NO(A)EL of 50 mg/kg bw/day (Clewell et al. 2011a). The effects were considered as non-adverse by the authors due to their reversibility. The result from Boberg and coworkers (2011) shows similar findings at 300 mg/kg bw/day and subsequently support a NOAEL of 50 mg/kg bw/day.

The increase in MNGs observed perinatally is generally no longer observable in adulthood after a gestational and lactational exposure (Boberg et al. 2011; Clewell et al. 2011b). No change in Inhibin B levels (indicator of Sertoli cell number and function) was reported but the mean testicular T content was reduced (63% of control level) at a high dose of 900 mg/kg bw/day on PND 90 without statistical significance (Boberg et al. 2011). However, degeneration of meiotic spermatocytes and Sertoli cells was reported at high doses by Masutomi and coworkers (2003; 2004) in adult rats after a peri/postnatal exposure. They also reported a decrease in number of corpora luteae in females. This indicates that peri-postnatal exposure may lead to permanent effects. DINP also decreased sperm count and motion/quality parameters after a four-week exposure of juvenile animals at dose level of 500 mg/kg bw/day (Kwack et al. 2009). A few cases of reproductive tract malformations in rat at high doses have been also been reported: 7.7% incidence of male reproductive tract malformations in Gray et al. (2000); two males with small testes and epididymides in treated animals in Boberg et al. (2011); fetal incidences of 2.7, 8.0, 13.3 and 7.1% at 0, 50, 250 and 750 mg/kg bw/day including slight hypospadias, unilateral enlarged testis, undescendent testis, and incomplete epididymis in Clewell et al. (2011b).

For further discussion on mode of action and the phthalate syndrome, see Chapter on Considerations on combined risk assessment of DINP and DIDP (and other phthalates).

Table 4.46 Foetal testicular T production, effects in Sertoli cells, gonocytes and sperm.

Study	Decreased foetal testicular T level/production, perinatal	Sertoli cells, gonocytes, perinatal	Permanent effects in T levels, Sertoli cells, gonocytes, sperm	Other relevant findings
Gray et al. (2000) 750 mg/kg bw/day GD14-PND3			Lack of spermatogenesis/ hypospermatogenesis associated with testis atrophy/fluid filled testis	7.7% of male reproductive tract malformations
Masutomi et al. (2003) 0, 400, 4000, 20000 ppm GD15-PND10			Degeneration of meiotic spermatocytes and Sertoli cells. Scattered cell debris in epididymal ducts.	↓ Corpora luteae. ↓ Number of live offspring. ↓ Pup body weight etc.
Borch et al. (2004) 750 mg/kg bw/day, gestation	Reduced production and T content at GD 21 (reduced to ~one third). T level in plasma was reduced by 25%.			

Lee et al. (2006a and b) 0, 40, 400, 4000, 20000 pm, GD15-PND21	No effects on serum T level on PND 7.			
Adamsson et al. (2009) 250, 750 mg/kg bw/day ED 13.5-17.5	No change in T content at ED 19.5. Increased testicular StAR, P450scc, GATA-4 and Insl3 mRNA levels on ED 19.5.			
Kwack et al. (2009) 500 mg/kg bw/day, 4 weeks from PND 28			↓ Sperm count. ↓ Sperm motion/quality parameters	
Boberg et al. (2011) 0, 300, 600, 750 and 900 mg/kg bw/day, GD7 – PND17.	Tendency towards reduction in production at all dose levels (no NOAEL) on GD 21.		Multinucleated germ cells (no NOAEL). ↑ Number of gonocytes with central location in seminiferous chords and ↑ chord diameters. One male at 600 and 750 had small testes and epididymides. ↓ Percentage of motile sperm with a NOAEL of 300 mg/kg bw/day. No effect on Inhibin B level. Mean testicular T content was 63% of control level at 900 mg/g bw/day.	Slight decrease in pup weight at higher doses.
Hannas et al. (2011b) 500, 750, 100, 1500 mg/kg bw/day, GD 14-18	Reduced testicular T production at and above 500 mg/kg bw/day (no NOAEL). Reduced testis StAR and Cyp11a gene expression (NOAEL of 750 mg/kg bw/day)			
Hannas et al. (2012)	Reduced T production with ED50 of 852 mg/kg bw/day. Several gene expressions affected in testes, most sensitives: Cyp11b1 with ED50 of 326 mg/kg bw/day, StAR with 597 mg/kg bw/day, Scarb1 with 796 mg/kg bw/day and Cyp17α1 with ED50 of 797 mg/kg bw/day.			

Clewell et al. (2011a) 0, 50, 250, 750 mg/kg bw/day, GD 12-19	↓ Testicular T on GD 19.	↑ Increase in multinucleated gonocytes on GD20 with NO(A)EL of 50 mg/kg bw/day. ↑ Leydig cell aggregates a the highest dose (NO(A)EL 50 mg/kg bw/day)		
Clewell et al. (2011b), targeted 0, 50, 250, 750 mg/kg bw/day (dietary), GD 12 to PND 14	No change in testicular T production during postnatal period	↑ Increase in multinucleated gonocytes on PND2. Large Leydig cell aggregates. (NO(A)EL 50 mf/kg bw/day)	No effects on T content on PND 49. A few cases of incomplete epididymides, interstitial edema in epididymides, undescendent testis, slight hypospadias. One high dose male had multinucleated gonocytes.	Decreased pup weight on PND 14. All effects recoverable.

Anogenital distance and nipple retention
The adjusted (normalized) AGD was reduced in males at all tested dose level and increased in females at the highest dose group (Lee et al. 2006b) with a LOAEL of 2 mg/kg bw/day (Table 4.47). In another study, a NOAEL of 750 mg/kg bw/day was determined for reduced male AGD (Boberg et al. 2011). In a study by Clewell et al. (2011b), AGD was reported to be reduced only on PND 14 at the highest dose level with a NOAEL of 250 mg/kg bw/day. There was no change in AGD in three studies (Gray et al. 2000, Masutomi et al. 2003; Clewell et al. 2011a). The results of Lee et al. (2006) would lead to a LOAEL of 2 mg/kg bw/day based on reduced AGD in males. However, there seem to be limitations in the study using pup as a statistical unit for analysis of AGD instead of litter and also taking into consideration that the reported change in AGD is very minor. Overall, it is considered that the LOAEL for reduced male AGD is around 750 mg/kg bw/day, as supported by a NOAEL of 750 mg/kg bw/day in Boberg et al. (2011) and a LOAEL of 750 mg/kg bw/day in Clewell et al. (2011b).

Nipple retention is another measure of anti-androgenicity. The finding may be permanent or transient. Based on the results from Boberg and coworkers (2011), the NOAEL for the permanent effect is 300 mg/kg bw/day. This is supported by increased nipples at 750 mg/kg bw/day reported by Gray and coworkers (2000). Nipple retention was not reported by industry (Clewell et al. 2011b) up to 750 mg/kg bw/day. Perinatal exposure to DINP seems not to consistently induce nipple retention. A NOAEL of 600 mg/kg bw/day may be set based on the results for this endpoint.

Sexual behaviour and related measurements
One research group reported a decreased sexual responsiveness in females at 2 mg/kg bw/day (no NOAEL identified) after peri-postnatal exposure (Lee et al. 2006a and b). Effects on male copulatory behaviour were not dose-dependent although the authors consider that as a whole there was a slight decrease (Lee et al. 2006b). This finding has not been confirmed by others, however finds some support from non dose-related gene expression changes in medial basal hypothalamus (including the ventromedial nucleus; VMH) that relate to sexual differentiation of the rat brain (Lee et al. 2006b). The measured genes grn and p130 are expressed in the area of sexually dimorphic pattern of the synaptic organisation. There was no NOAEL for males but a NOAEL of 200 mg/kg bw/day for females was seen for hypothalamic gene expression changes (Lee et al. 2006b). These gene expression parameters have not been evaluated in other studies after exposure to DINP. According to their results, DINP may affect organization

of the neuronal circuits in the VMH, but not in the hypothalamic medial preoptic area (MPOA), which is thought to be responsible for inducing the preovulatory GnRH surge. In line with this, no changes in gonadotropin releasing hormone (GnRH) or calbinding-D (CALB) mRNAs in MPOA or immunoreactive cell populations of LH, FSH or PRL were observed (Masutomi et al. 2004; Takagi et al. 2005).

FSH and LH serum levels were not changed in the study by Lee et al. (2006b). Furthermore there were no changes in the expression levels of ERα and ERß or steroid receptor co-activators (SRC-1, SRC-2; Takagi et al. 2005). Expression of SRC-1 or SRC-2 have been shown to play a role in the MPOA determining sex steroid-induced sexual behaviour in females (Apostolakis et al. 2002) and reduced SRC-1 protein in the neonatal rat hypothalamus have shown to trigger dysfunction of male behaviour in later life (Auger et al. 2000).

Other studies have reported a behavioural change (on a memory test) reflecting masculinisation of female brain as well as a reduction in progesterone receptor (PR) expression in MPOA further supporting assumptions of masculinisation of the female brain (Boberg et al. 2011; Takagi et al. 2005). On the other hand there seem to be no changes in volumes of sexually dimorphic nucleus of the preoptic area (SDN-POA), serum sex steroid hormone levels, estrous cyclicity, luteinizing hormone (LH), follicle stimulating hormone (FSH) or prolactin levels after peri-postnatal exposure to DINP (Masutomi et al. 2003; 2004; Lee et al. 2006a and b). In a Hershberger assay, there was a decrease in the weight of two androgen sensitive organs at 500 mg/kg bw/day indicating an anti-androgenic effect (Lee and Koo 2007). In other studies this was seen only at higher dose levels.

The results of Lee et al. (2006a and b) would lead to a LOAEL of 2 mg/kg bw/day based on reduced sexual behaviour in females. Female sexual behaviour is not a common parameter in a reproductive toxicity studies, and the results have not been confirmed by others. Furthermore the reporting is limited in Lee et al., and lordosis quotient in control animals is rather low. As a consequence the data are for the time being considered as supportive information rather than a solid base for NOAEL setting. However, the results on the female sexual behaviour cannot be disregarded and further clarifications of the relevance of the findings by Lee et al. are needed.

Pup body weight
Pup weight was reduced at and above 250 mg/kg bw/day with a NOEL of 50 mg/kg bw/day in the study provided by ExxonMobil (Study #2, Clewell et al. 2011b). The effect was not considered adverse by the authors. Slight decrease in pup weight was also reported by Boberg and coworkers but at high doses only (750 and 900 mg/kg bw/day).

Table 4.47 Findings in AGD, nipple retention and behaviour in studies evaluating these parameters.

Study	AGD	Nipple retention	Behaviour	Others
Gray et al. (2000) 750 mg/kg bw/day GD14-PND3	No change	2/52 of males had permanent nipples, 22% of the male pups had female like areolas/nipples		No effect in pup weights or litter size or androgen dependent organ weights.
Masutomi et al. (2003) 0, 400, 4000, 20000 ppm GD15-PND10	No change			Decrease in mean number of live offspring. Decrease in pup body weight etc.
Takagi et al. (2005) 0, 4000, 20000 ppm GD15-PND10			Reduced PR expression levels in MPOA (related to masculinisation of the brain)	

Lee et al. (2006a and b) 0, 40, 400, 4000, 20000 pm, GD15-PND21	Reduced male AGD, no NOAEL. Increased AGD in females at 20000 ppm		Reduced female sexual behaviour, no NOAEL. (reduced copulatory behaviour). Changes in hypothalamic gene expression	
Boberg et al. (2011) Hass et al. (2003), an abstract 0, 300, 600, 750 and 900 mg/kg bw/day, GD7 – PND17.	Reduced male AGD at 900 mg/kg bw/day PND21.	Increase in nipple retention with a NOAEL of 600 mg/kg bw/day (or 300) Nipple retention not fully recovered on PND90.		Slight decrease in pup weight at higher doses.
Clewell et al. (2011a) 0, 50, 250, 750 mg/kg bw/day, GD 12-19	No change on GD 20			
Clewell et al. (2011b) 0, 50, 250, 750 mg/kg bw/day (dietary), GD 12 to PND 14	No change on PND 2 or PND 49, reduced male AGD on PND 14.	No increase on PND 14 or 49. No reproductive tract malformation.		

Information in humans
Human studies suggest that perinatal phthalate exposure is associated with lower androgen levels and shorter AGD (Main et al. 2006; Swan et al. 2005; Swan 2008).

Binding potency in examined receptors
In vitro studies indicate that DINP has a low potency to elucidate oestrogenic and/or anti-oestrogenic effects as measured by ER receptor assays (Akahori et al. 2005; 2008; Takeuchi et al. 2005). DINP does not act as an agonist or antagonist for the AhR or AR receptors (Krüger et al. 2008; Takeuchi et al. 2005). Human constitutive androstane receptor CAR2 is especially sensitive to DINP (DeKeyser et al. 2011). Both Cyp2b6 and Cyp3a4 enzymes involving T metabolism are induced by CAR which may be one of the mechanisms by which DINP reduces foetal T levels.

Different phthalates examined showed different potencies with regard to E2-induced ER transactivation (Ghisari and Bonefeld-Jorgensen 2009). The effects of phthalates are rather weak in conditions mimicking the natural availability of the endogenous E2. DINP or the other phthalates do not activate PPAR expression in the foetal testes, and PPAR activation is likely not the mechanism behind the phthalate induced reduced testicular T levels and reproductive toxicity (Hannas et al. 2012).

In in vivo uterotrophic assay, DINP did not show oestrogenic properties (Akahori et al. 2008), but some anti-androgenicity was observed in the Hershberger assay (Lee and Koo 2007).

Proliferative and other hormonal effects
DINP reduced FSH stimulated estradiol production indicating a potential capacity to antagonize the stimulatory effects of FSH in ovarian granulose cells (Mlynarciková et al. 2007). DINP did not affect the basal progesterone production but it amplified FSH-stimulated progesterone release (Mlynarciková et al. 2007).

DINP may increase thyroid activity because it enhances iodide uptake in a rat thyroid cell line mediated by sodium/iodide symporter (NIS) (Wenzel et al. 2005; Breous et al. 2005).

DINP inhibits TH-dependent rat pituitary GH3 cell proliferation with and without T3 (Ghisari and Bonefeld-Jorgensen 2009). The effects of phthalates are rather weak in conditions mimicking the natural availability of the endogenous T3.

Risk assessments from other international organizations and bodies
Earlier international risk assessments do not refer to the later studies reviewed in this report with exposure during the critical window at gestation and/or perinatal period (in particular – Boberg et al. 2011, Hannas et al. 2011a, b and 2012)). They refer to several guideline compliant generation and developmental toxicity studies conducted previously, before the need to examine the possible effects of phthalates on masculinisation/sexual differentiation after exposure during a critical time window was realised. Based on these studies, most of the international agencies and bodies have come to similar conclusions on NOAEL/LOAEL values for reproductive toxicity. In spite of the slight differences between the effects and studies considered most relevant, or whether they have been used as a NOAEL/LOAEL for development or fertility, the LOAEL of 159 mg/kg bw/day based on decreased offspring body weight in the one- and two-generation reproductive toxicity studies reported by Waterman et al. (2000; also referred to as Exxon 1996a and b) is the lowest LOAEL proposed so far for reproductive toxicity.

The lowest NOAEL derived from the developmental toxicity studies was 100 mg/kg bw/day, which most international bodies referred instead to the NOAEL of 500 mg/kg bw/day for developmental toxicity (both from the same study; Waterman et al. 1999). It should be noted, that the standard developmental toxicity studies carried out with DINP in rats use the exposure period from GD 6 to GD 15 which only partly covers the sensitive period of differentiation of the reproductive system. In addition, the evaluation of the malformations and effects relevant to phthalate exposure are not easy to examine at the point of the termination of the study and/or not part of the standard study design. Thus, the standard study design is not sensitive for reproductive tract malformations. Also, the examination of postnatal endpoints is not part of the standard study design of a prenatal developmental toxicity study, and also not necessarily observable in standard one- or two-generation reproductive toxicity studies. For these reasons effects were seen only at higher doses. Recent studies use the more sensitive exposure period of GD 12-20 or even a more focused period of GD 14-18 and additional sensitive parameters have been examined during development. This enables identification of (to uncover) adverse effects at lower doses.

Summary of critical studies and effects

The database on DINP includes guideline and GLP compliant studies conducted using previous test guidelines. As a result, the prenatal developmental toxicity study by Waterman et al. (1999) may not have covered the sensitive period. Moreover, the prenatal developmental toxicity study (Waterman et al. 1999) and the one- and two-generation reproductive toxicity studies (Waterman et al. 2000) have not examined the most sensitive endpoints for characterising anti-androgenic activity (such as AGD, nipple/areolae retention, PPS, (fetal testis) T levels/production). For the reproductive toxicity endpoint, the same studies and the same endpoint considered providing relevant information in EU Risk Assessment (EC 2003a) are selected. For developmental toxicity, new relevant information has been provided from tailored studies concentrating on perinatal exposure and especially on exposure during critical time window of masculinisation. The studies considered critical and providing relevant NOAEL/LOAEL values are presented in Table 4.48.

Table 4.48 Critical studies and effects

Endpoint	Study	LOAEL (mg/kg bw/day) and critical effects	NOAEL (mg/kg bw/day)	Reference
Reproductive toxicity	One-generation reproductive toxicity study, dietary, rat	966 (1.5%), decreased live birth and survival indices	622 (1%)	Exxon (1996a) Waterman et al. (2000)
	104-week dietary study, mouse	742 (4,000 ppm), decreased testicular weight	276 (1,500 ppm)	Aristech (1995c)
Developmental toxicity	Prenatal developmental toxicity study, dietary, rat	500[/1,000[a]] skeletal (and visceral) variations	100[/500[a]]	Exxon (1994) Waterman et al. (1999)
	Two-generation reproductive toxicity study, dietary, rat	159 (0.2%), decreased body weight in offspring	No NOAEL	Exxon (1996b) Waterman et al. (2000)
	Tailored pre/perinatal developmental toxicity studies, exposure GD 12-19 or GD 12-PND 14	250 50% decrease in foetal testicular testosterone, increased MNGs[b]	50	Clewell et al. (2011a and b)

[a] The higher NOAEL/LOAEL values were used in EU Risk Assessment (2003a) but the lower NOAEL/LOAEL values were agreed by NTP-CERHR (2003a), US EPA (2005) and US CPCS (2010a) after a recalculation by the sponsor.
[b] MNGs = multinucleated gonocytes

4.4.9.1.5 Conclusions

A NOAEL of 50 mg/kg bw/day based on decreased T production/level and histopathological changes in foetal/pup testis at a LOAEL of 250 mg/kg bw/day is proposed (based on Clewell et al. 2011a and supported by Clewell et al. 2011b; Hannas et al. 2011a and b and 2012; Boberg et al. 2011).

The histopathological changes include increased multinuclear gonocytes/germ cells (MNGs) and Leydig cell aggregates in foetal/pup testis. These findings seem to be largely transient. Their biological implications, and whether they could affect reproductive health in adulthood (e.g., reduced germ cells), are currently unclear. Increase in MNGs was observed in two studies (Boberg et al. 2011; Clewell et al. 2011b).

The in vivo findings suggest that DINP has anti-androgenic potency but may also exhibit its effects through other modes of action. The decrease in testicular T levels seems to be transient and permanent changes were not generally seen in all studies with DINP. However, low incidences of permanent changes[33] after exposure to high doses at and above 500 mg/kg bw/day have been described. Most of these changes are likely to be linked to the reduced perinatal testicular T levels.

[33] I.e., reduced sperm count, reduced motility/quality parameters (Kwack et al. 2009); degeneration of meiotic spermatocytes and Sertoli cells, scattered cell debris in ducts in epididymis, decrease in number of corpora lutea (Matsutomi and coworkers 2003, 2004); reduction of motile sperm, low incidence of nipples/areolae, small testes and epididymides (Boberg et al. 2011; Gray et al. 2000).

Clewell et al. (2011b) reported only few effects near adulthood after dietary dosing. However, the incidences of findings from the positive control (DBP) were also lower than expected from studies using gavage dosing. These differences suggest that peak exposure (gavage dosing) during development may be critical. Furthermore, sufficient statistical power is required to detect low incidences of malformations/effects.

DINP causes low incidences of effects, similar to those observed with other phthalates, likely acting via the same modes of action, including androgen deficiency.

There are also results on changes in sexual dimorphic gene expression in hypothalamus and one study reporting reduced female sexual behaviour and reduced AGD at a low level of dietary exposure (40 ppm corresponding to 2 mg/kg bw/day; Lee et al. 2006a,b). Due to the limitations of the study, these results are considered to support the NOAEL value of 50 mg/kg bw/day rather than leading to a lower NOAEL.

4.4.9.2 DIDP

4.4.9.2.1 EU Risk Assessment conclusion

The following cites the 'Summary of toxicity for reproduction' from the EU Risk Assessment:
"In 42-44 day ~~year~~[sic] *old (pubertal) or adult rats there is no indication of organ reproductive effects evidenced by histological observation in repeated dose toxicity studies and the two-generation study. In the two-generation study decrease in mean percent normal sperm was observed but of low incidence and only in P1 generation. In pups (F1, F2 and in the cross fostering satellite group) decrease in testes weight and cryptorchidism in F2 high-dose offspring were observed likely due to the low body weight since no histopathological damages were observed in adult testes. There were no changes in Reproductive Indices. From those assays no adverse effects on fertility may be anticipated.*

In regard with reproductive toxicity DIDP is a developmental toxicant since decrease in survival indices was observed consistently in both two-generation studies (Exxon Biomedical Sciences, 1997b; 2000) leading to the NOAEL of 0.06% (Exxon Biomedical Sciences, 2000). The NOAEL of 0.06% (33 mg/kg/d DIDP) is taken into account in the risk characterisation.

In regard with developmental effects, skeletal variations are observed in the developmental studies at 1,000 mg/kg/d concurrently with slight signs of maternal toxicity and lead to a NOAEL of 500 mg/kg/d; in the two-generation rat study (Exxon Biomedical Sciences, 1997b) body weight decrease was observed in offspring partly related to lactation at the highest dose of 0.8% and leads to a NOAEL of 0.4% (253 to 761 mg/kg/d seeing that received doses are widely dependent on the period considered). Those NOAELs are considered for risk characterisation.

No effects were seen on fertility thus no classification according to the EU is needed. With regard to development decrease in survival indices mainly in F2 (day 1 and day 4) in the two-generation study as well as skeletal variations in developmental studies are not severe enough to justify a classification."(EC 2003b)

<u>Summary of examination of endocrine activity in the EU Risk Assessment</u>
According to the EU Risk Assessment (EC 2003b), DEHP, DINP and DIDP showed no activity in the different in vitro assays conducted to test the ability of binding to rodent or human oestrogen receptors or to induce oestrogen receptors-mediated gene expression (Harris et al. 1997; Zacharewski et al. 1998). In an uterotrophic assay/vaginal cell cornification assay with orally dosed rats, the response with uterine wet weight and vaginal cornification were both considered negative for the phthalates tested (DEHP, DINP and DIDP) – although the value of the test was questioned in relation to the uterine response (Zacharewski et al. 1998).

In the first two-generation reproductive toxicity study (Hushka et al. 2001 [Exxon 1997d]), some alterations in male reproductive development were found to be possibly indicative of a tendency of disturbance of masculinisation through an endocrine-mediated mechanism (change in sex ratio at the lowest dose, decreases of absolute but not relative testes weight in F1 and F2 offspring, cryptorchidism possibly related to delayed body weight gain). In a newer two-generation reproductive toxicity study (Hushka et al. 2001 [Exxon 2000]), there were no changes in developmental landmarks sensitive to hormonal disturbance at lower doses.

It was concluded that on the whole, no overt effect related to endocrine disruption of the reproductive system has been observed.

<u>Commentary to the EU Risk Assessment</u>
In this commentary, further details and clarifications to the summary of the EU Risk Assessment are given.

Reproduction toxicity of DIDP was evaluated based on information from 28-, 90-day repeated dose studies, an one-generation reproductive toxicity study, two two-generation reproductive toxicity studies and two prenatal developmental toxicity studies (a rat and mice study) and a range-finding study in rats (BIBRA 1986; Lake et al. 1991; BASF 1969b; Exxon 1997c; 1997d;

2000; 1995b; Harding 1987; BASF 1995). In addition, in vitro embryotoxicity studies were referred (Lee et al. 1974).

A decrease in offspring survival indices was observed consistently in two conducted two-generation reproductive toxicity studies with a NOAEL of 0.06% (corresponding to a dose level of 33 mg/kg bw/day according to EU Risk Assessment)(Hushka et al. 2001 [Exxon 2000]). The mode of action leading to decrease in offspring survival indices during neonatal period is not known. It may be related to paternal, maternal and/or developmental factors. Because the NOAEL is derived from the reduced F2 survival indices, it has been considered in some risk assessment reports that the lowest substance intake of 38 mg/kg bw/day during pregnancy of F1 females could be used instead of the lowest substance intake of 33 mg/kg bw/day of F1 males. However, because paternally-mediated effects cannot be excluded, 33 mg/kg bw/day is considered appropriate. The decreased offspring body weight at the highest concentration level of 0.8% leading to a NOAEL of 0.4% (corresponding to 253-761 mg/kg bw/day for females) was considered to be partly related to lactation (Hushka et al. 2001 [Exxon 1997d]). Skeletal variations (rudimentary lumbar ribs and cervical ribs) observed at 1,000 mg/kg bw/day concurrently with slight signs of maternal toxicity leading to a NOAEL of 500 mg/kg bw/day for both developmental and maternal toxicity (Waterman et al. 1999 [Exxon 1995b]). The developmental NOAEL of 500 mg/kg bw/day has been criticized and a NOAEL of 100 mg/kg bw/day has been supported/agreed (EFSA 2005b; CSTEE 2001b; NTP-CERHR 2003b). Similarly, the NOAEL of 200 mg/kg bw/day for skeletal variations based on another study (Hellwig et al. 1997) has not been accepted in all international evaluations. Due to increased incidence of total variations (skeletal and visceral variations) a NOAEL of 40 mg/kg bw/day has been concluded by NTP-CERHR (2003b) but not agreed by EFSA (2005b) or CSTEE (2001b). Justifications for lower NOAELs are presented in the chapter of assessment by NTP-CERHR.

It is to be noted that the dosing period in the developmental toxicity studies (Watermann et al. 1999; Hellwig et al. 1997) are not covering the most sensitive exposure period to induce the changes in sexual development described for certain phthalates which is 14-19 GD (Welsh et al. 2008). In addition, in the one- and two-generation reproductive toxicity studies, some parameters sensitive to endocrine disruption were not included in earlier studies but were included in the latest two-generation study on reproduction toxicity (Hushka et al. 2001 [Exxon 2000]). These include AGD and nipple retention and daily external examination before weaning. In addition, statistical power for some endpoints was increased (e.g., preputial separation) in the second two-generation reproductive toxicity study as compared to the earlier studies.

Table 4.49 Summary of reproductive toxicity studies and NOAEL/LOAEL values of DIDP referred to in EU Risk Assessment (EC 2003b). Differing NOAEL values proposed by other international bodies are presented as footnotes.

Species	Protocol/ doses	Results NOAEL/LOAEL	Test substance	References
Repeated dose studies				
Young rat Fischer 443	21-day in diet 0-0.3-1.2-2.5% (0, 264-304; 1042-1134;/1972-2100 mg/kg bw/day)	No testicular change	DIDP purity 99.84%	BIBRA (1986)
Rat Fischer 443	28-day in diet 0-0.02-0.05-0.1-0.3-1% (12; 57; 116; 353; 1287 mg/kg bw/day)	No testicular atrophy	DIDP (equal part by weight of Hexaplas (ICI), Jayflex	Lake et al. (1991)

			DIDP (Exxon) and Palatinol Z (BASF)	
Rat Sprague Dawley	90 days in diet 0-800-1,600-3,200-6,400 ppm	No histopathological changes in testis/ovaries	Palatinol Z (no further information)	BASF (1969b)
One-generation studies (oral)				
Rat Crl:CDBR	0-0.25-0.5-0-75-1% in diet (0; 132-264; 262-521; 414-776; 542-1014 mg/kg bw/day for males during premating period, 0; 165-479; 314-897; 500-1334; 631-1571 for females)	NOAEL for systemic toxicity 0.5% (262 mg/kg bw/day) in parents and 0.25% (165 mg/kg bw/day) for offspring based on decrease in body weight. NOAEL for fertility 1%	DIDP (assumed 100% pure)	Exxon Biomedical Sciences (1997e)
Two-generation studies (oral)				
Rat Crl: CDBR	diet 0-0.2-0.4-0.8% (0; 103-216; 211-437; 427-929 for males, 0; 127-379; 253-761; 508-1582 for females)	LOAEL for systemic toxicity in parents 0.2% (103 mg/kg bw/d) for minor liver changes NOAEL for fertility 0.8% (427 mg/kg bw/day) LOAEL for offspring 0.2% (103 mg/kg bw/day) based on decreased survival indices on PND 0 and 4 in F2. NOAEL for development 0.4% (253 mg/kg bw/day) based on decreased body weight in F1 and F2	DIDP (assumed 100% pure)	Exxon Biomedical Sciences (1997d); Hushka et al. (2001)
Rat Crl: CDBR	diet 0-0.02-0.06-0.2-0.4% (0; 11-26; 33-76;114-254; 233-516 for males, 0; 13-40; 38-114; 134-377; 254-747 for females	NOAEL for parental systemic toxicity 0.06% (33 mg/kg bw/day) based on liver and kidney changes in P1 animals[a] NOAEL for fertility 0.4% NOAEL for offspring 0.06% (33 mg/kg bw/day) for decreased survival indices in F2	DIDP (CAS No 68515-49-1, purity >99.7%)	Exxon Biomedical Sciences (2000); Hushka et al. (2001)
Developmental toxicity studies				
Rat Crl: CDBR	gavage in corn oil 0-100-500-1,000 mg/kg/d on GDs 6-15	NOAEL for dams 500 mg/kg/d based on transient decrease in body weight gain NOAEL for development 500[b] mg/kg bw/day based on significant increase of skeletal variation	DIDP (CAS No. 68515-49-1)	Exxon Biomedical Sciences (1995b); Nikiforov et al. (1995); Waterman et al. (1999)[b]

Rat Chbb:THOM, 7-10 pregnant rat per dose group	gavage in olive oil 0-40-200-1000 mg/kg bw/day on GDs 6-15	NOAEL for dams 200 mg/kg bw/day based on increase in liver weight (and vaginal heamorrhage and urine smeared fur) NOAEL for development 200[c)] mg/kg bw/day based on skeletal and visceral variations (rudimentary cervical and/or 14th ribs) Dilated renal pelvis and hydroureter were observed at all dose levels without dose-response and significance when litter incidences are compared.	DIDP purity 99.9% (CAS No. 26761-40-0 as cited in NTP-CERHR, 2003)	BASF (1995); Hellwig et al. (1997)[c]
Mouse CD-1	10 ml DIDP undiluted (e.g. 9,650 mg/kg bw/day) on GDs 6-13. Litter size, birth weight, neonatal growth and survival to PND 3 was recorded. Malformations were not systemically examined.	No adverse effects were noted	DIDP (no further information available)	Harding (1987)
Additional data				
chick embryos, cultured chick embryonic cells	Undiluted or Chick Ringer solution saturated with DIDP or 0,05 mg/ml	Undiluted DIDP solution caused lethality in chick embryos in ovo and in explanted streak stage chick embryos. At 0.05 mg/ml concentration, DIDP caused lethality in ovo or shortly before hatching. The most common abnormalities were twisting or clubbing of foot in hatched chicks, failure in brain and neural tube closure and affected somite formation in explanted streak stage chick embryos. On cultured chick embryonic cells, DIDP (0.05 mg/ml) caused morphologic alterations.	DIDP (no further information available)	Lee et al. (1974)

a Refers to the parental animals of the second generation
b These references describe the same study and NOAELs. Waterman has later agreed with a lower NOAEL of 100 mg/kg bw/day as described by NTP-CERHR (2003b) and supported by US CPSC (2010b). This lower NOAEL is also agreed with CSTEE (2001b) and EFSA (2005)
c A NOAEL of 40 mg/kg bw/day has been set by NTP-CERHR (2003b) based on foetal variations and supported by US CPSC (2010b)

4.4.9.2.2 Risk assessments from other international organizations and bodies

EU bodies

CSTEE 2001b

The Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE 2001b) supported a NOAEL of 500 mg/kg bw/day for maternal toxicity based on a prenatal developmental toxicity study (Waterman et al. 1999). However, the CSTEE does not agree with the EU Risk Assessment in using of 500 mg/kg bw/day as a NOAEL for developmental toxicity and proposes a NOAEL of 100 mg/kg bw/day based on the study by Waterman et al. (1999). CSTEE supports the developmental NOAEL of 100 mg/kg bw/day based on data presented by the NTP-CEHRH Monograph (2003b) which includes the NTP-CERHR Expert Panel Report where the statistical re-evaluation shows that the NOAEL of 100 mg/kg bw/day is more appropriate based on the incidence of cervical and accessory 14th ribs. From the other prenatal developmental toxicity study (Hellwig et al. 1997), a maternal and developmental NOAEL of 200 mg/kg bw/day is identified in EU Risk Assessment. However, the NTP-CERHR Expert Panel concluded in their report that a NOAEL of 40 mg/kg bw/day was based on increase in affected foetuses per litter with variation. The incidence of hydroureter and dilated renal pelvis occurred in all groups and is thought to account partially for the reported increase in affected foetuses. CSTEE agrees with a developmental NOAEL of 200 mg/kg bw/day because the effects on renal pelvis may be transient and no renal effects were observed in the two-generation reproductive toxicity study. An overall evaluation of prenatal studies suggests a maternal NOAEL of 500 mg/kg bw/day and a developmental NOAEL of 100-200 mg/kg bw/day, and applying a conservative approach a NOAEL of 100 mg/kg bw/day is proposed by CSTEE.

Regarding findings from a two-generation study where a decrease in offspring survival indices was noted, the CSTEE supports a NOAEL of 33 mg/kg bw/day for offspring toxicity as suggested in the EU Risk Assessment. The CSTEE does not agree with the EU Risk Assessment that a NOAEL of 253-761 mg/kg bw/day (based on the first of the two two-generation reproductive toxicity studies) should be used for the body weight decrease and prefers a lower NOAEL of 127-151 mg/kg bw/day for gestation and 166-377 mg/kg bw/day for lactation, as concluded by NTP-CERHR Expert Panel [comment from evaluator: probably referring the substance intake values from the second two-generation study, although then the range for a NOAEL should be 127-150 mg/kg bw/day)]. Finally, the CSTEE concludes that DIDP should be considered a developmental toxicant. For fertility, CSTEE agrees that DIDP does not affect fertility at doses up to 928 mg/kg bw/day based on the two-generation reproductive toxicity study and on the repeated dose studies in rats at doses up to 2100 mg/kg bw/day.

CSTEE mentions that DIDP was not able to bind to the rodent or human oestrogen receptors, to induce oestrogen receptor-mediated gene expression, or to stimulate cell proliferation in vitro. CSTEE also notes that DIDP neither significantly induce vaginal cornification nor increase uterine weight. Finally, CSTEE points out in a two-generation reproductive toxicity study with rats, lack of nipple retention and normal anogenital distance (AGD) in male offspring were noted when exposed to DIDP up to 295 mg/kg bw/day (0.4% in diet) during gestation. This study does not indicate an anti-androgenic activity at the doses tested.

EFSA 2005b

The European Food Safety Authority (EFSA) did not carry out a new extensive risk assessment to come to its opinion on use of DIDP in food contact materials (EFSA 2005b). EFSA set the reproduction NOAEL of 33 mg/kg bw/day which is based on a decrease of F2 offspring survival. There is no indication of effects on reproductive organs from histological observation in repeated dose toxicity studies and DIDP did not affect fertility in rats in one-and two-generation reproduction toxicity studies. With respect to developmental effects, skeletal variations (including rudimentary lumbar ribs and supernumerary cervical ribs) were observed in developmental studies in rats at 1,000 mg/kg bw/day concurrently with slight signs of maternal toxicity. A NOAEL of 500 mg/kg bw/day for maternal toxicity and a NOAEL of 100 mg/kg bw/day for developmental effects were established based on prenatal developmental

toxicity studies (Waterman et al. 1999; EFSA mistakenly refers to two-generation rat studies (Exxon 1997b; 2000).

ESFA's conclusion on NOAEL of 100 mg/kg bw/day for development is based on CSTEE opinion (2001b) on EU Risk Assessment where CSTEE refers to NTP-CERHR Expert Panel Report (2003b) which disagrees with a developmental NOAEL of 500 mg/kg bw/day and considers a NOAEL of 100 mg/kg bw/day more appropriate. The NOAEL of 100 mg/kg bw/day is based on a statistical re-evaluation of the data indicating an increase at 500 mg/kg bw/day of skeletal variation, rudimentary lumbar ribs and supernumerary cervical ribs. EFSA did not make any comment with regard to endocrine effects.

SCHER 2008
The Scientific Committee on Health and Environmental Risks (SCHER) adopted an opinion on phthalates in school supplies and concluded that there were no indications of reproductive organ effects of DIDP in the available repeated dose toxicity studies.

The United States

NTP-CERHR 2003b
NTP-CERHR Monograph including the Expert Panel Report concluded that DIDP may cause adverse developmental effects and could adversely affect human development if levels of exposure were sufficient high, but DIDP does not affect reproduction. As there is lack of human data, animal studies have been addressed on both development and reproduction. These studies reported that exposure of pregnant rats to relatively high doses of DIDP causes abnormal development of the foetal skeleton, and reduced weight gain and survival of pups. DIDP exposure was also associated with abnormalities of the urinary tract. The data also show that exposure during lactation can contribute to reduced weight gain in pups.

NTP-CERHR Expert Panel concurred author's interpretation of the maternal NOAEL of 500 mg/kg bw/day based on prenatal developmental study in rats (Waterman et al. 1999). However, NTP-CERHR selected a developmental NOAEL of 100 mg/kg bw/day based on incidence of cervical and accessory 14th ribs instead of a NOAEL of 500 mg/kg bw/day selected by the authors of the study. After statistical re-analysis of the skeletal variations by the sponsor of the study (Waterman), consistent results with the Expert Panel's interpretation supporting a NOAEL of 100 mg/kg bw/day were obtained (Table 4.50).

Table 4.50 Mean percent of pups in litter with skeletal variations after in utero exposure to DIDP (according to NTP-CERHR report after a re-evalution of data using the generalized estimating equation approach to linearized model). The original values reported by Waterman et al. (1999) are added for clarity by the evaluator and indicated in parenthesis as percentage of foetuses/litters affected (values attaining statistical significance in original analysis are in bold and in new analysis with asterisk(s)).

Parameter	Oral dose levels of DIDP			
	0 mg/kg bw/day	100 mg/kg bw/day	500 mg/kg bw/day	1,000 mg/kg bw/day
Skeletal variations	19.8	20.6	31.9*	64.1**
Rudimentary lumbar ribs	8.4 (8.2/40.4)	9.4 (9.0/36.4)	21.9* (**21.2**/62.5)	51.9** (**52**/**95.8**)
Supernumerary cervical ribs	1.1 (1.0/8.0)	3.1 (2.3/18.2)	6.2* (**6.2**/25)	10.2** (**9.2/41.7**)

* p≤0.05, **p≤0.01

Waterman also provided the NTP-CERHR Expert Panel with the following benchmark doses (95% CI): 188 (169) mg/kg bw/day for rudimentary lumbar ribs; 258 (238) mg/kg bw/day for Skeletal variants; and 645 (515) mg/kg bw/day for supernumerary cervical ribs.

In another prenatal developmental toxicity study, the Expert Panel agreed with the maternal NOAEL of 200 mg/kg bw/day based on increased liver weight and vaginal haemorrhage at 1000 mg/kg bw/day (Hellwig et al. 1997). However, the Expert Panel did not support the developmental NOAEL of 200 mg/kg bw/day proposed by the authors due to increased incidences of rudimentary cervical ribs and increased accessory 14th ribs at 1000 mg/kg bw/day, but agreed with a NOAEL of 40 mg/kg bw/day based on the increased foetal variations at 200 mg/kg bw/day. In this study, an increased incidence of hydroureter and dilated renal pelvis occurred in all treatment groups. This finding was considered by the Expert Panel as a sign of delay in maturation because it occurred in the absence of reduced foetal weight. The Expert Panel notes that LOAELs for developmental toxicity occur at doses at which there were no demonstrable maternal effects. The data for these findings according to the original publication are presented at the discussion chapter of all DIDP studies.

Developmental toxicity was observed and replicated in 2 two-generation reproductive toxicity studies in rats. The results revealed a NOAEL of a range of 38-44 mg/kg bw/day (gestational) and 52-114 mg/kg bw/day (lactational) for adverse effects on pup growth or survival. NTP-CERHR notes that these effects may be due to prenatal and/or lactational exposures to DIDP and uses maternal DIDP consumption values to set the NOAEL [Evaluator's comment: EU Risk Assessment uses the NOAEL of 33 mg/kg bw/day based on the lowest paternal exposure level].

There were no adverse structural or functional reproductive effects in 2 two-generation reproductive toxicity studies up to doses of 427-929 and 508-927 mg/kg bw/day (0.8% in diet; substance intake during premating period) in males and females, respectively. A reduced length of oestrous cycles and reduced relative ovary weight was observed at 0.8% in F0 females without effects in F1 adults. Systemic effects in parental animals included increased kidney and liver weights at and above 0.2%/0.4% dietary levels, and dilated renal pelvis and renal casts at 0.8% in males. An increase in relative seminal vesicle weight in F1 males and relative epididymis weight in F0 and F1 males at 0.4% dose was not considered adverse because of unaffected reproductive function and no histopathological effects. Hormonally-mediated endpoints such as anogenital distance and nipple retention in males were not affected up to 0.4% in diet. The age of preputial separation was increased in F2 males at 0.4% dietary level. Four F2 pups had undescendent testes at 0.8% dose level, probably related to delayed development. The NOAEL for reduced F2 pup survival was 0.2%. The highest dose of 0.8% was identified as the NOAEL for reproductive toxicity.

US CPSC 2010b

The memo on the assessment of potential toxicity associated with DIDP was provided by the US Consumer Product Safety Commission's (US CPSC) Health Sciences staff (2010b). CPSC staff assesses a household product's potential health risks to consumers under the Federal Hazardous Substances Act (FHSA). The assessment of hazard is risk based and the memo represents the hazard identification step of the risk assessment process.

In one subchronic toxicity study only, the relative testes weight was increased at the high dose of DIDP (BIBRA 1986), however, these studies have not examined if exposure prior to puberty may affect the testes. US CPSC staff memo summarises the reproductive effects based on repeated dose (BIBRA 1986) and multi-generation studies (Hushka et al. 2001) as follows: "*In summary, reproductive effects of DIDP include a significant decrease in ovary weight and significant increases in relative testes, epididymis and seminal vesicle weight without histological changes. There was a non-reproducible increase in age of offspring vaginal opening. There were no effects on mating, fertility, or gestational indices in any generation. There was a small but significant decrease in the number of normal sperm of treated males,*

and an increase in the length of the estrous cycle in the F0 females treated with 0.8% DIDP." They refer to the NOAEL for fertility as set at 0.4% by the authors (233-635 mg/kg bw/day for males and 271-645 mg/kg bw/day for females as reported by NTP-CERHR 2003b).

For developmental toxicity, based on prenatal developmental toxicity and multi-generation studies (Hellwig et al. 1997; Waterman et al. 1999; Hushka et al. 2001), US CPSC staff assessment concludes as follows: *"In summary, DIDP treatment led to increase incidences of minor skeletal variations. Offspring survival was affected and decreased pup body weight was observed at 0.2 and 0.4% DIDP in the F1 and F2 generations. DIDP is considered a probable toxicant under the FHSA based upon these developmental effects."* US CPSC staff refers to the developmental NOAEL of 40 mg/kg bw/day and maternal NOAEL for 200 mg/kg bw/day set by the Expert Panel for the Centre for the Evaluation of Risks to Human Reproduction (NTP-CERHR 2003b) based on the study of Hellwig et al. (1997) with foetal variations. The NOAEL of 100 mg/kg bw/day as set by the Expert Panel for the NTP-CERHR (2003b) based on skeletal variations at 500 mg/kg bw/day was also agreed by the authors of the study (Waterman et al. 1999) after a reanalysis and they also provided the panel with bench mark doses, the lowest being 188 mg/kg bw/day for rudimentary lumbar ribs.

The memo refers to overall NOAEL and LOAEL of 0.06 and 0.2%, respectively, for offspring survival effects (approximately 50 mg/kg bw/day and 165 mg/kg bw/day as calculated by Hushka et al. 2001). A developmental NOAEL was set at 0.06% by the authors (38-44 mg/kg bw/day and 52-114 mg/kg bw/day during pregnancy and lactation respectively as calculated by Hushka et al. 2001). In F2 pups the pup survival decreased on PND 1 and 4 at 0.2 and 0.4% DIDP. The F2 pup body weight decreased on PND 14 (females) and on PND 35 (males) at 0.2 and 0.4% dietary concentration of DIDP. The LOAEL for liver hypertrophy eosinophilia in F1 and F2 pups was 0.4%.

Based on a Hershberger assay (Lee and Koo 2007), US CPSC staff concluded that DIDP does possess anti-androgenic activity. At 500 mg/kg bw/day DIDP caused a decrease in ventral prostate and seminal vesicle weight leading to a NOAEL of 100 mg/kg bw/day.

US CPSP staff calculated that a reproductive ADI (acceptable daily intake) based on fertility using the range 233-645 mg/kg bw/day (0.4% in diet) divided by the safety factor of 100 [10 (rat to human) x 10 (sensitive population)] is 2.3-6.5 mg DIDP/kg. A developmental ADI using the dose of 40 mg/kg bw/day divided by the safety factor of 100 [10 (animals to human) x 10 (sensitive population)] is 0.4 mg DIDP/kg.

Industry

ECPI 2011a

European Council of Plasticizers and Intermediates (ECPI 2011a) reviewed endocrine data relevant to human health for selected phthalates, DIDP among them. Using the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals ("OECD Conceptual Framework"), ECPI considers there are sufficient data to conclude that DIDP is not an endocrine disrupting substance for mammals. The key conclusions by level of the OECD Conceptual Framework for DIDP are presented below:

Level 1 Sorting & prioritization based upon existing information

DIDP has a rich safety dataset available. These data have previously been collated and evaluated by international regulatory authorities and assessed for their potential risk to human health and the environment. These reviews have concluded that DIDP is not dangerous so should not be classified as hazardous under current EU regulations.

Level 2 In vitro assays providing mechanistic data
No significant responses were observed with DIDP in any of the in vitro assays. Taken as a whole, the available data indicate that DIDP does not have significant interactions with the oestrogenic or androgenic receptors. It is noteworthy that in vitro data need to be evaluated very carefully as the tests may have involved either substances which for all practical purposes do not exist under in vivo conditions or may have employed non-physiological conditions.

Level 3 In vivo assays providing data about single endocrine mechanisms and effects
The data collected support the conclusion that DIDP does not cause adverse endocrine effects in in vivo screening studies. DIDP shows no significant adverse effects in the Uterotrophic Assay (for oestrogenic effects), and no consistent significant adverse effects in the Hershberger Assay (for anti-androgenic effects).

Level 4 In vivo assays providing data about multiple endocrine mechanisms and effects
Sufficient in vivo data exist for DIDP to demonstrate that DIDP does not induce endocrine mediated chronic toxicity to non-reproductive tissues in rodents or non-human primates.

Level 5 In vivo assays providing data on effects from endocrine & other mechanisms
Based on the comprehensive two-generation reproductive studies and the developmental study, it can be concluded that DIDP is not an endocrine disruptor as defined by the Weybridge, IPCS and REACH guidance definitions. The adverse health effects mediated via an endocrine mechanism of cryptorchidism, hypospadias, and significant testicular pathology which are seen with DBP in laboratory animals are not seen with DIDP.

ExxonMobil 2011a
ExxonMobil Chemical Company, in its comments to the Consumer Product Safety Commission (US CPSC) Chronic Hazard Advisory Panel (CHAP), concluded that DIDP does not affect reproduction or is an endocrine disruptor (ExxonMobil 2011a). ExxonMobil refers to studies on both DINP and DIDP, but merely information related to DIDP is presented in the following paragraphs.

ExxonMobil illustrates extensive developmental and reproductive toxicity data for DIDP whose findings do not show DIDP to cause cryptorchidism, hypospadias, or gross reproductive tract malformations (Hushka et al. 2001). AGD and nipple retention were also not affected. ExxonMobil points out that there is no strong evidence of effects on sperm or fertility; sperm count was not affected after exposure to DIDP (Kwack et al. 2009). However, the study also mentions a statistically significant decrease in sperm motion/quality parameters.

Some effects in androgen sensitive tissue weight were reported by Lee and Koo (2007), but the conclusion was that DIDP did not induce consistent changes in androgen sensitive tissues. ExxonMobil believes that the data do not meet the Organisation for Economic Co-operation and Development (OECD) or Environmental Protection Agency (EPA) criteria for being classified as having a positive result since not all tissues were effected and no dose-response was observed.

To conclude, ExxonMobil considers that "*there is no scientific basis for including DIDP in a cumulative risk assessment based on "rat phthalate syndrome." The weight of the evidence approach indicates that DIDP does not cause the same effects observed LMW phthalates characteristic of the "rat phthalate syndrome" (hypospadias, cryptorchidism, decreased AGD, nipple retention, changes in androgen sensitive tissue weight and infertility). The LMW phthalates are classified in the EU as reproductive and developmental toxins whereas DIDP is not*".

The conclusion that DIDP is not an endocrine disrupter is based on the following data:

1) DINP and its monoester metabolite, MiNP, do not bind to androgen receptors in in vitro tests. Several studies (Harris et al. 1997; Zacharewski et al. 1998; Breous et al. 2005; Wenzel

et al. 2005) examined the ability of DIDP to bind to androgen (AR) and oestrogen receptors (ER) as well as modulate active iodine uptake in the thyroid. No significant responses were observed with DIDP in any of the in vitro assays. On the other hand, under in vivo conditions the DIDP is likely metabolized to its monoester which is not oestrogen receptor agonists (Koch and Angerer 2007; McKee et al. 2002).

2) DIDP did not meet the criteria established by OECD (using the "OECD Conceptual Framework") for classification as an androgen antagonist based on results from an in vivo study, and

3) In studies designed to see malformations of the male rat reproductive tract, minor effects have been observed following gavage exposure at very high doses, however, no effects on androgenic sensitive endpoints have been observed at even higher levels of exposure via the diet. Various short-term exposure studies (Ghisari and Bonefeld-Jorgensen 2009; Waterman et al. 1999) shown in the review are informative and have identified particular endpoints of interest including T synthesis, nipple retention, AGD, and epididymal malformations. However, ExxonMobil believes that those results do not invalidate the conclusions from the comprehensive two-generation reproductive toxicity studies (Waterman et al. 2000 [for DINP]; Hushka et al. 2001), which fully assess all critical aspects. No significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices were noted in these studies. Mean days of gestation were unaffected by treatment as well as the mean sex ratio of the treated offspring when compared with controls. DIDP did not induce nipple retention, affect AGD, induce hypospadias or cryptorchidism or induce gross male reproductive tract malformations (Hushka et al. 2001).

Commentary to ExxonMobil 2011a
The assessment stresses the importance of functional fertility measurements over indication of affected sperm parameters. However, humans produce less sperm as compared to rats and in cases of subfertility the decrease in quality may be critical (see section 4.4.9.1)

In the second two-generation reproductive toxicity study there was no difference in vaginal patency, in contrast to the results in the first two-generation reproductive toxicity study at dietary concentration of ≥ 0.4% (Huskha et al. 2001). These findings were not considered biologically significant by the authors. In the first two-generation study there were increases in absolute or relative or male reproductive organ weight and reductions in female reproductive organ weight in the first and/or second generation and the 0.4 and/or 0.8% dietary concentration levels. The percentage of live births in F1 was decreased at high dose levels as well as the survival index on PND 4. For F2 generation, the offspring survival was reduced at all exposure levels on PND 1 and 4. In addition, pup body weights were reduced on PND 0 at the high dose level and weight gain was reduced during the postnatal period.

The critical findings from the first two-generation reproductive toxicity study were confirmed in the second two-generation reproductive study conducted at lower dose levels (up to 0.4%). There were no findings in F1 pups but the survival of F2 pups on PND 1 and 4 was significantly reduced at 0.2 and 0.4% dietary concentrations with a NOAEL of 0.06% (~50 mg/kg bw/day) and a LOAEL of 0.2% (~165 mg/kg bw/day). In addition, there was a decrease in pup body weights during postnatal period but this does not lead to a lower NOAEL. The PPS was slightly delayed in F2 males at 0.4% (1.2 days) but not considered adverse by the authors. Taken into account the measured feed consumption and the lowest mean measured dose, a NOAEL of 33/38 mg/kg bw/day can be set for the perinatal findings in F2 animals using paternal/gestational feed consumption rate.

It should be also noted that the authors propose a value of 86 mg/kg bw/day as a theoretical NAEL based on reduced F2 offspring survival at a dose of 108 mg/kg bw/day using a 95% lower boundary value. The dose of 108 mg/kg bw/day is derived using a modified probit model for the combined data with the number of live pups as a covariate. This approach is not

considered in EU Risk Assessment (described in published article and probably not in original study report).

4.4.9.2.3 New studies

The studies conducted after the EU Risk Assessment (EC 2003b) are presented as divided according to the updated OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals (OECD Conceptual Framework) based on the draft OECD guidance document (GD) 150 (OECD 2011). This approach aims to a clear presentation of the different type of studies and their results. The OECD Conceptual Framework should only be regarded as a system for categorising information and study types. The Levels should not be regarded as a study strategy that should be followed.

Briefly, the different assays are divided into the following levels:
Level 1: Existing data and non-test information
Level 2: In vitro assays providing data about selected endocrine mechanism(s) /pathways
Level 3: In vivo assays providing data about selected endocrine mechanism(s) /pathways
Level 4: In vivo assays providing data on adverse effects on endocrine relevant end-points
Level 5: In vivo assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organisms

For the purpose of this assessment, the information from the new studies is presented in two sets; information from Level 4 or 5 assays and information from Level 2 or 3 assays.

A) Information on reproductive toxicity and integrity of endocrine systems from Level 4 and 5 assays according to OECD Conceptual Framework
There are no new Level 4 or 5 guideline compliant studies following standard test guidelines and good laboratory practices (GLP) conducted with DIDP such as two-generation reproductive toxicity studies or prenatal developmental toxicity studies. The first new in vivo study on DIDP evaluates the effects in young adult animals and another study addresses the possible androgenic/anti-androgenic mechanism.

Published in vivo animal studies

Kwack et al. 2009
DIDP did not affect sperm count after a 4-week exposure of juvenile rats at 500 mg/kg bw/day (oral gavage). DIDP did not significantly lower the sperm counts but reduced the motility, straight-line velocity, curvilinear velocity, straightness and linearity of the epididymal sperm motion. Liver weight was significantly increased at this dose level but testis weight was unchanged (slight decrease but no statistical significance; 0.664 vs. 0.770 g). From the haematological and clinical chemistry parameters only the platelet count and serum alkaline phosphatase (ALP) were significantly increased.

For comparison, DEHP decreased sperm count (~ 70%), motility, average path velocity and straight-line velocity. In addition, DEHP increased liver and thymus weights and decreased testis weights and increased serum glucose and calcium. Based on the phthalate diesters and monoesters examined, the adverse effects on sperm parameters were greater with phthalate diesters than monoesters according to the authors.

Hannas et al. 2012
To define the relative potency of several phthalates to genomic biomarkers of male developmental effects, DIDP (CAS No 26761-40-0) was administered by gavage at dose levels of 0, 500, 750, 1000 or 1500 mg/kg bw/day on gestation days (GDs) 14-18. DIDP had no effect on ex vivo foetal testicular testosterone (T) production. DIDP and phthalates positive for anti-androgenic activity as measured by ex vivo foetal testicular T production were further

measured for effects on gene expression levels in foetal testes (e.g., Cyp11b1, Scarb1, StAR, Cyp11a1, Cyp17a1, Insl3 and Hsd3b). DIDP only significantly reduced expression of Wnt7a (the lowest dose of a significant change was 900 mg/kg bw/day). The overall potency of the individual phthalates was: DPeP>DHP>DIBP≥DHeP>DINP and DIDP were not active in reducing T production or affecting gene expression examined. The overall sensitivity of each gene endpoint and T production for the other phthalates was Cyp11b1 >StAR =Scarb1 >Cyp17a1 =T production >Cyp11a1 =Hsd3b =Insl3 >Cyp11b2.

In addition to gene expressions referred to above, the examined phthalates did not affect PPAR-related genes in foetal testes. The anti-androgenic phthalates act through a similar mode of action but the proximate molecular target is still unclear and DIDP seem not to have anti-androgenic activity in this study.

New studies provided by industry
There are no new studies with DIDP provided by the industry.

Information in humans
There are no specific new studies addressing effects of DIDP in humans.

B) New information on endocrine disruption potency from Level 2 and 3 assays according to OECD Conceptual Framework
A Hershberger assay and several new in vitro experiments measuring endocrine disruption potency of DIDP are listed and shortly described in this chapter.

In vivo studies (Level 3 assays)

Lee and Koo 2007
Anti-androgenicity of DIDP was examined in a Hershberger assay at dose levels of 20, 100, or 500 mg/kg bw/day (Lee and Koo 2007) for 10 days. Weights of five androgen sensitive organs (glans penis, seminal vesicles, ventral prostate and levator ani/bulbocavernosus muscles (LABC)) were recorded in castrated male rats after co-treatment with testosterone and DIDP. Seminal vesicle weight and ventral prostrate weight were significant decreased by DIDP at dose level of 500 mg/kg bw/day.

Commentary to Lee and Koo 2007
There was a decrease in seminal vesicle and ventral prostate weights. Based on the test guideline, it is enough if there are changes in weights of two tissues, the result should be considered as a positive. In this study, the effects were observed at the highest dose group of 500 mg/kg bw/day. Due to the effect at a high dose group only and only in two tissues it can be concluded that the anti-androgenic potency of DIDP seem to be lower than that for DEHP (ventral prostate weight decreased at 20 mg/kg bw/day, seminal vesicle weight >100 mg/kg bw/day and LABC at 500 mg/kg bw/day) or DINP (LABC weights decreased at 500 mg/kg bw/day, and seminal vesicles at > 20 mg/kg bw/day). US CPSC staff (2010) suggested in their memo that DIDP may have anti-androgenic properties based on the results of this study.

In vitro studies (Level 2 assays)

Mlynarcikova et al. 2007
DIDP did not affect dose-dependently the basal progesterone production in porcine ovarian granulosa cell culture; DIDP increased progesterone levels at the lowest concentration tested (10^{-8} M). DIDP also amplified FSH-stimulated progesterone release into the culture medium. Basal oestradiol production was not affected by DIDP but the FSH-stimulated oestradiol production was inhibited after the treatment with DIDP.

Commentary to Mlynarcikova et al. 2007
Similarly to DINP, DIDP may have a potential capacity to cause anti-oestrogenicity and to affect progesterone-mediated effects.

Akahori et al. 2008
Akahori and coworkers (2008) examined a series of chemicals in a human ERα binding assay and compared the results to observations from an in vivo uterotrophic assay performed according to the OECD Test Guideline 440 and in compliance with good laboratory practices (GLP). The relative binding affinity (log RBA) value of -3.46 for DIDP was below the cut-off level (-2.63) that could induce oestrogenic/ anti-oestrogenic activities in the uterotrophic assay. In line with this, DIDP exhibited neither oestrogenic nor anti-oestrogenic responses in the in vivo assay (nor did DINP or DEHP). The discrepancies between in vitro and in vivo assays in phthalates (some ER-mediated activities in in vitro assays but no oestrogenic response in in vivo models) are probably caused by the deactivation of phthalates to mono alkyl phthalates (Harris et al. 1997; Picard et al. 2001; Zacharewski et al. 1998).

Krüger et al. 2008
The effects of DIDP on the aryl hydrocarbon receptor (AhR) and the androgen receptor (AR) were measured using luciferase reporter gene expression bioassay in recombinant mouse Hepa1.12cR cells (AhR-CALUX) and in transient transfected Chinese Hamster Ovary (CHO-K1) cells (AR-CALUX). DIDP induced AhR activity weakly (3.78-fold above the solvent control at 0.1 mM; also DEPH had only a weak effect; 1.7-fold increase in activity). DIDP did not react as an agonist in the androgen reporter gene assay in the tested dose range (10^{-10} – 10^{-4} M). The authors propose that DIDP, as well as DEHP and DBP, could be involved in the altered reproductive development in males due to their weak agonistic AhR activity. DIDP, DINP and DEHP did not affect the AR consistently with other reports (Roy et al. 2004; Takeuchi et al. 2005).

Takeuchi et al. 2005
Takeuchi and coworkers (2005) characterized the activities of the human ERα, human ERß and human androgen receptor (AR) using a reporter gene assay in Chinese Hamster Ovary (CHO) cells. DIDP (as well as DINP) did not show any oestrogenic/anti-oestrogenic or androgenic/anti-androgenic activity at the tested concentrations (up to 10^{-5} M). For comparison, DEHP induced ERα mediated oestrogenic activity, antagonized ERß and was not active via AR (showing 20% of the agonistic activity of 10^{-9} M E2 at 5.5 x 10^{-6} M and 20% of the antagonistic activity of 10^{-10} M E2 via ERß).

Harris et al. 2007, Turan et al. 2005
In another in vitro study (Harris et al. 2007) there were indications that DIDP may negatively affect the sulphate supply pathway which could potentially lead to increased levels of free hormones and decreased capacity for detoxification via sulphate conjugation. A potential negative effect on the sulphate pathway was also seen in an in vitro study by Turan and coworkers (2005).

Wenzel et al. 2005; Breous et al. 2005
The effects of DIDP and other phthalates on the basal iodide uptake in a rat thyroid cell line and the responsible mode of action were studied (Wenzel et al. 2005; Breous et al. 2005). DIDP enhanced iodide uptake in a rat thyroid cell line (FRTL-5) at concentrations of 0.1-1 mM but not at lower concentrations (Wenzel et al. 2005). The effects of DIDP (as well as other active phthalates) on iodine uptake were inhibited by a specific symporter inhibitor (perchlorate; 30 µM) indicating that the increased basal iodine uptake was mediated through sodium/iodide symporter (NIS). DIDP, DINP, and DOP seem to be of approximately similar potency, DEHP a more potent and BBP less potent and DBP was not active.

In further examinations of mode of action, DIDP induced up-regulation of the human NIS (hNIS) promoter construct (N3) in the presence of TSH (Breous et al. 2005). TSH induced

activation of promoter and enhancer (N3 + NUE) was slightly decreased by DIDP. The response of the hNIS promoter construct (N3) as well as the promoter and enhancer construct (N3+NUE) were investigated at 1 mM concentration of DIDP in the presence of 1.5 mU/ml TSH using PC C13 rat thyroid cell line (Breous et al. 2005).

For comparison, other examined phthalates had diverging effects: BBP and DOP stimulated the transcriptional activity of N3 whereas DEHP and DINP were not active and DBP had an inhibitory effect. DINP, BBP, DEHP and DOP had the same effect to TSH induced activation of N3 + NUE as DIDP, whereas DBP abolished the activation.

In line with the up-regulation of N3, mRNA level of rat sodium/iodide symporter was increased by DIDP (as well as by BBP and DOP; but was unaffected by DEHP, DINP and DBP). The slightly lowered TSH-induced transcriptional activities of the promoter and enhancer (N3 + NUE) may result of the interference with accessory factors, e.g., adenylyl cyclase, according to the authors. In conclusion, DIDP seems to enhance iodide uptake in thyroid as a consequence of sodium/iodide symporter transcriptional activation. The demonstrated stimulation is not very strong, but the accumulation of phthalates may contribute to thyroid hyperfunction, according to the authors.

Ghisari and Bonefeld-Jorgensen 2009
DIDP stimulated the thyroid hormone (TH)-dependent rat pituitary GH3 cell proliferation (T-screen) at concentration of 10 µM without T3, and no effect was observed in the presence of T3 (Ghisari and Bonefeld-Jorgensen 2009). DIDP had no oestrogenic effect measured in MVNL cells transfected with an oestrogen receptor (ER).

For comparison, several other phthalates (BBP, DEHP, DBP, and DOP) also stimulated the proliferation without T3 (although less than T3), but DINP inhibited. Most of the phthalates inhibited cell proliferation in the presence of T3 (DINP, DEHP, DBP, DOP) but BBP had a stimulatory effect. For oestrogenic effect, DEHP, DOP and DINP were inactive without E2, whereas BBP and DBP enhanced weakly the ER transactivation. In the presence of E2, BBP and DBP further enhanced the E2-mediated response. However, at concentrations above 10 µM, BBP, DBP and DEHP inhibited the E2-induced transactivation. The findings indicate that in conditions mimicking the natural situation (with endogenous hormone) the effects of different phthalates are less than the endogenous hormones and also varied depending of the phthalate. DIDP seems to stimulate GH3 cell proliferation in the absence of T3 and seems to have no oestrogenic effect.

4.4.9.2.4 Discussion

New Studies
There are only two new in vivo studies addressing reproductive toxicity of DIDP since EU Risk Assessment (2003b). The first study describes effects in sperm motility after a 4-week exposure period indicating slight effects in spermatogenesis and or maturation of sperm at 500 mg/kg bw/day (Kwack et al. 2009). Mating examinations in other studies revealed no adverse effect on functional fertility (reproductive performance) and percentage of progressively motile sperm was not affected by DIDP treatment in two-generation reproductive toxicity study.

The second study assesses androgenic/anti-androgenic properties of DIDP and indicates a low potency for anti-androgenic effects (Hannas et al. 2012). DIDP did not reduce foetal testicular T production or affect the gene expression levels in foetal testis. This is very different from DINP which reduced the foetal testicular T production and reduced the gene expression levels of genes involved in steroidogenesis and masculinisation. In a Hershberger assay, DIDP showed slight anti-androgenicity by reducing weights of two out of five androgen sensitive organs at 500 mg/kg bw/day (Lee and Koo 2007).

In in vitro studies, DIDP was involved in progesterone release in granulosa cells, was not oestrogenic and showed contradictory results for anti-oestrogenicity (Mlynarcikova et al. 2007; Akahori et al. 2008; Takeuchi et al. 2005; Ghisari and Bonefeld-Jorgensen 2009). DIDP did not affect AR but had a weak agonistic AhR activity (Kruger et al. 2008; Takeuchi et al. 2005). In in vivo uterotrophic assay DIDP was not oestrogenic or anti-oestrogenic (Akahori et al. 2008). It may affect the sulphate supply pathway leading to increase in the availability of free hormones and decreased capacity for detoxification via sulphate conjugation (Harris et al. 2007; Turan et al. 2005). In addition, DIDP enhanced iodide uptake in thyroid cell line and had TH-like effects in pituitary cells (Wenzel et al. 2005; Breous et al. 2005; Ghisari and Bonefeld-Jorgensen 2009). DIDP had a similar potency to induce iodide uptake than DINP, DEHP being more potent. Different phthalates seem to exhibit differ molecular mechanism leading to various effects – stimulatory, inhibitory or no effects – on certain endocrine parameters.

Studies evaluated in EU Risk Assessment
Two-generation reproductive toxicity studies referred in EU Risk Assessment indicate that DIDP affect reproduction at dietary concentration of 0.2% (corresponding to 114-377 mg/kg bw/day) based on decreased survival of F2 pups on PND 1 and 4 leading to a NOAEL of 0.06% (corresponding to 33 mg/kg bw/day)(Hushka et al. 2001). The reason for reduced neonatal survival of pups is not clear/reported and not examined further. It may be related to paternal, maternal and/or developmental exposure. Relationship to maternal behaviour is also plausible because there was also increase in cannibalism in P1 females (second generation females). However, it is not possible to discriminate whether early mortality is related to the development/vitality of the offspring or maternal behaviour or ability to produce enough milk. Poor condition of pups may trigger cannibalism in dams.

The NOAELs for other endpoints from reproductive studies were higher; the NOAEL for reduced body weight of the offspring was 0.4% (corresponding to 253 mg/kg bw/day) based on LOAEL of 0.8% and that for skeletal variations 500 mg/kg bw/day (Exxon 1997b; Hushka et al. 2001) according to EU Risk Assessment. However, EFSA (2005), CSTEE (2001b), NTP-CERHR (2003b) and US CPSC staff (2010b) proposed a lower NOAEL based on prenatal developmental toxicity studies; a NOAEL of 100 mg/kg bw/day based on increased incidence of skeletal variations (Waterman et al. 1999) and NTP-CERHR and US CPSC staff propose also a NOAEL of 40 mg/kg bw/day based on foetal variations based on the results of Hellwig et al. (1997)(see Table 4.51).

Table 4.51 Critical visceral and skeletal findings after in utero exposure to DIDP from the study of Hellwig et al 1997. Number of foetuses (litters) affected are shown.

Parameter	Oral dose levels of DIDP			
	0 mg/kg bw/day	40 mg/kg bw/day	200 mg/kg bw/day	1,000 mg/kg bw/day
Total skeletal variations	28 (10)	27 (8)	20 (5)	47 (10)
- accessory lumbar vertebra			1	
- rudimentary cervical ribs	1			15 (6)
- accessory ribs	1		1	21(8)
Dilated renal pelvis	4 (4)	14 (8)	14 (5)	15 (8)
Hydroureter	0	3 (3)	5 (3)	8 (3)
Number (and %) of foetuses with variations	32 (25%)	41 (37%)	34 (38%)	62 (43%)
Affected foetuses with variations (mean %)	24.3 %	37.2%	38.4%*	44.2%*

* $p \leq 0.05$

As indicated in the table above, the mean percentage of foetuses with variations (affected foetuses) increased at all dose levels reaching statistical significance at 200 mg/kg bw/day and above. Foetuses with any skeletal variation were increased only at the highest dose level and number of foetuses with variations increased at all dose levels, both without statistical significance. There is no dose-response in number of foetuses with dilated renal pelvis but a slight increase in number of foetuses, but not litters, with hydroureter. CSTEE (2001b) considers effects in renal pelvis potentially transient because there were no such observations in two-generation reproductive toxicity studies. On the other hand, NTP-CERHR (2003b) considers these findings as a sign of delay in maturation because they occurred in the absence of reduced foetal weight. It can be concluded, that there is no unanimous agreement on the relevance of these findings and in this evaluation these findings are considered not to be leading effects for a NOAEL but rather supportive to the selected NOAEL of 33 mg/kg bw/day based on reduced survival index of F2 pups in two-generation reproductive toxicity studies. The kidney findings may be suggestive of hydronephrosis after a longer exposure period covering the whole pregnancy and may compromise the health of neonatal pups.

ExxonMobil (2011a) stresses the importance of functional fertility measurements over indication of affected sperm parameters. However, humans produce less sperm as compared to rats and in cases of subfertility, the decrease in quality may be critical. Thus, the effects in sperm motility observed by Kwack and coworkers (2009) may represent a relevant effect even if not confirmed by mating trial at the same dose levels.

In the two-generation reproductive toxicity studies, there were findings in vaginal patency and PPS at dietary concentration of ≥ 0.4% but were not considered biologically significant by the authors (Huskha et al. 2001). However, in the second two-generation study with also higher statistical power, no change in vaginal patency but a delay in PPS (1.2 days) was observed in F2 males at 0.4% dietary concentration of DIDP. There were increases in absolute or relative or male reproductive organ weight and reductions in female reproductive organ weight in the first and/or second generation at 0.4 and/or 0.8% dietary concentration levels. The percentage of live births in F1 was decreased at high dose levels as well as the survival index on PND 4. For F2 generation, the offspring survival was reduced at all exposure levels on PND 1 and 4 in the first study and a NOAEL of 0.06% (~50 mg/kg bw/day) and a LOAEL of 0.2% (~165 mg/kg/bw/day) could be determined based on the second study. In addition, pup body weights were reduced on PND 0 at the high dose level and weight gain was reduced during the postnatal period but did not lead to a lower NOAEL. A NOAEL of 38 mg/kg bw/day can be set for the neonatal findings in F2 animals (reduced survival) using gestational feed consumption rate and a NOAEL of 33 mg/kg bw/day based on feed consumption of F1 males during premating period.

The authors of the two-generation reproductive toxicity study propose a value of 86 mg/kg bw/day as a theoretical no adverse effect level (NAEL) based on reduced F2 offspring survival at a dose of 108 mg/kg bw/day using a 95% lower boundary value. The dose of 108 mg/kg bw/day is derived using a modified probit model for the combined data with the number of live pups as a covariate. This approach was not considered in the EU Risk Assessment.

Summary of critical studies and effects

Information on DIDP includes guideline and GLP compliant studies which may not have examined the most sensitive period and/or endpoints. E.g., prenatal developmental toxicity studies indicate increase in skeletal and visceral variations but the exposure period has been shorter than in the relevant test guidelines today and not covering the whole critical time window for male masculinisation (up to GD 19). This may lead to underestimation of the NOAEL-values. A re-evaluation using a new statistical analysis of the incidences of skeletal and visceral variations in prenatal developmental toxicity studies has been conducted and CSTEE (2001b) has supported to use those for skeletal variations (Waterman et al. 1999) instead of higher NOAEL values presented in EU Risk Assessment but not for foetal variations based on

findings reported by Hellwig et al. (1997). In two-generation reproductive toxicity studies, additional parameters for endocrine disruption properties were included and for some parameters the statistical power was also increased by increasing the number of animals examined. However, new studies especially addressing potential effects leading to androgen deficiency during foetal period indicate that DIDP does not have such activity (Hannas et al. 2012). The studies considered critical and providing relevant NOAEL/LOAEL values are presented in Table 4.52.

Table 4.52 Critical studies and effects

Endpoint	Study	LOAEL (mg/kg bw/day) and critical effects	NOAEL (mg/kg bw/day)	Reference
Reproductive toxicity/ developmental toxicity	Two-generation reproductive toxicity study, dietary, rat	114-377[a] (0.2%)[b] Decreased survival index in F2	33 (0.06%)[b]	Hushka et al. (2001) [Exxon 2000]
	Two-generation reproductive toxicity study, dietary, rat	127-151[c] (0.2%, gestational) 166-377[c] (0.2%, lactational) Adverse effect on pup growth or survival index	38-44[c)] (0.06%, gestational) 52-114[c] (0.06%, lactational) (for developmental toxicity)	Huskha et al. (2001) [Exxon 2000]
	Prenatal developmental toxicity study	500/1000[d] Increased skeletal variations	100 [500[d]]	Waterman et al. (1999)
	Prenatal developmental toxicity study	200/1000[d] Increased skeletal and visceral variations	40 [200[d]]	Hellwig et al. (1997)
	Targeted developmental toxicity study	No LOAEL Reduction of ex vivo foetal testicular T production	1500	Hannas et al. (2012)

[a] The range of the lowest and highest substance intake in males and females
[b] Dietary concentration in parenthesis
[c] The range of the lowest and highest substance intake in P0 or P1 females as cited in NTP-CERHR Monograph (2003b)
[d] The higher values have been reported in EU Risk Assessment (EC 2003b). After a reanalysis NTP-CERHR (2003b) agreed with the lower values (also referred by US CPSC 2010b) which were also supported by CSTEE (2001b) and EFSA (2005b) for the Waterman et al. (1999) study but not for Hellwig et al. (1997) study

4.4.9.2.5 Conclusions

The most critical effect for DIDP is the decreased survival of F2 pups observed in both two-generation studies with rats. The LOAEL for this effect is 114 mg/kg bw/day leading to a NOAEL of 33 mg/kg bw/day (Hushka et al. 2001).

DIDP did not induce substantial anti-androgenic activity in available studies; in particular it did not reduce foetal testicular T levels or affect gene expression levels related to masculinisation during critical time window during development. However, DIDP was anti-androgenic in the Hershberger assay, with a lower potency than DEHP.

Thus, DIDP seems to have a different toxicological spectrum and/or potency regarding reproductive toxicity than several other phthalates, such as DINP, DEHP and DBP which potentially cause androgen deficiency during male development. The most sensitive reproductive effect for DIDP, reduced neonatal survival in the second generation, is observed only at high dose for e.g., DINP. The most sensitive effect for DINP, reduced foetal testicular T levels, is not observed with DIDP.

However, the ability to induce skeletal and visceral variations in prenatal developmental toxicity studies is equal for both DIDP and DINP. The developmental NOAEL for skeletal variations is 100 mg/kg bw/day and for foetal variations 40 mg/kg bw/day for DIDP.

4.4.10 Considerations on combined risk assessment of DINP and DIDP (and other phthalates) [34]

4.4.10.1.1 EU Risk Assessment

The EU Risk Assessments for DINP and DIDP (EC 2003a,b) considered exposure including all routes, pathways, and sources of exposure to DINP and DIDP separately (aggregated exposure[35]). The EU Risk Assessments did not consider combined risks from DINP and DIDP (or other phthalates). However, the EU Risk assessment for DINP did contain a combined risk assessment for the substances DINP-1 (CAS 68515-48-0) and DINP-2 (CAS 28553-12-0). In a similar manner combined risk assessment was carried out for two substances commonly termed DIDP (CAS No 68515-49-1 and 26761-40-0).

4.4.10.1.2 Other international assessments

EFSA 2005a,b
In the opinions concerning DINP and DIDP in food contact materials of 2005, the European Food Safety Authority (EFSA 2005a,b) noted that DINP and DIDP are "*mixtures that overlap chemically with each other and cannot analytically be distinguished clearly if present in a mixture*". For this reason it was proposed to establish a group restriction for DINP and DIDP for migration from food contact materials.

EFSA 2008
The opinion of the European Food Safety Authority (EFSA 2008) concerning methodologies for cumulative and synergistic risks from pesticides stated that "*ideally, risk assessments for chemicals, whether individually or combination, should consider all sources (e.g., plant protection products, veterinary drugs, human medicines), pathways (e.g., food, drinking water, residential, occupational) and routes (ingestion, dermal, inhalation) of exposure that could contribute materially to a person's total exposure*." The proposal is that grouping of compounds in cumulative assessment group (CAG) can be based e.g., on chemical structure, mechanism of pesticidal action, more refined criteria like common toxic effect or ultimate toxic mode of action. Thus, based on EFSA's proposal, e.g. anti-androgenicity could be seen as an appropriate criterion for grouping.

SCHER/SCENIHR/SCCS 2011
The Scientific Committee on Health and Environmental Risks (SCHER), the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), and the Scientific Committee on Consumer Safety (SCCS) issued a joint opinion "Toxicity and Assessment of Chemical Mixtures" (SCHER/SCENIHR/SCCS 2011). It is concluded that chemicals with common modes of action may act jointly leading to combined effects larger than the effects of each individual component. A major knowledge gap is the lack of exposure information and the limited number of chemicals for which there is sufficient information on their mode of action. There are no defined criteria how to characterise a mode of action for data-poor chemicals. Importantly, it is concluded that if no mode of action information is available, the dose/concentration addition method should be preferred over the independent action approach.

US EPA 2009
In the Action Plan US Environmental protection Agency (12/30/2009) concluded that available information including the data on cumulative effects of mixtures supports EPA's concern for potential human health hazard following exposure to phthalates. Several human studies describe associations of exposure of some phthalates with adverse reproductive outcomes and

[34] "Combined exposure" includes all routes, pathways, and sources of exposure to multiple chemicals (SCHER/SCENIHR/SCCS 2011).
[35] "Aggregated exposure" includes all routes, pathways, and sources of exposure to a given chemical (SCHER/SCENIHR/SCCS 2011).

developmental effects which are similar to those in the rat. The pathway for masculinisation in the foetus is highly conserved in all mammals. Thus, the reproductive developmental effects observed in rat studies are potentially relevant to humans. Recent studies in animals indicate that all mixtures of several active phthalates examined were cumulative for all endpoints. In these studies endpoints such as testosterone (T) production, foetal mortality, and male and female reproductive development later in life were examined. A major cumulative hazard assessment of phthalates is planned (CPSC/CHAP) with estimated complete report in 2012. In addition, EPA is conducting its own cumulative hazard assessment and screening certain phthalates for their endocrine disrupting properties.

NRC 2008
The National Research Council in his report on "Phthalates and cumulative risk assessment – The task ahead" from the Committee on the Health Risks of Phthalates notes that only three of the seven phthalates known to cause phthalate syndrome in rats have toxicity values following EPA's hierarchy (USA National Research Council 2008). The value for DEHP is the only one based on reproductive toxicity, the values for DEP and DMP are not based on reproductive toxicity. The phthalates listed to cause phthalate syndrome were: DBP, DIBP, BBP, Di-n-pentyl phthalate, DEHP, DCHP and DINP. For DBP, a proposed (acute, short-term, subchronic, chronic) oral reference dose is 0.3 mg/kg bw/day based on NOAEL of 30 mg/kg bw/day and an uncertainty factor of 100. The critical effect is a decrease in foetal T from rat developmental oral gavage study. For DMP EPA has developed a screening value of 0.1 mg/kg bw/day, a subchronic RfD with a uncertainty factor of 3000. It is based on a LOAEL associated with increased absolute and relative liver weight and decreased serum and testicular testosterone in weanling male rats. There were no adverse effects of DMP on reproductive outcomes or foetal development. The authors observed that several phthalate esters may have a common endpoint related to developmental and reproductive toxicity.

Industry

ExxonMobil 2011a
The following cites the comments of ExxonMobil to the CHAP/CPSC:
"There has been speculation or an assumption that the combination of phenomena associated with exposure to low molecular weight phthalates in laboratory rodents, "rat phthalate syndrome," can be extended to include high molecular weight phthalates and is relevant to humans. Proposed key events critical to the induction of the hypothesized "rat phthalate syndrome" include a decrease in fetal testosterone and insl3 (Gray and Foster, 2003; National Research Council, 2008). It is important to again emphasize that the mechanisms underlying these effects remained ill-defined. A decrease in fetal testosterone levels has been observed in two studies with DINP (Boberg et al. 2011; Borch et al. 2004); however, it appears to be a transient effect (Boberg et al. 2011).

Furthermore, there is a strong disconnection between this observed hormone change and the lack of predicted adverse phenotypes. The most sensitive phenotypic endpoints for the identification of "rat phthalate syndrome" are decreased anogenital distance and nipple retention (Carruthers and Foster, 2005; Gray et al. 2009; National Research Council, 2008; Wilson et al. 2007). While Boberg et al. (2011) reported a significant decrease in anogenital distance in males gestationally exposed to DINP (900 mg/kg/day) on post natal day 13 (approximately 6%), there was no difference between treated animals and controls on post natal day 90; the effect was transitory.

Additionally, there was no effect on nipple retention at either time point. No effects on AGD or nipple retention were observed in the definitive two-generation reproductive toxicity test on DIDP (Hushka et al. 2001). Additionally, both DINP and DIDP have been shown not to induce hypospadias, cryptorchidism, or alter the androgen sensitive tissues. Furthermore, in the definitive two-generation reproductive toxicity tests, DINP and DIDP had no effect on fertility

or developmental parameters. Overwhelmingly, the data clearly indicate that both DINP and DIDP do not induce the adverse effects hypothesized to be part of "rat phthalate syndrome". Therefore, the applicability of the "syndrome" for hazard assessment is not supported for either substance. Limited research suggests that DINP induces a reduction in fetal testosterone synthesis. However, use of decreased testosterone as the sentinel event predictive of adverse effects is problematic as DINP does not induce the effects consistent with the hallmarks of the "rat phthalate syndrome." In addition, species specific differences in sensitivity to phthalate induced disruption in testosterone are clear.

Recent and developing evidence indicates that humans are more similar to mice in that both seem to be refractory to phthalate induced testosterone reductions. Therefore, the relevance of this endpoint for human hazard or cumulative risk assessment is highly questionable. The two hazard index screens further support the previously discussed observation that all phthalates are not toxicologically equivalent. Even when an inappropriate endpoint is used, DINP has been shown to be a minimal contributor to any cumulative assessment on phthalates due to its low toxicity and very low exposure whereas LMW phthalates are seen to drive the risk associated with phthalate-induced effects on the male reproductive tract.

The published screening assessments described above based on the points of departure on various effects on the male reproductive tract, or effects presumed to presage male reproductive tract effects, which is an overly conservative approach. The point of departure used in both published assessments for DINP was a reduction in testosterone. Detailed analysis of the full manifestation of the "rat phthalate syndrome" indicates a multifactorial basis; therefore, a mere reliance on decreased testosterone synthesis as a predictive marker is likely simplistic and inaccurate for the purposes of estimating human risk. In addition, species specific differences in sensitivity to phthalate-induced disruption in testosterone are clear. Humans are more similar to mice in that both seem to be refractory to the androgen modulation. Therefore, the relevance of this endpoint for human hazard or cumulative risk assessment is questionable and should not be used to include DINP in a cumulative risk assessment based on male reproductive effects. DIDP has not and should not be included in a cumulative risk assessment based on any "rat phthalate syndrome" effects or a decrease in testosterone as there is no evidence to suggest DIDP induces any of these effects. Therefore, inclusion of DIDP based on any male reproductive tract effect (i.e. any "rat phthalate syndrome" effect) or a decrease in testosterone is unjustifiable.

Areas of limited evidence need to be highlighted and incorporated into any conclusions regarding cumulative risk. Cumulative risk assessment based on adverse health outcomes is a new area for risk assessors and screening methodologies help to characterize "worst case scenarios". However, these methodologies incorporate a number of untested assumptions including dose addition at human relevant doses, steady state exposure levels, and the absence of additional interactions which either increase the effects (synergy) or diminish the effects (antagonism) of a single chemical. In addition, factors such as the ability to adapt and compensate for as well as repair damage are largely ignored in current cumulative assessments. Without consideration of these data gaps, the characterization of risk is largely inaccurate and does not serve to inform rationale and scientific decisions regarding regulation of products."

Discussion on mode of action and combined risk assessment

It has been proposed to consider the simultaneous or sequential (combined) exposure of different substances in risk assessment of phthalates. The scientific basis for this would be that the substances share the same mode of action and/or affect the same endpoint. In case of phthalates and other substances suspected to affect masculinisation/feminisation and development, the anti-androgenicity (or androgen deficiency) has been proposed to be used as one criterion to allow building a group for combined (cumulative) risk assessment, but also

other endpoints have been considered. There are divergent opinions on what is needed to accept that substances fit into the same category. It is easily acceptable that the same mode of action through the same molecular mechanism can lead to cumulative effects and hence the necessity to consider such substances together. On the other hand, there are mechanisms that may saturate, like enzymes, transporters or receptors. However, in case of different molecular mechanism, even if the endpoint would be the same, grouping is not self-evident because different molecular mechanisms may in principle lead to opposite effects, which may overrule each other. Dose-addition models are generally proposed to be used for substances acting through a same mode of action/mechanisms.

Endocrine disrupters may affect human reproductive health, which is largely under hormonal control. Phthalates are one group of chemicals considered as potential endocrine disruptors, which may cause reproductive health problems as well as other health consequences if exposures are sufficiently high. The pathways of the critical action of androgens during foetal life are highly conserved and operate as they do in experimental animals (NRC 2008). In humans and all mammals the normal differentiation of the male reproductive tract during foetal period is androgen dependent. In humans, adverse effects related to androgen insufficiency are described in case of 5α-reductase deficiencies or alteration in AR structure and function (reviewed by Brinkmann 2001; Sultan et al. 2002). Disturbed androgen action causes male pseudohermaphroditism, and can results in a wide range of undervirilization from external feminization to infertility (NRC 2008).

Human Testicular Dysgenesis Syndrome (TDS)
In humans, a testicular dysgenesis syndrome (TDS; i.e. a failure of normal in utero development of the testis) has been associated with a number of human male reproductive deficits, including decreased semen parameters, increased incidence of cryptorchidism (non-descendent testes) and hypospadias (malformation of the penis in which the urethra does not open at the tip of the penis) and increased incidence of testicular (germ cell derived) cancer (Skakkebæk et al. 2001; Virtanen et al. 2005; Sharpe and Skakkebæk 2008). According to the hypothesis these effects share a common aetiological origin and follow a reduced androgen activity during the foetal critical window. Androgen insufficiency affects Sertoli and Leydig cells leading to impaired germ cell production, reproductive tract malformations and testicular cancer. Phthalate exposure has been associated with reduced anogenital distance (AGD) in humans (Swan et al. 2005; Swan 2006; 2008) and lower androgen levels in male newborn due to phthalate exposure (Main et al. 2006). Limited information from in vitro and xenotransplantation studies indicates that human foetal testis may be sensitive to certain phthalates; e.g. MEHP reduced the number of germ cells by inducing apoptosis in vitro (Lambrot et al. 2009) and DBP increased MNGs in xenotransplanted foetal testis (Heger et al. 2012).

The rat is a good animal model for this syndrome because it is possible to induce all the elements of testicular dysgenesis syndrome, except testicular germ cell cancers, by exposing pregnant rats to chemicals causing androgen insufficiency. The parallel syndrome with the hypothesized human testicular dysgenesis syndrome in rats is called "the phthalate syndrome" (e.g., Fisher et al. 2003; Schumacher et al. 2008).

Rat phthalate syndrome
In rats, the effects due to androgen insufficiency comprises non-descendent testes (cryptorchidism), malformations of external genitals (similar to hypospadias), poor semen quality and malformations of other sex organs (epididymides, vas deferens, seminal vesicles, prostate) and testicular injury together with permanent changes (feminization) in the retention of nipples/areolae (sexually dimorphic structures in rodents) and demasculinization of the growth of the perineum resulting in a reduced ADG. This spectrum of effects in rat is called the "phthalate syndrome" (e.g., Foster 2006; NRC 2008, Hannas et al. 2011b; Kortenkamp et al. 2011). The response is a continuum of manifestation of low incidences of mild changes at lower doses to more severe malformations and high incidences at higher doses. For instance,

AGD and nipple/areolae retention may be observed at lower doses whereas high incidences of reproductive tract malformations are seen at higher doses. The general potency of response of the three most examined phthalates on reproductive development is DEHP > DBP > BBP (Foster 2006). DBP has also been shown to cause testicular Leydig cell adenomas of developmental origin (Mylchreest et al. 1999; 2000).

The associated effects to the phthalate syndrome results from a combination of 1) abnormal Leydig cell aggregation (Mahood et al. 2005; Mylchreest et al. 2002) and gonocyte proliferation (Mylchreest et al. 2002), 2) a decrease in insulin-like hormone (Insl3) levels (McMinnell et al. 2005; Wilson et al. 2004), 3) disrupted foetal testicular testosterone (T) production (Parks et al. 2000; Scott et al. 2009, Welsh et al. 2008), and associated alterations in genes involved in androgen synthesis (Hannas et al. 2012; Shultz et al. 2001; Thompson et al. 2004).

Mode of action of the Phthalate Syndrome
The precise mechanism by which phthalates exert their toxicity is not clear. Several modes of action have been hypothesized one being anti-androgenicity or more precisely androgen deficiency. Androgen deficiency-related developmental effects may be caused by reducing androgen receptor-mediated effects (AR antagonisms), effects on T synthesis, and/or inhibiting dihydrotestosterone (DHT) formation from T (5α-reductase inhibitors). In addition, aromatase inhibitors reduce the conversion of T to oestrogen which is critical for brain sexual dimorphic development. Masculinisation of the brain occurs late in gestation in primates and perinatally in rodents (Arnold and Gorski 1984; McCarthy and; Konkle 2005; as cited Scott et al. 2009). Many of the phthalates, but not all, seem to reduce the foetal testicular T production (e.g., Hannas et al. 2011a and b; 2012), but other mechanisms are also involved.

Induction of apoptosis of germ cells without an effect on testicular T production in human foetal testis in vitro was reported by Lambrot et al. (2009). Flutamide, which also disrupts androgen action is different in effects from DBP (MacLeod et al. 2010). The responses of reduced androgen levels and AR antagonism would be similar and not likely always to be separated from each other. The responses after reduced androgen activity depends whether the effects of DHT or T are compromised although there are overlapping effects. AR antagonists do not suppress T synthesis and lead to weaker effects on T dependent tissue development (such as epididymis). Malformations dependent on DHT, such as hypospadias, are less frequent after exposure to substances which primarily affect T production (NRC 2008). Thus, depending on the mechanism(s) by which a chemical suppresses androgen activity, the spectrum of effects may differ. In addition, combinations of variety of other modes of action broaden the spectrum of effects. The chemicals blocking the AR or interfering with the conversion of T to DHT seem to have more pronounced effects on genital malformations, retained nipples and decreased AGD as compared to those chemicals that lower the T levels by interfering with the uptake of the steroid hormone precursors.

The onset of undisturbed gametogenesis and steroidogenesis is fundamental for the reproduction in the adult as discussed by Lambrot et al. (2009). The number of germ cells formed during foetal life is essential for fertility (indicated in germ-cell deficient mutant mouse and mice lacking the proliferation of germ cell zone; Lu and Bishop 2003). Androgens and insulin-like factor 3 (Insl3) produced by foetal Leydig cells control masculinisation of the reproductive tract and genitalia (Jost et al. 1973; Kubata et al. 2002). Leydig cells may be the primary target of phthalates because high doses reduce steroidogenesis and Insl3 mRNA (e.g., Hannas et al. 2012). Leydig cells produce T that is necessary for Sertoli cells to support the spermatogenesis. Leydig cells produce also Insl3 that facilitates for the first phase of testes descent (Foster 2006). Decreased androgen and Insl3 synthesis in Leydig cells lead to abnormal cellular differentiation, such as multinucleated gonocytes (MNGs), abnormal Sertoli cell-gonocyte contact and apparent foetal Leydig cell hyperplasia and Leydig cell aggregation (reviewed by e.g., Howdeshell et al. 2008b).

Leydig cells
Foetal and adult Leydig cells arise from distinct lineages and differ in their structure and function. The foetal Leydig cells are responsible for foetal and neonatal masculinisation and the adult Leydig cells are required for pubertal masculinisation (reviewed by Scott et al. 2009).

The effect on Leydig cells has been proposed to be caused by interactions between the phthalate monoesters and members of the peroxisome proliferator-activated receptor (PPAR) family of transcription factors, since they are expressed in adult Leydig cells. However, it has been shown that PPARα and PPARγ pathways are not involved in phthalates mode of action in foetal testis (Hannas et al. 2012). Hannas et al. (2012) did not specifically test the effects of PPARβ agonist on foetal T production, but they conclude that there is currently no evidence to suggest that PPARβ is involved in testicular toxicity.

The morphological changes in Leydig cells (foetal Leydig cell hyperplasia or large aggregates of foetal Leydig cells at the end of gestation) are preceded by significant decrease in foetal T production (Parks et al. 2000; Shultz et al. 2001; Mylchreest et al. 2002; Lehmann et al. 2004). The clustering may be due to abnormal cell migration (reviewed by Howdeshell et al. 2008b). Decreased T production by Leydig cells may reduce the proliferation of Sertoli cells leading to lower number of Sertoli cells available at the end of the masculinisation. Proliferation of Sertoli cells is driven by T primarily during the late phase of pregnancy (reviewed by Scott et al. 2009). Because Sertoli cells support germ cell production, this may lead to a decrease in sperm counts.

Disruption of seminiferous cord formation and germ cell development leads to the appearance of large MNGs in late gestation (Mylchreest et al. 2002; Barlow and Foster 2003; Kleymenova et al. 2005). The MNGs disappear postnatally but the disturbance in gonocyte proliferation and delayed germ cell maturation may lead to reduced number of spermatogonia and sperm count (Sharpe 2008). Phthalate induced MNGs may be caused by disrupted interactions of the gonocytes and Sertoli cells (Ferrara et al. 2006; Klymenova et al. 2005). This may be a separate mechanism than delayed early gonocyte development (as induced by DBP) and separate from reduced T synthesis (reviewed by Howdeshell et al. 2008b).

Abnormalities in foetal gonocyte development are precursor events to germ cell cancer in humans (Sharpe and Skakkebæk 2008). Rats do not show germ cell tumors but develop another type of testicular tumors after phthalate exposure, interstitial Leydig cell adenomas (Mylchreest et al. 1999, 2000). Leydig cell adenomas are fairly common in humans but called micronodules (Holm et al. 2003 as cited in Foster 2006).

T synthesis
The initiation mechanism of foetal testicular T production is unclear because stimulatory hormone release (LH) does not start until ED 17.5-18 in rats (Aubert et al. 1985; Livera et al. 2006) following a foetal T peak. Autonomous or paracrine regulation may play a critical role during embryonic days of 15.5 – 17.5 (Scott et al. 2009).

Reduced foetal testicular T levels may be caused by low uptake of steroid hormone precursors into foetal Leydig cells, but other mechanisms are also possible. Low testicular androgen synthesis and T levels at critical time points disturb the development of the Wolffian duct system into the vas deference, epididymis and seminal vesicles and may cause malformations of internal reproductive organs such as testes and epididymides (Barlow and Foster 2003). Lower T concentrations also affect the development of DHT-dependent tissues such as the prostate and external genitalia.

Phthalate exposure may change expression of genes related to cholesterol transport and steroidogenesis (see Table 4.53 for an example). The genes affected include steroid acute regulator protein (StAR), side chain cleavage enzyme (P450scc), Cyp17 (P450c17), and 3β-hydroxysteroid dehydrogenase (3βHSD). However, decrease in T levels precedes the decrease

in these genes. Thus, as a primary effect, phthalates may decrease cholesterol availability for steroidogenesis. No effects have been observed on mRNA for 17β-hydroxysteroid dehydrogenase (17βHSD), FSH or LH receptors (Barlow et al 2003; as cited in Foster 2006). Recent studies support the hypothesis that phthalate exposure reduces testicular T production by interfering with cholesterol regulation (Hannas et al. 2012). Anti-androgenic phthalates reduce StAR, scavenger receptor class B type 1 (Scarb1; SRB1), and Dhcr7 gene expression (Hannas et al. 2012; Plummer et al. 2007). SRB1 and StAR are involved in transport of cholesterol into the cell and mitochondria, respectively (Plummer et al. 2007; Thompson et al. 2004), and Dhcr7 mediates the final step in cholesterol formation. Steroidogenic factor 1 (SF1, NR5A1) regulates expression of downstream steroidogenic enzymes, and also regulates expression of SRB1 and HMG-CoA synthase and HMG-CoA reductase which converts intracellular C2-acetyl units to cholesterol. In addition, it has a critical role in the development of the adrenal gland and testes in both mice and human (reviewed by Scott et al. 2009).

AR protein reduction (no change in mRNA) has been associated with failure of the epididymal ducts to coil (Mylchreest et al. 2002; Barlow and Foster 2003). Further studies are needed on the molecular mechanisms to understand how phthalates affects steroidogenesis.

Table 4.53 Effects of DBP on T biosynthesis on gestation day (GD) 19 in foetal Leydig cell as an example of anti-androgenicity. The percentages are relative gene expression ratios (% of controls) after exposure to 500 mg/kg bw/day of DBP for mRNA expression of each protein, or the reduction in overall T production, asterisks indicates significantly different from control p< 0.05. Based on Barlow et al. 2003; as cited in Foster 2006, Hannas et al. 2012).

Target	Location/Function	Magnitude of effect (% of control)
HDL cholesteryl ester	Starting material for cholesterol synthesis /extracellular	-
acetate	Starting material for cholesterol synthesis/intracellular	-
Dhcr7 (7-dehydrocholesterol reductase)	Enzyme mediating the final step in cholesterol production	
Cholesterol	Starting material for steroid hormone synthesis	Not measured
SRB1	Leydig cell membrane, transports cholesterol into the cell	41%*
StAR	Carries cholesterol from outer mitochondrial membrane to the inner mitochondrial membrane	34%*
P450scc (Cyp11a)	Converts cholesterol to pregnenolone	5%*
3βHSD	Converts pregnenolone to progesterone at smooth endopasmic reticulum	52%*
Cyp17a1	Converts progesterone to 17-α hydroxyprogesterone and androstenedione	59%*
17βHSD	Converts androstenedione to T	142%*
T	End product of the steroidogenesis	10%
Insl3	Leydig cell gene product important to the initial stages of testicular descent	25%*

It has been shown that although the T concentration in the foetal testes returns to normal after excretion of the metabolites of DBP, the induced malformations persist into adulthood following in utero exposure to 500 mg/kg bw/day (Thompson et al. 2004; Barlow et al. 2004; Barlow and Foster 2003).

Role of dihydrotestosterone (DHT)
Low testicular T levels lead also to low DHT levels as DHT is converted from T by 5α-reductase. DHT is a more potent androgen than T and is essential for development of prostate and external genitalia (Foster 2006). Low DHT levels may cause hypospadias and in rats also smaller AGD and retained nipples. The growth of the perineum to produce the longer AGD in males as compared to females is also dependent on DHT (Foster 2006). AGD is a sexually dimorphic trait in laboratory rodents and humans; rodent males exhibit a distance 2 – 2.5 fold greater than females. Androgens are responsible for normal AGD elongation in neonatal males (Clemens et al. 1978; Hotchkiss et al. 2007; Imperato-McGinley et al. 1985, 1986 (as cited in Foster 2006)). Androgen receptor antagonists induce a decrease in AGD in males. DHT also induces the normal apoptosis of nipple anlagen in males resulting in the lack of nipples.

Nipple retention in males is considered as a sensitive endpoint for androgen deficiency (anti-androgenic effect and reduction in foetal T production). The development of the rodent nipple is sexually dimorphic (Kratochwil 1971; Kratochwil and Schwartz 1976) but begins similarly in both sexes in utero. In the developing rodent males, DHT produced locally from foetal T causes regression of the nipple anlagen (Imperato-McGinley et al. 1986; Kratochwil 1977, 1986). The reduction in foetal testicular T production has been associated to disrupted regression and leading to transient or even permanent nipples/areolae in male rats (Foster 2006).

Insl3
Reduced levels of Insl3 are associated with gubernacular defects and cryptorchidism, a failure of testicular descent into the scrotum (Adham et al. 2000; Nef and Parada 1999; Zimmermann et al. 1999). Insl3 is produced by Leydig cells and it induces the gubernacular cord to differentiate and mature helping testes descent from the kidney area to the inguinal region, but may not be essential for spermatogenesis as such (Zimmermann et al. 1999). The latter phase of testis descent is androgen dependent. Androgens regress the cranial suspensory ligament. In the absence of Insl3, the gubernacular cord involutes and in the absence of T the cranial suspensory ligaments develops as in the untreated female rodent foetus (Howdeshell et al. 2008a). Decrease in Insl3 gene expression may be related to the increased incidence of cryptorchidism after foetal exposure to phthalates (Foster 2006). The Insl3 gene is associated with Leydig cell differentiation and T levels which peak in foetal rat testes, which decrease after birth, rise again at puberty but decrease in old animals (Paust et al. 2002).

Disruption of Insl3 action causes complete failure of testicular descent such as in knockout mice (Nef and Parada 1999; Adham et al 2000; Nef et al. 2000). In humans, polymorphisms of the Insl3 receptor have been reported to be associated with cryptorchidism (Ivell and Hartung 2003). DIBP, DEHP and DIHP are roughly equipotent in reducing foetal testicular T production and Cyp11a, StAR and Insl3 gene expression, similar to the potencies observed for reproductive malformation endpoints (Hannas et al. 2011b). These phthalates are more potent than DINP but less potent than DPeP in this respect. There is, however, only quantitative and not qualitative difference between DINP and DIBP, DIHP, DEHP and DPEP (Hannas et al. 2011b).

Litter size and offspring mortality
Several phthalates (DIBP, DEHP, DBP) increases postimplantation foetal loss and reduced number of live foetuses at birth at high doses (750-1500 mg/kg bw/day) when administered during gestation (Saillenfait et al. 2006; Moore et al. 2001; Ema et al. 2000; Mylchreest et al. 1998). However, exposure period seems to affect the results and foetal mortality is not always induced (Gray et al. 2000; Parks et al. 2000). Exposure to DBP by oral gavage from weaning through life reduced litter size at similar high dose levels (500-1000 mg/kg bw/day) that

caused a reduction in serum progesterone levels in dams on GD 13 in rats (Gray et al. 2006). This indicates that affected pregnancy maintenance may be associated with disruption of maternal ovarian steroidogenesis at midpregnancy.

Offspring mortality observed at similar dose levels that decrease AGD (or postnatal malformations) after exposure to DPEP at 100 mg/kg bw/day is not a common finding with phthalates (Hannas et al. 2011a). A NOAEL of 33 mg/kg bw/day was determined for reduced offspring viability for DPEP. This indicates that DPEP acts also via other mechanisms than anti-androgenicity too (Hannas et al. 2011a). For DIDP reduced offspring viability without anti-androgenic effects has been reported at 117 mg/kg bw/day with a NOAEL of 33 mg/kg bw/day for F2 offspring in a two-generation reproductive toxicity study (Hushka et al. 2001).

Critical time period for male reproductive development
The critical time period for inducing malformations seems to be GDs 15-19 in rats. The foetal testis is more sensitive than pubertal or adult testis to phthalate exposure (Gray et al. 2000; Mylchreest et al. 2000; Lehmann et al. 2004). In utero exposure of rats to certain phthalate esters during the period of masculinisation (GD 14-18) causes malformations in reproductive tissues of male offspring by reducing critical hormones during this period (Foster 2006, Gray et al. 2000; Howdeshell et al. 2008b; Hannas et al. 2011a, b; 2012). The T production begins on GD 14.5 to 15.5 in the rat (Habert and Picon 1984; Warren et al. 1972, as cited in Scott et al 2009) and starts a critical time period of "masculinization programming window" for androgen influence necessary for morphological differentiation of the male genitalia (Scott et al. 2009).

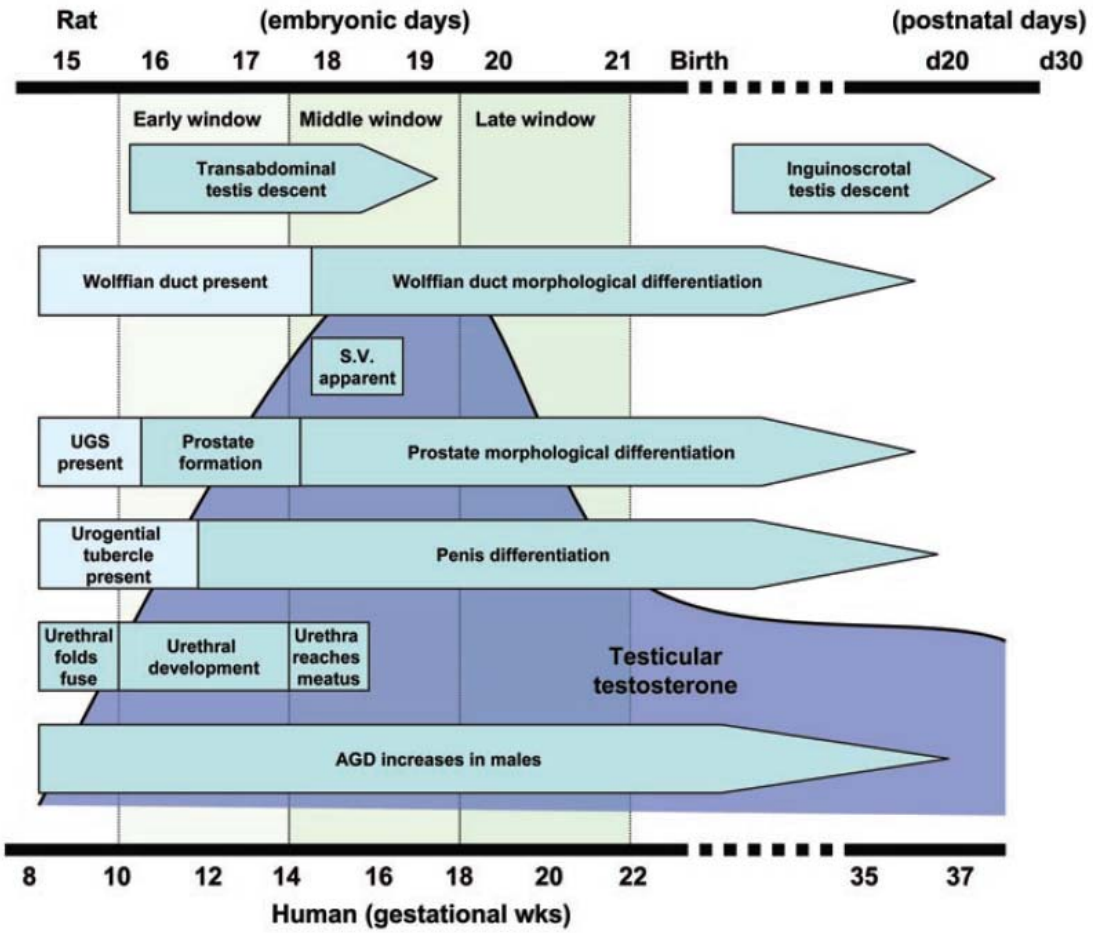


Figure 4.8 Comparison of timing of male reproductive tract development in the rat and human in relation to foetal testicular testosterone concentration. The curve indicates the changes in foetal testicular testosterone concentrations which peaks at the middle time window of the masculinisation programming window occurring between ED15.5 and ED 19.5 in the rat. S.V., seminal vesicles. Source: Welsh et al. 2008 Reproduced with permission; copyright 2008, Clinical Investigation.

The peak of the testicular T production begins on GD 14.5 to 15.5 (ED 15) in the rat and peaks around ED 19 in rats whereas in humans the T production begins after gestation week 8 and peaks on gestation week 17 (See Figure 1 above; Welsh et al. 2008). After the peak T levels stay at a lower level from ED 21 in the rat and gestational week (GW) 22 in human. The transabdominal testis descent, prostate formation, beginning of penis differentiation, urethral development, and beginning of AGD increase are developmental events during the early window of the critical period (ED 15.5-17.5 in the rat; GWs 10-14 in humans). During the middle window of the T peak (ED 17.5-19.5 in the rat; GWs 14-18 in human), the transabdominal testis descent continues, Wolffian duct morphological differentiation begins, seminal vehicles appear, prostate morphological differentiation begins, penis differentiation continues, urethra reaches meatus, and AGD continues to increase. During the late window of the T peak, the T levels decrease (ED 19.5-21 in the rat; GWs 18-22), Wolffian duct morphological differentiation continues, as well as prostate morphological differentiation, penis differentiation, and increase in AGD continues. All these developmental events continue after the late window (postnatal period in the rat) and in addition, inguinoscrotal testis descent begins.

It has been shown that without sufficient T action during the masculinisation programming window disorders such as lack of formation of penis, malformation of penis, cryptorchidism, underdeveloped prostate and reduced anogenital distance and penis length may follow. If the androgen action is blocked after the masculinisation window, the masculinisation process is not affected but it may lead to a shorter penis or reduced testis size (reviewed by Scott et al. 2009).

Phthalates can cross the placenta (Fennell et al. 2004) and have been measured in amniotic fluid in human studies (Silva et al. 2004; as cited in NRC 2008). Phthalates have also been measured in breast milk (Parmar et al. 1985; Dostal et al. 1987; as cited in NRC 2008). Studies of urine samples of pregnant women indicate that foetuses may also be exposed to phthalates (Adibi et al. 2008; Wolff et al. 2008). Foetuses and small children have different metabolic capacities than adults, and urinary concentrations of oxidized metabolites are more prevalent in children than in adults (Koch et al. 2004; CDC 2005; Koch et al. 2005a; as cited in NRC 2008). The lack of oxidized metabolites in amniotic fluid may be a consequence of immature expression of some enzymes by foetuses. Concentrations in maternal and foetal serum are similar to those in amniotic fluid, and all three compartments have lower concentrations than those in urine (Silva et al. 2004; Calafat et al. 2006; Silva et al. 2007b; as cited in NRC 2008).

Sensitivity of different species/strains
Phthalates have affected testis or indicated adverse reproductive outcomes in several species after in utero or pubertal exposure: rats, mice, hamsters, ferrets, guinea pigs, rabbits, fish, and frogs (Gray et al. 1982; 2000; Ward et al. 1998; Lake et al. 1976; Higuchi et al. 1999; 2003; Patyna et al. 1999). To reveal the effects of exposure during perinatal or pubertal period is necessary and sufficient number of animals should be evaluated to get enough statistical power to detect effects with low incidence.

Rat was shown to be the most sensitive species in inducing of testicular toxicity of DBP and DEHP (Gray et al. 1982; as cited in NRC 2008). Guinea pig showed approximately similar sensitivity, mouse being much less sensitive and hamster resistant. The reason for these differences was suggested mainly to be pharmacokinetic but there may be also other

contributing factors. In utero exposure to DBP results in MNG formation and an increase in seminiferous tubule diameter both in rats and mice, however, only rats exhibit suppression of testicular steroidogenesis and T synthesis and Insl3 production (Gaido et al. 2007). This may be due to a species specific effect of DBP exposure on foetal Leydig cell SREBP2 activity; however the underlying mechanism is unknown (Johnson et al. 2011).

Wilson et al. (2007) reported that DEHP administered at 750 mg/kg bw/day to dams during GD 14-18 resulted in a higher rate of epididymal lesions (an androgen-dependent tissue) in SD rats than in Wistar rat offspring (67% in SD vs 8% in Wistar), whereas the same exposure caused a higher incidence of gubernaculums lesions (an Insl3-dependent tissue) in Wistar than SD rat offspring (0% in SD vs 64% in Wistar). The phenotypic differences in epididymal and gubernaculums development are likely due to tissue specific strain differences in the androgen and Insl3 signalling pathways rather than differential effects of DEHP on foetal T production and Leydig cell Insl3 gene expression (Hannas et al. 2012). There was only a small strain effect on StAR and Cyp11 expression.

Similarly, different strain sensitivity has been reported between SD and Long-Evans (LE) rats after pubertal exposure to DEHP (Noriega et al. 2009). The onset of puberty and reduction of androgen-dependent tissue weights were of greater magnitude in LE than SD rats at 300 and 900 mg/kg bw/day. On the contrary, alterations in testis histopathology were more severe in SD than in LE rat at 300 and 900 mg/kg bw/day but still qualitatively similar.

The rat is generally considered as a good model of human male reproductive toxicity (NRC 2008). However, as reviewed by Scott et al. (2009), there are also notable differences between rat and human. One difference between rat and humans is the principle form of circulating cholesterol, starting material for T synthesis. HDL is the primary source in rats and is taken up by the SRB1/HDL receptor on the Leydig cells. LDL is the primary source in human and is taken up by the LDL receptor on the Leydig cells. Cholesterol can be also synthesised de novo from acetate or conversion of intracellular C2-acetyl units. Due to several available sources to obtain cholesterol, blockade of one of these routes tends to be without major effect as also indicated by knockout of SRB1 in mice.

For steroidogenesis, free cholesterol has to be transported from the outer to inner mitochondrial membrane which is facilitated by StAR. At inner mitochondrial membrane Cyp11a enzyme starts the steroidogenic pathway. Steroidogenesis is fully dependent on availability of cholesterol at inner mitochondrial membrane and, thus, absence of StAR protein during the masculinisation programming results in wholly female genitalia (as reviewed by Scott et al. 2009). SF1 and LH-mediated activation of cAMP-dependent pathways regulate StAR (in human). In addition, ERK1/2 and the translocator protein TSPO are also involved in regulating StAR (reviewed by Scott et al. 2009).

Several enzymes are involved in synthesis of T from cholesterol). These can be divided into two categories; the cytochrome P450 enzymes and the HSD enzymes. Each P450 gene is the product of a single gene but HSD enzymes have several isoforms, each the product of a distinct gene. The first step in steroidogenesis, conversion of cholesterol to pregnenolone by Cyp11a is the rate-limiting step and homozygotes totally lacking Cyp11a do not survive. The rest reactions in steroidogenesis take place in endoplasmic reticulum. There are species differences in regulation of Cyp11a activity indicated between rats and mice postnatally, mouse Cyp11a activity likely being more susceptible to perturbation (reviewed by Scott et al. 2009). In neonatal rat, hCG stimulate Cyp17 mRNA levels but this is down-regulated in adults. It is thought that this is one way to ensure adequate T synthesis in foetal Leydig cells for masculinisation in rodents and human (reviewed by Scott et al. 2009).

Pregnenolone will be further converted via Δ4 or Δ5 pathways to androstenedione, the precursor of T. The Δ4 pathway occurs via progesterone and its intermediate 17α-hydroxyprogesterone while the Δ5 pathway occurs via pregnenolone and its intermediates,

17α-hydroxypregnenolone and DHEA. The preference for either the Δ4 or Δ5 pathway may be both species- and age-dependent (reviewed by Scott et al. 2009). Species differences in preferred pathway are likely to depend upon relative substrate affinity of the Cyp17 enzyme. In human the Δ5 pathway predominates whereas in the rat, the Δ4 pathway is preferred. The rat Cyp17 readily cleavages both the Δ4 and Δ5 C21 steroids but human Cyp17 has less 17,20-lyase activity than rat Cyp17. However, there is flexibility in the pathways that can be used. As noted in Table 4.53, DBP inhibits most Cyp11A, the rate limiting step similar to human and rats, converting cholesterol to pregnenolone and the other measured enzymes. On the other hand, 3βHSD activity, which is needed anyway whether Δ4 or Δ5 route is preferred, is HSD3B2 enzyme in humans but HSD3B1 enzyme in all other species to date (reviewed in Scott et al. 2009). DBP inhibits also this activity in rats. The type of the 17βHSD involved in foetal Leydig cell is likely the same as in the adult cells, type 3, although a possible role of type 5 17βHSD cannot be dismissed.

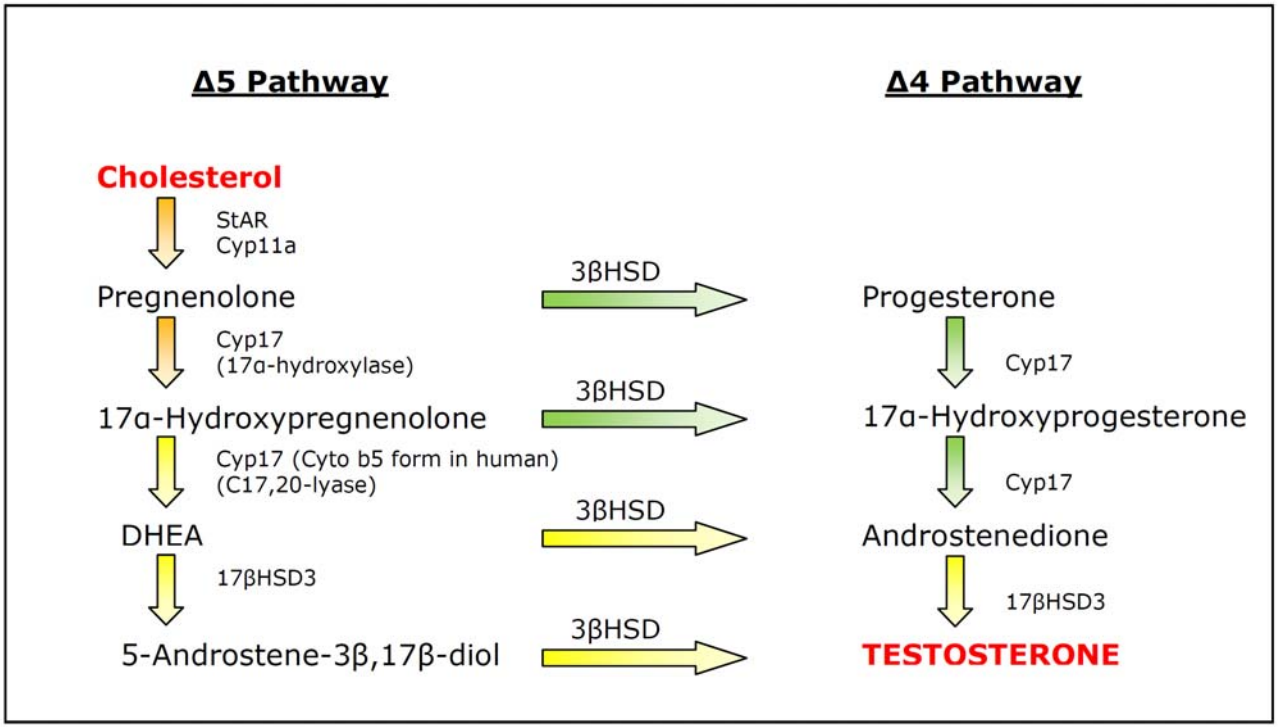


Figure 4.9 Main components of the steroidogenic pathway in foetal Leydig cell. The yellow colour shows the preferred pathways in human, the green in the rat and orange in both species. Cyp17 17,20-lyase activity is weak in human when 17α-hydroxyprogesterone is the substrate. Note the flexibility in the pathways. Modified from Scott et al. (2009).

In foetuses, the control of steroidogenesis differs (reviewed by Scott et al. 2009). The T production during the critical time window of masculinisation (GD 15.5-17.5) is largely LH-independent in rats. T production is LH independent until at least GD 19 of foetal life (reviewed by Scott et al. 2009). The negative feedback mechanisms triggering increased LH secretion in response to reduced T production does not function before (Huhtaniemi and Toppari 1995). Thus, suppression of steroidogenesis may not lead to compensatory change by LH at least until end of gestation. The regulation of steroidogenesis in the foetal rat is either autonomous or paracrine before the onset of LH secretion even functional LH receptors are available at ED 14.5 (reviewed by Scott et al. 2009). The identities of putative paracrine factors that stimulate steroidogenesis in the rat are unclear, several have been proposed, and e.g., retinoic acid which stimulates T production in human foetal testis, inhibits steroidogenesis in rat testis (reviewed by Scott et al. 2009).

Human foetal T production begins around gestational week 8 and for most of the foetal life the human testis is exposed to chorionic gonadotropin (hCG), a hormone with similar effect to that of LH but not produced by rodents, that might potentially override or compensate for inhibiting effects of MBP (or other phthalates) on steroidogenesis (Lambrot et al. 2007). By gestation week 12, hCG begins to decline and LH levels are seen to rise and peak around week 16. However, hCG is two to six times more potent than LH on a weight basis and may continue to stimulate steroidogenesis at weeks 15-20 (reviewed by Scott et al. 2009). LH-mediated drive is essential for T production in human testis although at weeks 7-10 it may be partially or completely LH/hCG-independent (the LH receptor is first reported around week 10 in human testis with maximal binding capacity between weeks 15-20). The available data indicate that steroidogenesis in human testis could be regulated by hCG, LH, or paracrine factors such as retinoic acid and that humans and rodents all seem to masculinise normally in the absence of a pituitary or pituitary LH (reviewed by Scott et al. 2009).

It is inconclusive whether the feedback mechanisms of the HPT axis are established in the first 6 months in boys (Pierik et al. 2009). In a study on the regulation mechanism of the hypothalamus-pituitary-testis (HPT) axis in boys less than 6 month, the inhibin B – FSH feedback loop seem to be functioning. There are indications that T production in the neonatal rat, human and marmoset is completely LH dependent (Grumbach 2005; Habert and Picon 1982; Mann and Fraser 1996). As a consequence, suppression of steroidogenesis in neonatals could lead to negative feedback and a compensatory increase in LH secretion and enhanced steroidogenesis (Lambrot et al. 2007). In boys with cryptorchidism, disturbed Leydig cell function as indicated by lower T and T not bound to steroid hormone-binding globulin were found (Pierik et al. 2009). However, the exact details of foetal T regulation in rats and humans have not yet been clarified.

In marmosets, a nonhuman primate, no testicular effects were observed after 13 weeks exposure to DEHP during adulthood (Kurata et al. 1998). There is one study reporting effects on developing Leydig cells and reduced T concentration in the neonatal marmoset (Hallmark et al. 2007). The relevance of the marmoset model has been questioned because the endocrine system including the testes in marmosets has some unique features that have not been observed in rodents, Old Word primates, and humans (Li et al. 2005). Most of the studies of nonhuman primates do not show effects of phthalates on adult testicular function but there are several studies suggesting an association of phthalate exposure and male reproductive effects in human populations (reviewed in Matsumoto et al. 2008).

There is only very limited data addressing effects by phthalates on the human foetal Leydig cells or suppression of T production. MEHP (a metabolite from DEHP) had no effect on basal or LH-stimulated T and did not affect proliferation and apoptosis of Sertoli cells in human foetal testes (from foetuses during 7-12 weeks of pregnancy; Lambrot et al. 2009). The mRNA expression of anti-Müllerian hormone was reduced as well as the number of germ cells was also reduced (via increased apoptosis). After 3 days of treatment with 0.1 mM MEHP reduced the number of germ cells by 40% in cultures human testis due to increase in their apoptosis (Lambrot et al. 2009). MBP had no effect on human foetal testis explants culture (Hallmark et al. 2007). Intratesticular T levels, P450scc expression as well as Leydig cell aggregation were measured. However, the authors questioned the utility and validity of the in vitro system because the in vivo effects of DBP/MBP were not reproduced in vitro in the rat. Hallmark et al. (2007) concluded that the findings suggested that DBP/MBP suppress steroidogenesis by foetal-type Leydig cells in primates as in rodents, but this cannot be studied in vitro. In newborn marmosets, a single dose of 500 mg/kg bw/day MBD suppressed blood T level 5 hours later. A treatment for 14 days resulted in increased Leydig cell volume per testis consistent with MBP induced inhibition of steroidogenesis followed by compensatory Leydig cell hyperplasia/hypertrophy (Hallmark et al. 2007). Newborn male marmosets exhibit a neonatal T rise comparable to human males (Grumbach 2005; Mann and Fraser 1996; McKinnell et al. 2001) and the Leydig cells producing T are thought to be foetal-type Leydig cells rather than adult-type Leydig cells (Huhtaniemi and Toppari 1995).

There are indications that in rodents, the androgen pathway is not involved in germ cell number. Phthalates induced MNG in rodents after DBP gavage only from 19.5 post conception (Ferrara et al. 2006). However, this is in contrary to the findings with DINP where MNGs were observed after exposure on GD 12-19 (Clewell et al. 2011a).

Human foetal testes have also been xenotransplanted within the renal subcapsular space or under the dorsal skin of a nude rat host followed by up to three days exposure to DBP (Heger et al. 2012) or 4 or 21 days to DBP or MBP (Mitchell et al. 2012). Results indicate that the steroidogenic gene expression was highly variable but without statistical significant change (testosterone production of the foetal testes was not measured; Heger et al. 2012) and reduced serum testosterone and seminal vesicle weight without statistical significancy measured from limited number (3-4) of host mice dosed with MBP (Mitchell et al. 2012). A rapid increase in MNGs per total number of germ cells was reported (Heger et al. 2012). In conclusion, limited data on effects on human foetal testes suggest effects such as reduction in number of germ cells and increase in MNGs but no clear effect on T biosynthesis.

A significant reduction in foetal T production has been considered as a key event in phthalate induced effects in male reproductive tract. There are some differences between human and rat steroidogenesis, but the processes underlying male development are remarkably similar and, thus, rats are seen as an appropriate model. The critical enzymes involved in steroidogenesis are identical in rats and humans and all mammals are believed to have parallel activation mechanisms for androgen dependent processes. It is possible, thus, that a sufficient human exposure may cause similar anti-androgenic effects in human foetuses as those observed in animals.

Dose-response curve for decrease in male ADG on PND 2 parallels the dose-response curve of reduced foetal testicular T production. A reduction in prenatal T production occurs at lower doses than a reduction in AGD in neonatal male rat. This has been shown e.g., with DPEP, the so far most potent phthalate inducing androgen deficiency related effects (Hannas et al. 2011a). Based on the results with DPEP, a reduction of foetal testicular T production by 80% correlated with a 20% reduction in ADG at the dose level of 100 mg/kg bw/day, which already affected pup viability (from implantation to PND2)(Hannas et al. 2011a). The study by Hannas et al. (2011a) support the hypothesis that foetal testicular T production is a more sensitive endpoint for anti-androgenic effects of phthalates than genomic and early postnatal endpoints and the notion of using reduction in foetal testicular T production as a critical effect in the risk assessment (Hannas et al. 2011a).

Phthalate metabolism is qualitatively similar among species (NCR 2008). The rate of metabolism may vary by species and by diester structure, especially the length and saturation of the alkyl side chain of the diester. At present, there is not enough information to conclude whether or not phthalates exert inhibitory effects on steroidogenesis in the human foetal testis because of conflicting data and the supporting evidence is indirect (AGD)(Scott et al. 2008).

Effects in females
Considering females, a similar phenomenon to the testicular dysgenesis syndrome has not been identified and female foetuses have been predominantly unaffected after treatment by DEHP, BBP and DBP. At very high doses, ovarian granulosa cell function has been affected (Lovekamp and Davis 2001; Lovekamp-Swan and Davis 2003). Exposure to DBP at 500 mg/kg bw/day may decrease progesterone levels and cause failure to maintain pregnancy (Gray et al. 2006). Endocrine-related health outcomes in humans include precocious puberty, female fecundity, polycystic ovary syndrome, fertility, endometriosis, uterine fibroids and hormonal cancers. Higher serum phthalate concentrations have been associated with precocious thelarche (premature breast development) and precocious puberty in human (reviewed by Kortenkamp et al. 2011) as well as with endometriosis (Reddy et al. 2006).

Justifications for combined risk assessment of phthalates showing androgen deficiency (anti-androgenicity)

It has been proposed that substances having the same mode of action or the same adverse outcome should be evaluated using combined risk assessment (cumulative risk assessment) due to concern of additive or even synergistic mixture effects following parallel or sequential exposure from different sources. The dose additive approach has been proposed in many studies to fit better to the data than other models in case of phthalates (e.g., Howdeshell et al. 2008a, b; Benson 2009) and other anti-androgenic substances (e.g., Kortenkamp and Faust 2010).

The most prominent effects of several phthalates seem to be due to androgen deficiency. However, this does not concern all the phthalates and in addition other mode of actions play also a role. For instance, DINP belong to the phthalates displaying anti-androgenicity although it has a lower potency than DEHP or DBP. DIDP on the other hand does not reduce testicular T production even at high doses (Hannas et al. 2012). However, DIDP reduces early postnatal survival in F2 generation at 114 mg/kg bw/day (Hushka et al. 2001) with an unknown mode of action which may be related developmental, paternal or maternal factors or even due to developmental exposure of F1 animals affecting maternal behaviour because F2 animals were affected at lower dose levels than F1 animals. DINP did affect postnatal survival in one- and two-generation reproductive toxicity studies only at high doses (~1100 mg/kg bw/day; Waterman et al. 2000).

Thus, it seems obvious that all phthalates could not be included into the same group for combined risk assessment. It would for example be difficult to justify combining DINP and DIDP into the same group of adverse outcomes even if their structural similarity. Considering anti-androgenicity, DINP was only 2.3 fold less potent than DIBP, DIHP and DEHP in reducing foetal testicular T production after exposure on GDs 14-18 (Hannas et al. 2011b) and could be justified to be grouped with anti-androgenic phthalates (see Table 4.54). DEHP, DBP, DIBP, DIHP, DINP, DPeP and BBP all produce similar reproductive alterations in male offspring but with different potency (Saillenfait et al. 2008; McKee et al. 2006; Borch et al. 2004; Gray et al. 2000; Hannas et al. 2011a, b; 2012). T production has been shown to be the most sensitive foetal testicular endpoint for several phthalates, such as DEHP, DINP and DPEP, but for some phthalates, other testicular endpoints may be more sensitive, such as Cyp11a expression is a sensitive endpoint for DIBP (Hannas et al. 2012). It seems that DINP differs only quantitatively in potency but not qualitatively from DIBP, DIHP, DEHP and DPEP based on foetal endocrine alterations (gene expressions and T production)(Hannas et al. 2011b).

Table 4.54 Comparison of some key effects (parameters) and sensitiveness of selected phthalates. Phthalate dose mg/kg bw/day is not otherwise stated.

Parameter	NOAEL/LOAEL/ED50 (mg/kg bw/day)					
	DEHP	DBP	DIBP	BBP	DINP	DIDP
Foetal testis T production (ex vivo)	100/300/383 Howdeshell et al. (2008a), Hannas et al. (2011a) BMD1SD=142 BMDL1SD=67 Benson (2009) -/-/347 (Wistar) -/- /426 (SD) best fit 380 Hannas et al. (2011b) -/300/- Borch et al. (2004, 2006b)	10/100/- Lehman et al. (2004) 100/300/399 Howdeshell et al. (2008a) BMD1SD=139, BMDL1SD=104 Benson (2009)	100/300/466 Howdeshell et al. (2008a) BMD1SD=136, BMDL1SD=80 Benson (2009) -/-/374 Hannas et al. (2011b) -/-/305 Hannas et al. (2012) -/600/- Borch et al. (2006a)	100/300/464 Howdeshell et al. (2008a) BMD1SD=133, BMDL1SD=102 (Benson 2009)	-/-/852 Hannas et al. (2011b, 2012)	No effect up to 1500 Hannas et al. (2012)

Foetal testis T concentration	-/300/- Borch et al. (2004, 2006a)	10/50/- Lehmann et al. (2004)	-/600/- Borch et al. (2006a)		50/250/- Clewell et al. (2011a)	
Magnitude of the reduction of foetal T production/ concentration	50% at 300 mg/kg bw/day (production; Hannas et al. (2011b)) 70% at 300 mg/kg bw/day (concentration; Borch et al. (2004))	61% at 50 mg/kg bw/day (concentration; Lehmann et al. (2004))	56% at 300 mg/kg bw/day (production; Hannas et al. (2011b)) 91% at 600 mg/kg bw/day (concentration; Borch et al. (2006a)) 96% at 600 mg/kg bw/day (production; Borch et al. (2006a))		50% at 250 mg/kg bw/day (concentration; Clewell et al. (2011a)) 30% (production at 500 mg/kg bw/day; Hannas et al. (2012)) 74% at 750 mg/kg bw/day (concentration; Borch et al. (2004))	
Leydig cell insl3 gene	-/-/534 (Wistar) -/-/589 (SD) best fit for both 569 Hannas et al. (2011b)	-/1000/- Wilson et al. (2004)	-/-/393 Hannas et al. (2012)	-/1000/- Wilson et al. (2004)	-/-/1488 Hannas et al. (2012)	No effect up to 1500 Hannas et al. (2012)
Testis StAR	-/-/296 (Wistar) -/-/443 (SD) best fir for both 405 Hannas et al. (2011b)	10/50/- Lehmann et al. (2004)	-/-/191 Hannas et al. (2011b) -/-/295 Hannas et al. (2012) -/600/- (protein; Borch et al. (2006a))		-/-/901 Hannas et al. (2011b) -/-/597 Hannas et al. (2012)	No effect up to 1500 Hannas et al. (2012)
Testis Cyp11a	-/-/555 (Wistart) -/-/574 (SD) best fit for both 569 Hannas et al. (2011b)		-/-/171 Hannas et al. (2011b) -/-/339 Hannas et al. (2012)		-/-/1356 Hannas et al. (2011b) -/-/1148 Hannas et al. (2012)	No effect up to 1500 Hannas et al. (2012)
AGD	100/300/- Gray et a. (2009) 135/405/- Andrade et al. (2006)	-/500/- Clewell et al. (2011b) 50/500/- Lee et al. (2004) 100/500/- Barlow et al. (2004); Mylchreest et al. (2000) 148/500/- Ema et al. (1998) 259/555/- Mylchreest et al. (1998;1999) 331/712/- Zhang et al. (2004)	-/600/- Borch et al. (2006a)	250/750/- Tyl et al. (2004) 50/250/- Aso et al. (2005) 100/500/- Nagao et al. (2000) BMD1SD=240, BMDL1SD=130 Benson (2009)	750/900/- Boberg et al. (2011) No effect Clewell et al. (2011a, b) -/2/- Lee et al. (2006a, b)	
PPS	5/15/- Andrade et al. (2006)			100/500/- Nagao et al. (2000)		
Areolas/nipples	135/405/- Andrade et al. (2006)	50/100/- Mylchreest et al. (2000) BMDL10=40 US EPA (2006)		250/750/- Tyl et al. (2004)	300-600/600-750/- Boberg et al. (2011)	

Multinucleated gonocytes	45/135/- Andrade et al. (2006)	-/500/- Kleymenova et al. (2005) 50/100/- Ferrera et al. (2006)	-/600/- Borch et al. (2006a)		-/300/- Boberg et al. (2011) 50/250/- Clewell et al. (2011)	
Leydig cell aggregates		-/1.5/- Lee et al. (2004)	-/600/- Borch et al. (2006a)		50/250/- Clewell et al. (2011)	
Small and absent reproductive organs	3-5/14-23/- NTP (2004) BMD10=42, BMDL10=27 Benson (2009)					
Neonatal survival	Some effect at 900? Howdeshell et al. (2008a)	Slight increase at 600? Howdeshell et al. (2008a)	300/600/- Howdeshell et al. (2008a)	300/600/- Howdeshell et al. (2008a)	750/1100/- Waterman et al. (2000)	33-38/134/- Huskha et al. (2001)

AGD = anogenital distance
ED50 = effective dose with 50% of the maximum response
PPS = preputial separation
BMD10 = bench mark dose for a 10% change
BMDL10 = the corresponding 95% lower confidence for above
BMD1SD = bench mark dose for a 1 standard deviation decrease

Benson (2009) has defined relative potency factors to certain anti-androgenic phthalates (Table 4.55). For three of the phthalates (dipentyl phthalate (DPP), DIBP, BBP) the reduced foetal testicular T production was selected as the most sensitive effect for reference dose (RfD) calculation. For DBP and DINP, the foetal testicular T concentration was the sensitive effect. Decrease in foetal testicular T production due to DEHP exposure was at similar level than that for DBP (ED50 approximately 390 mg/kg bw/day; Howdeshell et al. 2008a), however, the most sensitive effect was small or absent male reproductive organs at 14-23 mg/kg bw/day from a two-generation reproductive toxicity study (NTP 2004). Benson (2009) calculated the lower confidence limit for a one standard deviation decrease using bench mark dose method (BMDL1SD values) for the most sensitive effects of various phthalates when data proved to be appropriate for that purpose. To obtain molar concentrations for relative potency factor calculation, the RfD as mg/kg bw/day was divided by molecular weight. For DINP, a NOAEL or dose-related data for BMD calculation was not available. Thus, an uncertainty factor (UF) of 1000 was used to divide the LOAEL for DINP to derive RfD. Benson (2009) calculated a RfD of 0.8 mg/kg bw/day for DINP based on a LOAEL of 750 mg/kg bw/day (the only dose level studied by Gray et al. 2000 and Borch et al. 2004). By using the NOAEL value of 50 mg/kg bw/day (Clewell et al. 2011a) and an UF of 100, a RfD of 0.5 mg/kg bw/day can be derived. This would lead to higher relative potency factor for DINP, but would be still smaller than the relative potency factor for DBP.

Table 4.55 Relative potency factors of certain anti-androgenic phthalates (according to Benson 2009)

Phthalates	Relative potency factor	RfD (µmol/kg bw/day)	RfD (mg/kg bw/day)	POD/UF	POD	Critical effect and reference
DPP	1.26	0.548	0.2	17/100	BMDL1SD	↓ foetal T production Howdeshell et al. (2008a)
DEHP	1.00	0.692	0.3	27/100	BMDL10	Small or absent male reproductive organs NTP (2004)

DBP	0.64	1.08	0.3	30/100	NOAEL	↓ foetal T Lehmann et al. (2004)[a]
DINP	0.39	1.79	0.8	750/1000	LOAEL	↓ foetal T Borch et al. (2004); Gray et al. (2000)
DIBP	0.24	2.88	0.8	80/100	BMDL1SD	↓ foetal T production Howdeshell et al. (2008a)
BBP	0.21	3.27	1.0	102/100	BMDL1SD	↓ foetal T production Howdeshell et al. (2008a)

[a] In the original publication, the NOAEL is 10 mg/kg bw/day
POD = Point of departure
UF = Uncertainty factor
RfD (µmol/kg bw/day) = 1000x RfD (mg/kg bw/day)/mol wt (mg/mmol)
BMD10 = bench mark dose for a 10% change
BMDL10 = the corresponding 95% lower confidence for above
BMD1SD = bench mark dose for a 1 standard deviation decrease

Effects on thyroid

In case of the thyroid, weak effects have been reported on iodide uptake for certain phthalates. DINP, DIDP, DEHP and DOP significantly enhanced iodide uptake, whereas BBP augments the uptake but that at toxic concentration and DBP had no effect (Wenzel et al. 2005; Breous et al. 2005). The molecular mechanisms may differ: DIDP, BBP and DOP enhanced transcriptional activity of promoter N3, whereas DEHP and DINP had no effect and DBP even reduced the activity. In addition, phthalates enhanced promoter and enhancer (N3 + NUE) activity in the following order: DIDP, BBP, DEHP, DOP and DINP, and DBP had a decreasing effect. Only DIDP, BBP and DOP seem to increase the mRNA levels of rNIS, and DEHP, DINP and DBP had no effect.

The TH-like effects on rat pituitary GH3 cells were also different: BBP, DEHP, DBP, DIDP and DOP increased slightly the proliferation whereas DINP decreased proliferation in conditions without T3. In the presence of T3, BBP increased proliferation, DIDP had no effect, and DEHP, DBP, DOP and DINP decreased cell proliferation. This indicates that a combined risk assessment may not be easily justifiable to include all phthalates without considering potentially different mode of actions.

Other endpoints

Some of the phthalates, such as BBP and DBP, have a weak oestrogenic activity in in vitro studies. DIDP, DINP, DEHP and DOP seem not to be active. Certain phthalates, such as DEHP, have suggested affecting also female reproductive health but as whole the effects of phthalates on reproduction in females have been studied much less than in males (reviewed by Lyche et al. 2009). In granulosa cell culture, MEHP decreased estradiol production by reducing the levels of aromatase, which may be due to induction of PPARα and PPARγ (reviewed by Lyche et al. 2009). Both DINP and DIDP also reduced FSH-stimulated oestradiol production in granulosa cell culture, but had no effect on the basal production (Mlynarciková et al. 2007). For both males and females, other relevant human health endpoints concerning endocrine disruption such as developmental neurotoxicity, thyroid system, arylhydrocarbon receptor signalling and obesity have not been clearly associated with phthalate exposure (reviewed by Kortenkamp et al. 2011).

There might be also combined liver effects from DINP, DIDP and DEHP. The NOAEL for spongiosis hepatis is 15 and 147 mg/kg bw/day for DINP and DEHP respectively. For DIDP, a

LOAEL of 22 mg/kg bw/day is established for spongiosis hepatis. As discussed in section 4.4.8, increased incidences of MNCL are seen with DINP and DIDP. Since the available information does not allow to draw definite conclusions concerning their relevance, combined effects were not considered for this endpoint.

4.4.10.1.3 Conclusion

Based on the available information from in vitro studies, different phthalates seem to exhibit various effects – stimulatory, inhibitory or no effects – on certain endocrine parameters. Phthalates having the same mode of action or the same adverse outcome are likely candidates for combined risk assessment. However, the mode of action should always be carefully considered in selecting candidates for combined risk assessment.

DINP has anti-androgenic properties and it could be appropriate to include this substance in a combined risk assessment of phthalates with anti-androgenic properties. DIDP, on the other hand, does not have similar properties/potency and it would not be justified to group DIDP in a combined risk assessment of phthalates on the basis of anti-androgenic properties.

There seem to be sufficient grounds to assess combined effects of DINP and DIDP (as well as DEHP and possibly other substances) on the basis of liver toxicity (spongiosis hepatis).

4.4.11 Derivation of DNELs

4.4.11.1 Considerations on absorption

Based on a study from Sjöberg et al. (1985) which seemed to show a greater absorption of DEHP by the oral route in young rats compared to older ones, the ECHA draft report (ECHA 2012a) differentiated between adults and children, assuming that the absorption rates in children are higher (100%) than in adults (50%). As a consequence, a lower DNEL for children was derived than for adults. This endpoint modification step was in line with the EU Risk Assessments for DINP, DIDP and DEHP (EC 2003a,b; EC 2008). The RAC opinion on the Danish restriction proposal on four phthalates of 15 June 2012 had also assumed 100% absorption in children (ECHA 2012b).

However, as discussed in section 4.4.1, in its opinion on the draft review report, RAC (ECHA 2013a) considered that humans orally absorb DINP and DIDP 100%. This has as a logical consequence that children cannot absorb more than adults.

According to the ECHA guidance[36], an endpoint modification is necessary to correct for the species differences in absorption for deriving oral DNELs when there are differences in absorption between experimental animals and humans. In this case, adult rats absorb about 50% whereas humans around 100%. This equals to an endpoint modification with a factor of 2. RAC (ECHA 2013a,b) considered that a modification of the dose descriptor with a factor of 2 is justified. RAC noted however, that the estimated absorption rate of 50% in adult rats might underestimate the actual absorption at low dose levels, in particular given the contribution of biliary excretion, and that therefore the modification of the dose descriptor with a factor of 2 might be considered to be conservative.

With regard to the assumption for inhalation, RAC (ECHA 2013b) agreed with the assumption in the ECHA draft report of 75% absorption in adults and 100% absorption in children. The assumption of 100% absorption in children could be considered conservative.

4.4.11.2 DINP

Table 4.56 summarises the key dose descriptors for DINP per endpoint, based on the assessment in the previous sections.

Table 4.56 Key dose descriptors for DINP per endpoint

Endpoint	Route of exposure	Dose descriptor	Qualitative assessment
Acute toxicity	oral, dermal and inhalation		Low acute oral, dermal and inhalation toxicity.
Irritation/Corrosivity	skin/eyes		Very slight skin and eyes irritant, with effects reversible in short time
Sensitisation	dermal/inhalation		Lack of intrinsic sensitising potential. DINP seems to show adjuvant properties.

[36] As indicated in the ECHA guidance R.8, Appendix R.8.2-2, the default situation, in the absence of information, is to assume the same bioavailability for experimental animals and humans for a particular exposure route. However, when available information indicates that at the relevant level of exposure humans absorb less (or more) than experimental animals, the dose descriptor needs to be corrected for this difference in bioavailability.

Repeated dose toxicity	oral	NOAEL of 15 mg/kg bw/day Exxon (1986)	Significant increases of incidence of spongiosis hepatis together with other signs of hepatotoxicity
Mutagenicity	in vitro/in vivo		Negative
Carcinogenicity	oral	/	DINP is carcinogenic to rodents. The renal tumors are related to alpha-2u-globulin and are generally not considered relevant for humans. Increased incidences of MNCL in rats remain difficult to interpret in the light of the high and variable background incidences and the unclear relevance to humans. As a reasonable approach it would be possible to conclude that the carcinogenicity findings further strengthen the selected NOAELs for repeated dose toxicity.
Reproductive toxicity	oral	NOAEL of 50 mg/kg bw/day for reduced foetal testosterone level Clewell et al. (2011a); LOAEL of 159 mg/kg bw/day for decreased body weight in offspring Waterman et al. (2000); NOAEL of 100 mg/kg bw/day for skeletal variations Waterman et al. (1999)	Decreases foetal testicular testosterone concentration during critical time window of sexual differentiation and increased incidence of multinucleated gonocytes and Leydig cell aggregates (Clewell et al. 2011a,b). In two-generation reproductive toxicity study the offspring bodyweight was decreased and increased skeletal variations were observed in prenatal developmental toxicity study. Effects on fertility (decreased live birth and survival indices and decreased testicular weights) occur at higher dose levels (Waterman et al. 2000; Aristech 1995c).

From Table 4.56 it is clear that the most sensitive endpoints are repeated dose toxicity with a NOAEL of 15 mg/kg bw/day and reproductive toxicity with a NOAEL of 50 mg/kg bw/day.

For reproductive toxicity a NOAEL of 50 mg/kg bw/day was identified for reduced foetal testosterone level (Clewell et al. 2011a). In addition, a LOAEL of 159 mg/kg bw/day was identified for decreased body weight in offspring (Waterman et al. 2000). If an assessment factor of 3 is applied to the LOAEL[37], a NAEL of 53 mg/kg bw/day is obtained, which is close to the selected NOAEL of 50 mg/kg bw/day. A NOAEL of 100 mg/kg bw/day was identified for

[37] Chapter R.8 of the ECHA Guidance suggests to use an assessment factor between 3 as minimum for LOAEL to NAEL derivation, which is appropriate in the majority of cases.

skeletal and visceral variations derived from a prenatal developmental toxicity study (Waterman et al. 1999). This study was carried out according to an earlier version of the OECD test guideline where the exposure period did not cover the whole foetal development. Ossification continues until birth and beyond and more severe effects may be anticipated after a longer exposure period covering the whole pregnancy (as is the case with studies performed according to the current OECD guideline). Thus, an additional assessment factor could be applied to extrapolate to the exposure of full foetal period. By applying an assessment factor of 2 this would lead to a NOAEL of 50 mg/kg bw/day which would further support the selected NOAEL based on reduced testosterone levels in foetal testes.

The derived NOAEL for reproductive effects is in particular relevant to derive DNELs for pregnant women, i.e. to the developing foetus in the mother's womb. Naturally, the DNELs based on repeated dose toxicity are equally applicable to pregnant women (considered under adult population).

Small children may be sensitive to androgen deficiency due to immaturate HPG axis (Pierik et al. 2009) and therefore DNELs for reproductive toxicity for children were calculated as well. Pierik and coworkers (2009) did not find evidence on the negative feedback mechanism of T on LH in the first 6 months in boys.

DNELs for consumers have been derived for repeated dose toxicity as well as reproductive toxicity for the oral, dermal and inhalation route for adults and for children. ECHA used default assessment factors as described in ECHA guidance R.8.

CEFIC ECPI (2013) proposed to use an AF of 40, assuming that rat spongiosis hepatis is not relevant to humans, and that the toxicokinetics between humans and the rat are similar. CEFIC ECPI argued that thus the factor of 2.5 for remaining differences between species would not be necessary[38]. However, RAC noted (ECHA 2013b) that the relevance of spongiosis hepatis for humans has been questioned by some, while others have indicated that treatment-related lesions similar to spongiosis hepatic are described in human pathology (sinusoidal dilations or sinusoidal ectasia), but that the terminology differs. Moreover, as mentioned above, RAC considered that the absorption of humans was twice that of rats. In line with the opinion of RAC (ECHA 2013a,b), the default AF of 2.5 for remaining interspecies differences is applied in the DNEL setting.

Adults - repeated dose toxicity

Table 4.57 gives an overview of the derived DNELs for adult consumers exposed to DINP.

To correct for the species differences in absorption (adult rats absorb about 50% whereas humans around 100%), an endpoint modification with a factor of 2 was carried out to obtain the correct starting point for derivation of the oral DNEL. See also section 4.4.11.1.

The oral NOAEL rat (in mg/kg bw/day) was converted into an inhalatory NOAEC rat (in mg/m3) 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 (50% oral absorption in rats, 75% inhalation absorption in humans/rats). No allometric scaling factor was used.

[38] As a default, a factor of 4 is used to correct for differences in metabolic rate between the rat and humans (allometric scaling) and an additional factor of 2.5 for other interspecies differences, i.e. toxicokinetic differences not related to metabolic rate (small part) and toxicodynamic differences (larger part). In case substance-specific information shows specific susceptibility differences between species, which are not related to differences in basal metabolic rate, the additional factor of 2.5 for 'remaining differences' should be modified accordingly (see ECHA guidance R.8).

The standard respiratory volume for rats is 0.2 l/min/rat (sRV_{rat}) or for a 250g rat 0.8 l/min/kg bw or for 24 h of exposure 1.15 m^3/kg bw/day. Thus when using the formula below, the corrected inhalatory NOAEC = 15 mg/kg bw/day x 1/1.15 m^3/kg bw/day x 50/75 = 8.7 mg/m^3.

$$\text{corrected inhalatory N(L)OAEC} = \text{oral N(L)OAEL} \times \frac{1}{sRV_{rat}} \times \frac{ABS_{oral\text{-}rat}}{ABS_{inh\text{-}rat}} \times \frac{ABS_{inh\text{-}rat}}{ABS_{inh\text{-}human}}$$

$$= \text{oral N(L)OAEL} \times \frac{1}{sRV_{rat}} \times \frac{ABS_{oral\text{-}rat}}{ABS_{inh\text{-}human}}$$

The oral NOAEL rat (in mg/kg bw/day) was converted into a dermal NOAEL rat (in mg/kg bw/day) by correcting for differences in absorption between routes. In a dermal absorption study with rats, ca. 2-4% of dermally administered DINP was absorbed in 7 days (Midwest Research Institute 1983b in EC 2003a ; McKee et al. 2002). The study is non-guideline and non-GLP using 15 rats in 3 groups. From this study it is assumed that 4% is dermally absorbed in rats. It is recognised that in humans skin absorption is lower than in rats. Nevertheless, the EU Risk Assessment (EC 2003a) indicated that no precise estimate of the skin penetration was possible, however it could be concluded that there is very little penetration of DINP through intact human skin. It seems appropriate to consider a dermal absorption rate for humans of 4%.[39] It could be clarified that the actual data used for estimating both migration and absorption is from a study using PVC film plasticised with 14C labelled DEHP, applied to the shaved backs of male rats, was used to derive a dermal absorption of 0.024 µg/cm2/h (Deisinger et al. 1998). Although the dermal absorption figure of 4% is indeed considered realistic, the figure is merely chosen to be able to follow the "normal" approach in calculating DNELs and exposure. In the exposure assessment, the dermal absorption figure of 0.024 µg/cm2/h is divided by an absorption factor of 0.04 to give an estimated migration rate of 0.6 µg/cm2/h from a PVC foil of 0.51mm thickness with 40% DINP. This migration figure is subsequently used to calculate the external exposure. The assumption is thus effectively cancelled out in the risk characterisation ratio, and thus in principle any other figure would result in the same RCRs.

Table 4.57 Overview of derivation of repeated dose DNELs for adult consumers exposed to DINP

Route	Dose descriptor	Modification to obtain correct starting point	AF	DNEL
Oral	NOAEL = 15 mg/kg bw/day (2-year oral, rat)	bioavailability factor = 1/2 (50% rats, 100% adults) NOAEL = 7.5 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10	0.075 mg/kg bw/day

[39] ExxonMobil (2011b) also assumed a dermal absorption of 4%.

			Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	
Inhalation	NOAEL = 15 mg/kg bw/day (2-year oral, rat)	Inhalation NOAEC = 8.7 mg/m3	Overall AF = 25 Interspecies: 2.5 (AS of 1 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	0.35 mg/m^3
Dermal	NOAEL = 15 mg/kg bw/day (2-year oral, rat)	Absorption factor 50/4 (50% oral rats, 4% dermal humans) dermal NOAEL = 187.5 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	1.88 mg/kg bw/day

Adults – reproductive toxicity

Chapter R.8 of the ECHA Guidance on information requirements and chemical safety assessment recognises that in order to always cover the most sensitive person exposed to any chemical would require a very large default assessment factor. Since this is not workable, usually a default assessment factor of 10 is sufficient to protect the larger part of the population. The Appendix R. 8-12 mentions in addition the following:

> *"The methodology for the DNEL calculation for reproductive toxicity is similar to the methodology as described for repeated dose toxicity. However, reproductive toxicity also includes effects which may occur after one single exposure in a susceptible window during foetal development (e.g. malformations and functional deficits)."*
>
> *"A number of studies may provide relevant information in relation to reproductive toxicity. However, the different studies may provide different levels of certainty with respect to the evaluation of reproductive toxicity. Since reproductive toxicity is a complex endpoint expert judgement using an overall weight of evidence approach considering all available data is crucial when performing safety assessment for this endpoint. Therefore the choice of a specific assessment factor in relation to qualitative and quantitative uncertainties should be decided on a case-by-case basis."*

It is here assumed that an interspecies factor of 10 is sufficiently protective. It is assumed that transfer of DINP metabolites across placenta is 100%. This is supported by the measurements

of maternal and foetal plasma concentrations of MINP indicating a similar AUC-value at dose level of 50 mg/kg bw/day in rats (Clewell et al. 2011a).

Using the same standard respiratory volume for rats as indicated above for repeated dose toxicity (0.2 l/min/rat (sRV_{rat}) or for a 250g rat 0.8 l/min/kg bw or for 24 h of exposure 1.15 m3/kg bw/day) and the same formula, the corrected inhalatory NOAEC for reproduction is: 50 mg/kg bw/day x 1/1.15 m3/kg bw/day x 50/75 = 29 mg/m3.

Table 4.58 Overview of derivation of DNELs for reproductive toxicity in adults exposed to DINP

Route	Dose descriptor	Modification	AF	DNEL
Oral	NOAEL = 50 mg/kg bw/day (targeted developmental toxicity study)	bioavailability factor = 1/2 (50% rats, 100% adults and 100% mother to foetus) NOAEL = 25 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration: 1 Issues related to dose-response: 1 Quality of database: 1	0.25 mg/kg bw/day
Inhalation	NOAEL = 50 mg/kg bw/day (targeted developmental toxicity study)	Inhalation NOAEC = 29 mg/kg bw/day	Overall AF = 25 Interspecies: 2.5 (AS of 1 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	1.16 mg/m^3
Dermal	NOAEL = 50 mg/kg bw/day (targeted developmental toxicity study)	Bioavailability factor = 50/4 (50% oral absorption in rats/humans, 4% dermal humans) dermal NOAEL = 625 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	6.25 mg/kg bw/day

Children - repeated dose toxicity

Table 4.59 gives an overview of the derived DNELs for children exposed to DINP. 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: 15 mg/kg bw/day x 1/1.15 m^3/kg bw/day x 50/100 = 6.5 mg/m^3.

Table 4.59 Overview of derivation of repeated dose DNELs for children exposed to DINP

Route	Dose descriptor	Modification	AF	DNEL
Oral	NOAEL = 15 mg/kg bw/day (2-year oral, rat)	bioavailability factor = 1/2 (50% rats, 100% for children) NOAEL = 7.5 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	0.075 mg/kg bw/day
Inhalation	NOAEL = 15 mg/kg bw/day (2-year oral, rat)	Inhalation NOAEC = 6.5 mg/m3	Overall AF = 25 Interspecies: 2.5 (AS of 1 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	0.26 mg/m^3
Dermal	NOAEL = 15 mg/kg bw/day (2-year oral, rat)	Absorption factor 50/4 (50% oral rats, 4% dermal humans) dermal NOAEL = 187.5 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	1.88 mg/kg bw/day

Children - reproductive toxicity

Table 4.60 gives an overview of the derived DNELs for reproductive toxicity for children exposed to DINP. 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: 50 mg/kg bw/day x 1/1.15 m^3/kg bw/day x 50/100 = 21.7 mg/m^3.

Table 4.60 Overview of derivation of reproductive DNELs for children exposed to DINP

Route	Dose descriptor	Modification	AF	DNEL
Oral	NOAEL = 50 mg/kg bw/day (targeted developmental toxicity study)	bioavailability factor = 1/2 (50% rats, 100% for children) NOAEL = 25 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	0.25 mg/kg bw/day
Inhalation	NOAEL = 50 mg/kg bw/day (targeted developmental toxicity study)	Inhalation NOAEC = 21.7 mg/m3	Overall AF = 25 Interspecies: 2.5 (AS of 1 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	0.868 mg/m^3
Dermal	NOAEL = 50 mg/kg bw/day (targeted developmental toxicity study)	Absorption factor 50/4 (50% oral rats, 4% dermal humans) dermal NOAEL = 625 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	6.25 mg/kg bw/day

4.4.11.3 DIDP

Table 4.61 summarises the available dose descriptors for DIDP per endpoint, based on the assessment in the previous sections.

Table 4.61 Available dose descriptors for DIDP per endpoint

Endpoint	Route of exposure	Dose descriptor	Qualitative assessment
Acute toxicity	oral, dermal and inhalation		Low acute oral, dermal and inhalation toxicity.
Irritation/Corrosivity	skin/eyes		Very slight skin and eyes irritant, with effects reversible in short time
Sensitisation	dermal/inhalation		Lack of intrinsic sensitising potential. DIDP seems to show adjuvant properties however.
Repeated dose toxicity	oral	LOAEL of 22 mg/kg bw/day (Cho et al. 2008, 2010)	Significant increases of incidence of spongiosis hepatis together with other signs of hepatotoxicity in a 2-year study in rat
		NOAEL of 60 mg/kg bw/day[40] (BASF 1969b as cited in EC 2003b)	Dose-related increase of relative liver weights in a 90-day rat dietary study.
		NOAEL of 15 mg/kg bw/day (Hazleton 1968b as cited in EC 2003b)	Hepatic effects were observed in a 90-day study in dog. No statistical analysis was possible (n = 3/sex/dose).
Mutagenicity	in vitro/in vivo		Genetic toxicity: negative
Carcinogenicity			DIDP is carcinogenic to rodents. Liver neoplasms have been considered to be related to peroxisome proliferation. Carcinogenicity seen with peroxisome proliferators has traditionally considered not to be relevant to humans, however caution needs to be taken. Increased incidences of MNCL in rats remain difficult to interpret in the light of the high and variable background incidences and the unclear relevance to humans. As a reasonable approach it would be possible to conclude that the carcinogenicity

[40] As pointed out by CSTEE (2001b), a LOAEL of 55 mg/kg bw/day could be derived based on relative liver weights in males in the 90 days study in rats in stead of a NOAEL of 60 mg/kg bw/day (BASF 1969b). Thus, using a NOAEL of 60 mg/kg bw/day is a less cautious approach. This was not discussed by RAC (2013a,b).

			findings further strengthen the selected NOAELs for repeated dose toxicity.
Reproductive toxicity	oral	NOAEL of 33 mg/kg bw/day Hushka et al. (2001) NOAEL of 40 mg/kg bw/day Hellwig et al. (1997) NOAEL 100 mg/kg bw/day Watermann et al. (1999) NOAEL 52 mg/kg bw/day Hushka et al. (2001)	The mortality of neonatal F2 pups was increased and the finding was confirmed in a second two-generation reproductive toxicity study with a NOAEL of 33 mg/kg bw/day. NOAELs of 40 and 100 mg/kg bw/day have been derived for foetal variations from prenatal developmental toxicity studies. The NOAEL for reduced body weight of the offspring was 0.06% (corresponding to 52 mg/kg bw/day) based on LOAEL of 0.2%.

From Table 4.61 it is clear that the most sensitive endpoints are repeated dose toxicity and reproductive toxicity.

DNELs for consumers have been derived for repeated dose toxicity as well as reproductive toxicity for the oral, dermal and inhalation route for adults and for children. ECHA used default assessment factors as described in ECHA guidance R.8. CEFIC ECPI (2013) proposed not to apply the default factor of 2.5 for remaining interspecies differences. In line with the opinion of RAC (ECHA 2013a,b), the default AF of 2.5 is applied in the DNEL setting. See section 4.4.11.2 for more details.

Repeated dose toxicity

As discussed in section 4.4.6.2, the three key studies for repeated dose toxicity with DIDP each have certain limitations. RAC (ECHA 2013a) recommended a weight of evidence approach using a LOAEL of 22 mg/kg bw/day based on spongiosis hepatis in a 2-year study in rat (Cho et al. 2008, 2010), a NOAEL of 15 mg/kg bw/day based on hepatic effects in a 90 days study in dogs (Hazleton 1968b) and a NOAEL 60 mg/kg bw/day based on in a 90 days study in rats (BASF 1969).

To correct for the species differences in absorption (adult rats absorb about 50% whereas humans around 100%), an endpoint modification with a factor of 2 was carried out to obtain the correct starting point for derivation of the oral DNEL. See also section 4.4.11.1. It could be assumed here that this species differences in absorption would also exist with the dog, and that the correction could also be applied.

The oral DNEL after the modification of the dose descriptor can then be derived as follows (in-line with ECHA (2013b)):

- Dog 90 d study (Hazleton 1968b), corrected NOAEL 7.5 mg/kg bw/day
  - Interspecies factor:
    - a. AS (correction for differences in metabolic rate): 1,4
    - b. remaining differences: 2,5
  - Intraspecies factor general population: 10

Exposure duration 90 day - chronic: 6[41]
Issues related to dose-response: 1
Quality of whole database: 1
Total factor: 210
DNEL: 0.036 mg/kg bw/day

- Rat 90 d study (BASF 1969), corrected NOAEL 30 mg/kg bw/day
  Interspecies factor:
    c. AS (correction for differences in metabolic rate): 4
    d. remaining differences: 2,5
  Intraspecies factor general population: 10
  Exposure duration 90 day - chronic: 2
  Issues related to dose-response: 1
  Quality of whole database: 1
  Total factor: 200
  DNEL: 0.15 mg/kg bw/day

- Rat 2y study (Cho et al 2008, 2010), corrected LOAEL 11 mg/kg bw/day
  Interspecies factor:
    e. AS (correction for differences in metabolic rate): 4
    f. remaining differences: 2,5
  Intraspecies factor general population: 10
  Exposure duration 90 day - chronic: 1
  Issues related to dose-response: 3
  Quality of whole database: 1
  Total factor: 300
  DNEL: 0.037 mg/kg bw/day

The average of the 3 DNELs leads to an oral DNEL for repeated dose toxicity of 0.075 mg/kg bw/day.

To derive the DNELs for inhalation, a correction for differences in absorption between routes was carried out (50% oral absorption in rats, 75% inhalation absorption in adults and 100% in children). No allometric scaling factor was used. Using the standard respiratory volume for rats is 0.2 l/min/rat (sRV_{rat}) or for a 250g rat 0.8 l/min/kg bw or for 24 h of exposure 1.15 m^3/kg bw/day, and the formula below, the DNEL for inhalation can be calculated starting from the oral DNEL for adults as follows: 0.075 mg/kg bw/day x 4 (to neutralise factor for AS) x 2 (to neutralise modification of the dose descriptor) x 50/75 x 1/1.15 m^3/kg bw/day = 0.35 mg/m^3. For children this would be 0.075 mg/kg bw/day x 4 x 2 x 50/100 x 1/1.15 m^3/kg bw/day = 0.26 mg/m^3.

[41] The default assessment factor for sub-chronic to chronic extrapolation is 2 for a rat 90 day study. The lifespan of a Beagle dog is around 13 year; thus, a study duration of 90 days covers roughly 2% of its lifespan. As a comparison between dog and rat, a 28 day study (subacute) covers 4% of the lifespan and a 90 day study (sub-chronic) covers 12% of a rat's life. Thus, a 90 day dog study covers about half of the length of a subacute study in rats. This justifies a default assessment factor of 6 for subacute to chronic extrapolation for the 90 day dog study (see Table R. 8-5 in ECHA guidance R.8).

$$\text{corrected inhalatory N(L)OAEC} = \text{oral N(L)OAEL} \times \frac{1}{sRV_{rat}} \times \frac{ABS_{oral\text{-}rat}}{ABS_{inh\text{-}rat}} \times \frac{ABS_{inh\text{-}rat}}{ABS_{inh\text{-}human}}$$

$$= \text{oral N(L)OAEL} \times \frac{1}{sRV_{rat}} \times \frac{ABS_{oral\text{-}rat}}{ABS_{inh\text{-}human}}$$

To derive the DNELs for the dermal route (adults and children), the differences in absorption between routes needs to be corrected for. The maximum percentage of absorption may be estimated 4% of applied dose in 7 days by analogy with DINP (Midwest Research Institute, 1983 in EC 2003a; McKee et al. 2002). Similarly to DINP, it seems thus appropriate to consider a dermal absorption rate for humans of 4%. Starting from the oral DNEL for adults, the dermal DNEL for both adults and children can be derived as follows: 0.075 mg/kg bw/day x 2 (to neutralise modification of the dose descriptor) x 50/4 = 1.88 mg/kg bw/day.

The DNELs for repeated dose toxicity for DIDP are summarised in Table 4.62.

Table 4.62 DNELs for repeated dose toxicity with DIDP

	Repeated dose toxicity	
Route	Adults	Children
Oral (mg/kg bw/day)	0.075	0.075
Inhalation	0.35	0.26
Dermal	1.88	1.88

Reproductive toxicity
A NOAEL of 33 mg/kg bw/day was selected for reproductive toxicity.

Critical effects were observed in two-generation reproductive toxicity studies (decreased survival index and pup weight) and prenatal developmental toxicity studies (increased skeletal and visceral variations). A NOAEL of 100 mg/kg bw/day for skeletal variations and a NOAEL of 40 mg/kg bw/day for foetal variations were derived from prenatal developmental toxicity studies conducted according to a previous OECD test guideline where the exposure period did not cover the whole foetal development. Ossification as well as kidney development continues until birth and beyond and more severe effects may be anticipated after a longer exposure period. Thus, an additional assessment factor could be applied to extrapolate to the exposure of the full foetal period. By applying an assessment factor of 2 this would lead to NOAELs of 50 mg/kg bw/day and 20 mg/kg bw/day which would further support the selected NOAEL of 33 mg/kg bw/day based on reduced survival index of F2 pups.

The derived NOAEL for reproductive effects is in particular relevant to derive DNELs for pregnant women, i.e. to the developing foetus in the mother's womb. Naturally, the DNELs based on repeated dose toxicity are equally applicable to the pregnant women themselves (considered under adult population).

For small children, a DNEL for reproductive toxicity (developmental toxicity) has been derived as well. The observed decreased body weight of F2 pups during lactation in the 2-generation study with rats by Hushka et al. (2001) is considered the relevant effect for DNEL derivation for small children. The observed effects are thought to arise from postnatal exposure via feed starting from postnatal day 14 as well as lactational exposure. Lactational exposure to DIDP has not been quantified, but is considered to be low in comparison to foetal exposure. The neonatal dose has been approximated by measuring the concentrations of MiNP and its oxidative metabolites (MHiNP, MCiOP and MOiNP) in pooled plasma samples on PND 2 (Clewell et al. 2011b). MiNP and MCiOP were present at comparable levels and showed similar clearance rates in the maternal and foetal blood (Clewell et al. 2011a). A comparison of the results of this study with the study by Clewell et al. (2011b) is indicative of lower exposure of the pups compared to the foetus. Clewell et al. (2011a) found peak foetal plasma levels of 21 µM MiNP and Clewell et al. (2011b) found pup plasma levels of 0.02 µM (point measurement). The difference between the peak foetal plasma levels and measured pup plasma levels was much less drastic for MCiOP (20 µM and 1.7 µM). It has to be noted that the study by Clewell et al. (2011b) does not provide information concerning the changes of the metabolites in time and comparison of the data is therefore very difficult. It can be concluded however, that there clearly is exposure via milk as evidenced by a dose-related increase in DINP metabolites in pup plasma (Clewell et al. 2011b).

Increased neonatal mortality in Hushka et al. (2001) is considered to be related mainly to foetal exposure. However, some effects due to lactational exposure cannot be excluded. Thus, also lactational exposure may have partly affected the neonatal survival incidence and it is therefore considered that this finding supports the NOAEL for reduced body weight of pups. In fact, the NOAEL for both effects is 0.06%, but the lowest intake of DIDP by dams postpartum (lactation) is 52 mg/kg bw/day instead of 33 mg/kg bw/day which is the lowest intake during all exposure periods (premating, gestation and lactation).

Adults
To correct for the species differences in absorption (adult rats absorb about 50% whereas humans around 100%), an endpoint modification with a factor of 2 was carried out to obtain the correct starting point for derivation of the oral DNEL. See also section 4.4.11.1.

For the derivation of DNELs from the NOAEL based on increased neonatal mortality it was considered appropriate to apply an additional correction factor of 2 for severity of the effect.

Similarly to DINP, it is assumed that transfer of DIDP metabolites across placenta is 100%.

The corrected inhalatory NOAEC can be calculated as follows: NOAEC = 33 mg/kg bw/day x 1/1.15 m3/kg bw/day x 50/75 = 19.13 mg/m3.

Table 4.63 Overview of derivation of DNELs for reproductive toxicity in adults exposed to DIDP

Route	Dose descriptor	Modification	AF	DNEL
Oral	NOAEL = 33 mg/kg bw/day (for reduced neonatal survival in F2; two-generation reproductive toxicity study)	bioavailability factor = 1/2 (50% rats, 100% adults and 100% mother to foetus)	Overall AF = 200 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response	0.08 mg/kg bw/day

			(severity of effect): 2 Quality of database: 1	
Inhalation	NOAEL = 33 mg/kg bw/day (two-generation reproductive toxicity study)	Inhalation NOAEC = 19.13 mg/m3	Overall AF= 50 Interspecies: 2.5 (AS of 1 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response (severity of effect): 2 Quality of database: 1	0.38 mg/m3
Dermal	NOAEL = 33 mg/kg bw/day (two-generation reproductive toxicity study)	Absorption factor 50/4 (50% oral rats, 4% dermal humans) Dermal NOAEL = 412.5 mg/kg bw/day	Overall AF = 200 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response (severity of effect): 2 Quality of database: 1	2.06 mg/kg bw/day

Children -Reproductive toxicity
Table 4.64 gives an overview of the derived DNELs for reproductive toxicity for children exposed to DIDP. 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 = 52 mg/kg bw/day x 1/1.15 m^3/kg bw/day x 50/100 = 22.6 mg/m^3.

It was not considered appropriate to apply an additional correction factor of 2 for severity of the effect (neonatal mortality).

Table 4.64 Overview of derivation of reproductive DNELs for children exposed to DIDP

Route	Dose descriptor	Modification	AF	DNEL
Oral	NOAEL = 52 mg/kg bw/day (reduced body weight in F2 pups in two-generation reproductive toxicity study)	bioavailability factor = 1/2 (50% rats, 100% for children) NOAEL = 26 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	0.26 mg/kg bw/day

Inhalation	NOAEL = 52 mg/kg bw/day (two-generation reproductive toxicity study)	Inhalation NOAEC = 22.6 mg/m3	Overall AF = 25 Interspecies: 2.5 (AS of 1 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	0.904 mg/m^3
Dermal	NOAEL = 52 mg/kg bw/day (two-generation reproductive toxicity study)	Absorption factor 50/4 (50% oral rats, 4% dermal humans) dermal NOAEL = 650 mg/kg bw/day	Overall AF = 100 Interspecies: 10 (AS of 4 and remaining differences 2.5) Intraspecies: 10 Exposure duration : 1 Issues related to dose-response: 1 Quality of database: 1	6.50 mg/kg bw/day

4.5 Human health hazard assessment of physico-chemical properties

4.5.1 Explosivity

DINP and DIDP have no explosive properties (EC, 2003a and EC, 2003b).

4.5.2 Flammability

DINP and DIDP have a very low degree of flammability (flash point >200°C) (EC, 2003a and EC, 2003b).

4.5.3 Oxidising potential

DINP and DIDP have no oxidising potential (EC, 2003a and EC, 2003b).

4.6 Exposure assessment

4.6.1 Introductory remarks

Amec Environment & Infrastructure UK Limited carried out a consumer exposure assessment on behalf of ECHA under Framework contract No ECHA/2008/02 between ECHA and AMEC Environment & Infrastructure UK Limited (AMEC). The ECHA review report used this assessment as a basis.

Environmental release is calculated in the EU Risk Assessments for DINP and DIDP (EC 2003a,b). The values are relevant for the calculations of exposure of man via environment. It was considered outside the scope of the current review to update the estimations of exposure of man via environment in the EU Risk Assessments for DINP and DIDP. The values are discussed in Section 4.7.7.

General considerations on migration and exposure

DINP or DIDP are used in high volumes to make PVC soft and flexible. The soft PVC is used in a wide array of applications (see also section 4.2). The service-life of the PVC articles can range from very short to 50 years or more. (ECPI 2011b)

Phthalates are not covalently bound to the PVC matrix. The plasticiser molecules are intercalated between the polymer chains, where electrostatic plasticiser-plasticiser, plasticiser-polymer, and polymer-polymer interactions occur between the dipoles (Van der Waals forces). Plasticisers can be released by volatisation, extraction to a liquid, or by migration to a solid or semi-solid. The conditions of migration depend on the type of contact, contact duration, temperature, concentration difference, concentration level, simulant properties, molecular weight and structure (ECPI 2011b; INEOS ChlorVinyls 2012). It is important to consider that the actual driver for migration is determined by thermodynamics, i.e. a reduction of free energy (INEOS ChlorVinyls 2012). Phthalates are highly lipophilic, and therefore fatty simulants, such as olive oil, can produce significant migration in contrast with non-lipophilic media (INEOS ChlorVinyls 2012). For articles requiring a long service-life (e.g. flooring, cable), loss of plasticiser results in loss of mechanical performance and leads to product shrinkage and brittleness (ECPI 2011b).

Summary of the existing legal requirements

Entry 52 of Annex XVII to the REACH Regulation restricts DINP, DIDP and DNOP in toys and childcare articles which can be placed in the mouth by children (see Section 2).

According to Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food (consolidating food contact legislation, applicable as from 01/05/2011), the following restrictions apply to DINP and DIDP:

- SML(T)[42] = 60 mg/kg expressed as the sum of the substances (DEHP, DBP, BBP, DINP, DIDP, and 15 other substances)[43]
- SML(T) = 9 mg/kg expressed as the sum of the substances (DINP and DIDP)[44]
- DINP and DIDP shall only to be used as:

[42] 'total specific migration limit' (SML(T)) means the maximum permitted sum of particular substances released in food or food simulants expressed as total of moiety of the substances indicated (Article 3 of Commission Regulation (EU) No 10/2011).
[43] Group restriction 32
[44] Group restriction 26. According to Article 3 of Commission Directive 2007/19/EC, Member States had to ensure that the restrictions would apply by 1 June 2008.

(a) plasticiser in repeated use materials and articles;
(b) plasticiser in single-use materials and articles contacting non-fatty foods except for infant formulae and follow-on formulae as defined by Directive 2006/141/EC or processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC;
(c) technical support agent in concentrations up to 0,1 % in the final product.

Recurring main assumptions

Age groups
Children were assessed in age groups of 0-6 months, 6-12 months, and 12-18 months old. The reason for assuming these age groups stems from the available observation studies of mouthing behaviour of children (Table 4.67). It is clear that children of 0-18 months old have the longest daily mouthing duration of articles (excluding pacifiers). Juberg et al. (2001) reported a statistically significant shorter mouthing time in the age group 19-36 months as compared to the 0-18 months old children. From Smith and Norris (2002) it is also clear that the estimated daily mean mouthing of toys and other objects (excluding pacifiers and anatomy) is at its peak level at the age of 6-9 months. In this age category also the highest maximum mouthing of toys was observed (3h 47min) (Smith and Norris 2002).

Body weight
For the assumptions of the body weight for the different age groups for children, the Children's toys fact sheet (Bremmer and van Veen 2002) was used. This gave 6.21 kg for 0-6 months, 7.62 kg for 6-12 months and 9.47 kg for 12-18 months old children. Adult body weight was assumed to be 60 kg from the default body weight for females in the ECHA guidance R15.

4.6.2 Toys and Childcare articles

It is assumed that the oral and dermal daily exposure estimates from mouthing of articles by newborns and infants (0-3 years old) are equal for both DINP and DIDP. This was also assumed in the EU Risk Assessment for DIDP (EC 2003b), and as discussed in section 4.4.1 there are no new data indicating the contrary.

4.6.2.1 Exposure from mouthing

There has been extensive investigation of potential oral exposure to phthalates in PVC toys. Estimates of oral exposure to phthalates in toys have been based on experimentally determined rates of migration of phthalates from soft PVC toys combined with assessments of how long infants and newborns spend mouthing such objects each day. Most of the experimental determinations of phthalate migration have been conducted in in vitro studies employing a variety of protocols. There are also some data from adult volunteer studies in which volunteers have sucked or chewed PVC items and concentrations of phthalate measured in saliva.

Some mouthable parts of childcare articles[45] are made of PVC. The EU Risk Assessments (EC 2003a,b) did not include these articles explicitly in their exposure assessment. It is here assumed that migration rates from these mouthable parts of childcare articles are the same as for PVC toys. The mouthing times derived in the current review cover both toys and the mouthing of all other articles that can be placed in the mouth by children. This includes any PVC articles with DINP or DIDP that are not covered by the existing restriction for toys and childcare articles that can be mouthed by children.

4.6.2.1.1 EU Risk Assessment

The EU Risk Assessments (EC 2003a,b) based the estimates of children's exposure to DINP and DIDP from mouthing toys on the results of a study undertaken by RIVM (1998). The RIVM study was overseen by a "Consensus Group" of interested parties drawn from industry, health experts and regulatory authorities. The study included a human volunteers study to determine release if DINP into saliva; a child observation study to determine mouthing times; as well as the development of a routine laboratory method to determine the release rate from PVC toys.

The EU Risk Assessment for DINP (EC 2003a) combined a migration rate of 8.9 µg/10cm^2/min (53.4 µg/cm2/h) with a mouthing time of 3 h/day and an assumed body weight of 8 kg to derive an oral daily exposure estimate for newborns and infants (0-3 years old) to DINP in toys and teething rings of 200 µg/kg bw/day. The migration rate selected in the EU Risk Assessment was the highest individual estimate from the RIVM human volunteer study (see section 4.6.2.1.6). The assumed mouthing duration was based on the maximum mouthing time (excluding pacifiers but including other non-toy objects) in the observation study undertaken by RIVM.

The EU Risk Assessment for DIDP assumed that the oral daily exposure estimate for newborns and infants (0-3 years old) to DIDP was equal to DINP (EC 2003b).

4.6.2.1.2 Other Assessments

In addition to the RIVM (1998) assessment that also formed the basis of the estimated exposures to DINP and DIDP in the EU Risk Assessments, a number of other exposure assessments for DINP in toys have been published.

[45] Such as changing mats, pushchairs, high chairs, cribs, changing table pillows, carrying slings, breastfeeding pillows and car seats.

The studies by the US Consumer Product Safety Commission (US CPSC) and Sugita et al. (2003) are based on original data whereas other assessments were based on previously published information (Table 4.65).

Sugita et al. (2003) is a publication in Japanese: the reported information is based on the English abstract, figures and tables, as well as on ECPI (2009). Exposure estimates were generated using Monte Carlo simulation and therefore took account of all of the mouthing and migration data rather than being based on a single value for either.

Table 4.65 Published estimates of children's oral exposure to DINP in toys (µg/kg bw/day)

Study	Age in months	Toys, teethers and rattles		Pacifiers	
		Mean	95th Percentile (maximum)	Mean	95th Percentile
EU Risk Assessment (EC 2003a,b)	0-36 months	-	(200)		
RIVM (1998)	3-6	14.4	39.7 (112)		
	6-12	11.6	38.9 (204)		
	12-18	3.4	16 (89.4)		
	13-36	1.7	6.4 (30.2)		
Sugita et al. (2003)	3-10	14.8	35.7	21.4*	65.8*
US CPSC (2002)	3-12	2.91	10.71	4.75	24.55
	12-24	0.84	3.35	2.82	17.44
	24-36	0.28	1.25	1.71	5.41
CHAP (2001)	0-18		280		
	19-36		66		
Austrian Standards Institute Fiala et al. (2000)	-	31.25			
Health Canada [*2]	3-12	44	(320)	120	(640)
	13-36	39	(228)	62	(458)
Danish EPA Tonning et al. (2009)	24		(3.91)[*3]		

* Total toys and pacifiers
[*2] Based on RIVM (1998) migration measurements and 5th percentile bodyweights
[*3] Combined dermal and oral exposure – based on toy with highest measured migration rate of 5.5 mg/kg/h

There have been no recent evaluations of the potential exposure of children to DIDP in toys and exposure to DIDP in toys and pacifiers has not been subject to the same level of scrutiny as DINP. The EU Risk Assessment for DIDP assumed that the level of exposure to DIDP in toys would be equivalent to that calculated for DINP (i.e. 200 µg/kg bw/day). Published estimates of DIDP exposure arising from toys are much lower than for DINP (Table 4.66).

Table 4.66 Estimated maximum dose of DIDP µg/kg bw/day for a child of 8 kg mouthing toys for 3 hours/day with a mouthed surface area of 10 cm2 (from EU Risk Assessment, EC 2003b).

DIDP	Reference
17	CSTEE (1997a)
0.004	Artsana as cited in CSTEE (1997b)
<4	CEFIC-ECPI as cited in CSTEE (1998)
0.005	Artsana as cited in CSTEE (1997c)
19	Gesundheidsbescherming as cited in CSTEE (1997c)
7	Based on RIVM (1998) – CSTEE (1998a)

4.6.2.1.3 Mouthing times

The relevant studies of the mouthing behaviour of children are described in what follows. See also Table 4.67 for an overview of the data. The studies used either professional or parental observation. Smith and Norris (2003) compared results from professional and parental observations and found that the results are essentially the same.

Juberg et al. (2001)
Juberg et al. (2001) employed parental observations with US children aged 0 to 36 months. It could be mentioned that Juberg et al. (2001) is the largest available observation study. The study is available as a peer reviewed article (no full report was available).

In Phase III of the study, 168 children aged 4-21 months were observed for 5 non-consecutive days over a two month period (793 valid child observation days). Data was reported for 'pacifiers' and for 'non-pacifiers'[46]. The mean mouthing time for non-pacifiers was 36 ±48 min/day for the 4-21 month old children. The mean included 137 zero mouthing days[47] out of 793 observation days (17%). Three out of 168 children in Phase III showed no mouthing behaviour over 5 observation days. The maximum total mouthing time for non-pacifiers was longer than 300 min/day (based on the 5-day average).

Similar data were obtained from Phase I and II, with a mean for non-pacifier mouthing by 0-18 months old children (total of teethers, plastic toys, and other objects[48]) of 33 ±46 min/day (n=107) and a maximum above 200 min/day. The mean mouthing time for plastic toys and teethers was 23 min/day in this age group. There were 35 children that did not mouth in this age group (35/107 = 33%). For the 19-36 months old the mean was 5±14 min/day and a maximum of above 90 min/day (n = 110). There were 79 children that did not mouth in this age group (79/110 = 72%). Excluding zero mouthing days[47], the mean mouthing times were 70 min/day for 0-18 months old (48 min/day for plastic toys and teethers), and 56 min/day for 19-36 months old children.

Juberg et al. (2001) observed a considerable day-to-day variation in mouthing behaviour. The authors described a tendency that a short mouthing time for a child on one day would be higher on another day, and vice-versa for observations of long mouthing times. High frequency events with short duration were not recorded and might be a factor of underestimation. The

[46] According to the lead author (D. Juberg), the category 'non-pacifiers' also included mouthing of the anatomy (Juberg 2013).
[47] "zero mouthing days" are days in which the parents had not observed any mouthing during the entire day.
[48] According to the lead author (D. Juberg), the category 'non-pacifiers' also included mouthing of the anatomy (Juberg 2013). It might thus be assumed that the category 'other objects' includes mouthing of anatomy. However, it was not not known whether parents were asked to report mouthing of anatomy. The proportion attributable to mouthing of anatomy in the category 'other objects' was not reported.

authors reported that this was not a common observation among all participants. However, it is not clear from the study if parents were asked to report what they had not recorded. The question could be raised here whether asking parents to record mouthing times over a whole day can be expected to lead to additional unrecorded events[49].

The authors interviewed parents of 3 child participants with a consistently high mouthing time over 5 days, and reported that the behaviour would not occur always. The authors did not provide a similar explanation on the basis of follow-up interviews for the very many observed zero mouthing days. However, the authors pointed out that there were fewer children with zero mouthing days in 5 observation days in comparison with single day observations.

The Juberg et al. (2001) was used to set a plausible upper boundary of 3 h mouthing of toys in the Chronic Hazard Advisory Panel (CHAP 2001) risk assessment of DINP.

Smith and Norris (2003)
Smith and Norris (2003) studied 236 children aged 1-5 years in age by parental observation. The observation time per child was 5 hours split in 20 observations of 15 minutes (study by DTI). The results and the methodology were reported in great detail used (full report published). Participants were provided with a stopwatch that was started and stopped, but not reset, during the observation periods of 15 minutes (there were 20 such observation periods per child). This allowed to also account for very short mouthing behaviours. Methodology and results were reported in detail (the full report is published). Mean and maxima were reported for 12 age groups for 'dummy/soother', 'fingers', 'toys', 'other objects', and 'not recorded' (= unknown objects).

The highest average mouthing time for all toys was 39 minutes/day for children aged 6-9 months and the highest individual estimate was 227 min/day for a child aged 6-9 months. The highest mean mouthing time for all items was 119 min/day for 18-21 month old children. When pacifiers were subtracted from the total mouthing time, the highest mean value was 90 min/day for 3-6 month olds. When not considering pacifiers nor mouthing of fingers, the highest mean value was 63 min/day for 6-9 month olds).

The authors observed that 50% of the mouthed 'toys' and 'other objects' were made from plastic

Greene (2002)
The US CPSC staff (Greene 2002) carried out an observational study of children's mouthing activity in the Chicago and Houston metropolitan areas, as also reported in Babich et al. (2004). The latter publication contained some error however[50]. The study used trained

[49] Can parents observe their child continuously? While observing, parents need to attend to their daily tasks, which might hamper recording if recoring spans a long time. Perhaps there could be a phenomenon of 'recording fatigue' as well?

[50] A comment submitted during the public consultation pointed out that *"The CPSC (2002) [Greene 2002] reported the 95th percentile mouthing time for 3-12 month-olds to soft plastic toys, teethers, and rattles equal to approximately 17.9 minutes/day. The 95th percentile daily mouthing time to soft plastic toys alone was 7.1 minutes/day."* (ECPI 2012b). Since the comment presented figures from a different mouthing category, the comment did not point to any mistakes. However, after the public consultation, on 10 October and 9 November respectively ECPI (2012c) and CEFIC (2012) commented that the ECHA report contained an error in the mouthing time it used from Greene (2002) .

In ECHA's draft report, a 95th percentile of 127 min/day was calculated from Table 4 in Babich et al. (2004) for the category "All soft plastic items except pacifiers" for 3-12 months old. Babich et al. (2004) used the data from Greene (2002) as its source. Upon close examination, the peer reviewed publication by Babich, Greene and colleagues from 2004 contained several mistakes . As a consequence the 95th percentile used by ECHA from this study for the 3-11 month old children (127 minutes/day) was incorrect. Babich (2013) confirmed the error in Babich et al. (2004). The corrected figures are reported in the current document.

observers that monitored the mouthing activity of 169 children during 4 hours (twelve 20 minutes observations spread over 2 days). Data were reported for 3-11 months, 12-23 months and 24-36 months old children. The raw data had been processed by Greene (2002) as the data did not follow a known distribution. Bootstrapping (a resampling method) is used by Greene (2002) to generate a dataset with a normal distribution. Each resampling of the database gives a bootstrap sample. The procedure was repeated to generate 5000 bootstrap samples. From the resulting normalised data 95th percentiles were calculated. The summary statistics are reported in Table 4.67.

The categories were grouped as follows:

All Objects
- Non Pacifiers
  - Soft Plastic Objects
    - Soft Plastic Food Contact Items[4]
    - Soft Plastic Non Food Contact Items
      - Soft Plastic Toys, Teethers and Rattles
        - Soft Plastic Toys
        - Soft Plastic Teethers and Rattles
      - Other Soft Plastic[5]
  - Anatomy[6]
  - Toys, Teethers and Rattles, not soft plastic
  - Other Objects[7]
- Pacifiers

[4] Bottle, Drinking Cup/Straw, Fork.
[5] Clothing, Furniture, Other, unknown
[6] Hair, skin, fingers, hands
[7] Books, clothing, carpet and furniture, non soft plastic food contact items such as spoons and cups.

RIVM (1998)
RIVM (1998) employed parents' observation to determine the frequency and duration of mouthing events. The observation of 42 children lasted 15 minutes and were repeated 10 times in two different days. The mean total mouthing time (excluding pacifiers) for the 6-12 month age group was 44.0 ±44.7 min/day with a maximum mouthing time of 171.5 minutes. The mouthing of anatomy in this age group was 7.5 ±11.6 min/day.

RIVM (1998) did not include pacifier use in their estimates of exposure as it was considered that these are not usually made of PVC and contain no plasticisers.

As described above in section 4.6.2.1.1, the EU Risk Assessments (EC 2003a,b) used a mouthing time of 3 h/day based on the maximum total mouthing time (excluding pacifiers but including all other mouthing activity) in the observation study undertaken by RIVM (171.5 min/day).

Sugita at al. (2003)
Sugita at al. (2003) undertook a mouthing observation survey for 25 children. Estimated total mouthing times, including the use of pacifiers, ranged widely from 11.4 to 351.8 min/day (mean = 105.3 +/- 72.1 min/day). The mean of the total mouthing time without pacifiers was 73.9 +/- 32.9 min/day. The mean mouthing duration for all toys ranged from about 18-37 min/day across age categories (6-10 months).

Beamer et al. (2008)
In a relatively small study by Beamer et al. (2008) as reported in US EPA (2011) of 23 US farm workers' children[51] that employed video footage the median hand-to-mouth frequency was 15.2 events/hour and the median object-to-mouth frequency was 27.2 events/hour. The hourly mouthing duration was 1.2 and 2.2 min/h with the hands and objects, respectively. The

[51] Participants were 6- to 13-month-old infants or 20- to 26-month-old toddlers.

median mouthing duration with hands and objects was 2 seconds. Boys had higher contact frequencies while girls had longer contact durations.

Table 4.67 Summary of published estimates of mouthing times (minutes/day) in young children

Reference	Description	Age (months)	Mean mouthing time ±1 s.d. (min/day)	95th percentiles (Maximum)
Juberg et al. (2001)	Combined results from pilot and second phase data from western New York State (Phase I and Phase II) 1 day parental observation n = 107 + 110			
	Pacifiers	0-18	108 ±187 (all n=107) 221 (those mouthing n=52)	(>800)
	Teethers	-"-	6 (all n=107) 20 (those mouthing n=34)	
	Plastic toys	-"-	17 (all n=107) 28 (those mouthing n=66)	
	Other objects	-"-	9 (all n=107) 22 (those mouthing n=46)	
	Non-pacifier (total of teethers plastic toys and other objects, including anatomy)	-"-	33 ±46 (all n=107)	(> 200)
	Pacifiers	19-36	126 ±246 (all n=110) 462 (those mouthing n=52)	(> 700)
	Teethers	-"-	0 (all n=110) 30 (those mouthing n=1)	
	Plastic toys	-"-	2 (all n=110) 11 (those mouthing n=28)	
	Other objects	-"-	2 (all n=110) 15 (those mouthing n=18)	
	Non-pacifier (total of teethers plastic toys and other objects)	-"-	5 ±14 (all n=110)	(> 90)
	Phase III results n = 168 children,			

	most of these values (n = 146) corresponded to 5 valid parental observation days (793 valid child observation days in total)			
	Non-pacifier	4-21	36 ±48 (incl. 137 zeros in 793 observation days)	(>300)
Smith and Norris (2002)	5h (20 * 15 minutes) parental observation n = 236 children			
	Pacifiers	1-3 (n = 9) 3-6 (n = 14) 6-9 (n = 15) 9-12 (n = 17) 12-15 (n = 16) 15-18 (n = 14) 18-21 (n = 16) 21-24 (n = 12) 2y (n = 39) 3y (n = 31) 4y (n = 29) 5y (n = 24)	47.2 27.8 14.6 41.7 60.3 25.4 69.0 25.2 32.9 48.7 16.7 0.3	(175) (153) (100) (324) (212) (220) (318) (115) (217) (304) (322) (8)
	Finger	1-3 (n = 9) 3-6 (n = 14) 6-9 (n = 15) 9-12 (n = 17) 12-15 (n = 16) 15-18 (n = 14) 18-21 (n = 16) 21-24 (n = 12) 2y (n = 39) 3y (n = 31) 4y (n = 29) 5y (n = 24)	18.3 49.0 16.9 14.0 8.4 10.1 18.7 35.6 29.7 34.7 19.4 44.1	(51) (96) (77) (99) (36) (39) (81) (113) (148) (199) (171) (543)
	Toys	1-3 (n = 9) 3-6 (n = 14) 6-9 (n = 15) 9-12 (n = 17) 12-15 (n = 16) 15-18 (n = 14) 18-21 (n = 16) 21-24 (n = 12) 2y (n = 39) 3y (n = 31) 4y (n = 29) 5y (n = 24)	0.2 28.3 39.2 23.1 15.3 16.6 11.1 15.8 12.4 11.6 3.2 1.9	(1) (155) (227) (64) (44) (58) (33) (102) (126) (95) (21) (11)
	Other object	1-3 (n = 9) 3-6 (n = 14)	5.2 12.5	(28) (37)

		6-9 (n = 15) 9-12 (n = 17) 12-15 (n = 16) 15-18 (n = 14) 18-21 (n = 16) 21-24 (n = 12) 2y (n = 39) 3y (n = 31) 4y (n = 29) 5y (n = 24)	24.5 16.4 12.0 23.0 19.8 12.9 21.8 15.3 10.7 10.0	(70) (91) (63) (98) (66) (40) (178) (85) (77) (53)
	Total (all objects)	1-3 (n = 9) 3-6 (n = 14) 6-9 (n = 15) 9-12 (n = 17) 12-15 (n = 16) 15-18 (n = 14) 18-21 (n = 16) 21-24 (n = 12) 2y (n = 39) 3y (n = 31) 4y (n = 29) 5y (n = 24)	71.8 117.7 95.2 95.3 96.0 75.2 118.8 103.7 99.5 110.3 50.1 59.3	(212) (216) (317) (413) (257) (315) (412) (395) (462) (510) (329) (601)
	Total all objects without pacifiers [*2]	1-3 (n = 9) 3-6 (n = 14) 6-9 (n = 15) 9-12 (n = 17) 12-15 (n = 16) 15-18 (n = 14) 18-21 (n = 16) 21-24 (n = 12) 2y (n = 39) 3y (n = 31) 4y (n = 29) 5y (n = 24)	24.6 89.9 80.6 53.6 35.8 49.9 49.8 78.5 66.5 61.6 33.4 59.0	
	Total all objects without pacifiers and fingers [*2]	1-3 (n = 9) 3-6 (n = 14) 6-9 (n = 15) 9-12 (n = 17) 12-15 (n = 16) 15-18 (n = 14) 18-21 (n = 16) 21-24 (n = 12) 2y (n = 39) 3y (n = 31) 4y (n = 29) 5y (n = 24)	6.2 40.9 63.7 39.5 27.4 39.7 31.1 42.9 36.8 26.9 14.0 14.9	
Greene (2002)	4h (12 * 20 min) observations by trained observers n = 169			
	Pacifiers	3-11 (n = 54) 12-23 (n = 66) 24-36 (n = 49)	roughly 34[*1] roughly 26 roughly 18	roughly 195 roughly 199 roughly 48
	Non Pacifiers	3-11 (n = 54) 12-23 (n = 66)	70.1 47.4	134.4 121.5

		24-36 (n = 49)	37.0	124.3
	Anatomy	3-11 (n = 54) 12-23 (n = 66) 24-36 (n = 49)	roughly 24[*1] roughly 17 roughly 12	roughly 101 roughly 83 roughly 51
	Non Pacifiers - Anatomy	3-11 (n = 54) 12-23 (n = 66) 24-36 (n = 49)	roughly 46[*1,2] roughly 30 roughly 25	
	Soft plastic toys	3-11 (n = 54) 12-23 (n = 66) 24-36 (n = 49)	1.3 1.9 0.8	7.1 8.8 3.3
	Soft plastic items (except pacifiers)	3-11 (n = 54) 12-23 (n = 66) 24-36 (n = 49)	4.4 3.8 4.2	17.5 13.0 18.5
	All items	3-11 (n = 54) 12-23 (n = 66) 24-36 (n = 49)	roughly 101[*1] roughly 73 roughly 53	roughly 262 roughly 220 roughly 156
RIVM (1998); Groot et al. (1998) as cited in Bremmer and van Veen (2002)	10 times 15min, parental observation, extrapolated to time awake and not eating/day. n = 42			
	Pacifier	3-6 (n=5) 6-12 (n=14) 12-18 (n=12) 18-36 (n=11)	94.9 27.3 17.3 20.8	
	Toys meant for mouthing	3-6 (n=5) 6-12 (n=14) 12-18 (n=12) 18-36 (n=11)	3.4 ±5.1 5.8 ±11.4 0.0 ±0.1 0.0 ±0.0	
	Other toys	3-6 (n=5) 6-12 (n=14) 12-18 (n=12) 18-36 (n=11)	11.3 ±10.0 22.1 ±28.5 3.6 ±3.5 1.1 ±1.2	
	Non-toys	3-6 (n=5) 6-12 (n=14) 12-18 (n=12) 18-36 (n=11)	2.8 ±2.8 9.4 ±8.4 7.2 ±14.2 2.0 ±3.4	
	Fingers	3-6 (n=5) 6-12 (n=14) 12-18 (n=12) 18-36 (n=11)	20.5 ±18.8 7.5 ±11.6 5.8 ±14.9 6.3 ±9.1	
	Total	3-6 (n=5) 6-12 (n=14) 12-18 (n=12) 18-36 (n=11)	131.8 71.3 33.6 30.1	
	Total mouthing time, excluding pacifiers	3-6 (n=5) 6-12 (n=14) 12-18 (n=12) 18-36 (n=11)	36.9 ±19.1 44.0 ±44.7 16.4 ±18.2 9.3 ±9.8	(67.0) (171.5) (53.2) (30.9)
Sugita et al. (2003) n = 25	total mouthing time with pacifiers	6-10	105.3±72.1	(351.8)

	total mouthing time without pacifiers	6-10	73.9±32.9	(136.5)
Beamer et al. (2008) n=23	Hands	6-26	1.2 min/h	
	objects	-"-	2.2 min/h	

*[1] The values in Greene (2002) in minutes per hour were multiplied by 10 to obtain daily values (as supported by Babich et al 2004, footnote to table 4).
*[2] Calculated from the above

4.6.2.1.4 Discussion

Methodology
Two of the key studies used parental observations, whereas Greene (2002) used professional observation. Smith and Norris 2002 included a study with professional observations and concluded that the results of professional and parental observations are essentially the same. This conclusion is not necessarily valid for other studies using a different methodology, but differences as a consequence to the type of observer per se can thus be assumed to be small.

Bootstrapping is used by Greene (2002) to generate a dataset with a normal distribution. The mean and maximum values of the raw data were not reported and the influence of the bootstrapping method on these values was not discussed.

High frequency events with short duration were not recorded by Juberg et al. (2001) and might be a factor of underestimation. Furthermore in Juberg et al. (2001) parents were asked to record the duration in minutes, which may have led to rounding to the nearest minute. Moreover asking parents to record mouthing behaviour for the entire day would likely result in omissions of recording such behaviour, and in addition might lead to 'recording fatigue'. On the other hand, the observation over a whole day might also be seen as increasing the representativity for daily mouthing behaviour. In Smith and Norris (2002) and Greene (2002), a stopwatch was used that allowed to account also for very short mouthing behaviours.

The possible mouthing of items during the sleep time were not taken into account in any of the estimates.

Relevance of studies
The key studies have relatively small sample sizes. The short data collection periods may not represent behaviour of children over longer time periods. Two of the key studies (Juberg et al. 2001 and Greene 2002) were of US children and a small number of locations. The key studies are not necessarily representative for the EU child population.

The Juberg et al. (2001) is the largest in terms of observation time (793 full observation days in Phase III versus 4 hours per child in the Greene (2002) and 5 hours in the Smith and Norris (2003) and similar in terms of population sampled[52].

Discrepancy between results
There is a large discrepancy between the maximum value of 227 min/day for mouthing of toys by a child aged 6-9 months in Smith and Norris (2002) (with a mean of 39 min/day) and the 95th percentile of 17.5 min/day for 3-11 months old for mouthing of soft plastic items as calculated by a bootstrap procedure in Greene (2002). Furthermore, in contrast to the *95th*

[52] Using comparable age groups the keys studies made observations for 107 children in the age category 0-18 months in Juberg et al. (2001), 120 children in Greene (2002) in the category 3-23 months, and 113 children in the age category 0-24 months in Smith an Norris (2002).

percentile for soft plastic items in Greene (2002), the *mean* mouthing time for plastic toys and teethers in Juberg et al. (2001) was 23 min/day for 0-18 months old children. Excluding children that did not show mouthing activity, the *mean* mouthing times for plastic toys and teethers was 48 min/day in this age category. In addition, the category 'other objects' from Juberg et al. contained further plastic items mouthed by children not belonging to the 'plastic toy' or 'teether' categories.

Skewness

All studies indicate that the distribution of mouthing data is highly skewed. Many observations are from children that are either not mouthing at all, or very little, during the observation time. Juberg et al. (2001) reported that 33% of the observed children did not mouth in the age group of 0-18 months. For the 19-36 months the children that did not mouth amounted to 72%.

As Van Engelen et al. (2006) pointed out, it seems not appropriate to take into account the zero mouthing times when the goal is to protect the majority of children. In fact, it could be questioned whether it useful to characterise a reasonable worst case estimate for characterising the risk of mouthing articles in a population that does not show or shows extremely little such behaviour.

Excluding zeros in Juberg et al. (2001), the mean mouthing time for non-pacifier items went from 33 ±46 to 70 min/day for 0-18 months old (i.e. by a factor of 2) and from 5±14 to 56 min/day for 19-36 months old children (i.e. by a factor of 11). Greene (2002) nor Smith and Norris (2002) discussed the impact of excluding zeros.

Article categories

The category of articles legally covered by the existing restriction entry 52 for toys and childcare articles which can be placed in the mouth by children is different from any of the categories that are covered in the observation studies. It is thus not straightforward to select an appropriate figure for a typical mouthing scenario for the articles covered by the existing restriction. Furthermore, in order to estimate the total exposure from DINP and DIDP, an assessment is needed of exposure from other article groups that can be mouthed but do not fall under the scope of the existing restriction. Such articles could be children's clothing and footwear insofar they would be mouthed and contain flexible PVC (see section 2 concerning interpretations on the scope of the existing restriction) but also anything that is not specifically marketed for children.

Parents frequently determine accessibility to mouthed items (Juberg et al. 2001). The authors (Juberg et al. 2001) use this statement in the context of pacifier mouthing. It can however be assumed that such preference by parents would exist also for other article types (e.g. parents buying the articles and handing them over to the child). It also seems reasonable to assume that children would develop a preference for mouthing certain articles over others (favourite toys). As a consequence, mouthing might for some children be restrained to a few articles whereas other children might mouth very many different objects (that might contain or not contain DINP or DIDP).

Moreover, not all children in Europe or in all layers of society might have an abundance of toys or other articles to choose from. It might also be mentioned that although pacifiers should be excluded in the estimate of a reasonable worst case mouthing time for articles, in some populations pacifiers might be less common and thus the figures of mouthing other articles than pacifiers might be somewhat underestimated for those populations (mouthing of pacifiers might be at the expense of mouthing of other items).

The following background information to the selection of an appropriate mouthing time could be noted:

- *The mouthing time used in the opinion of RAC on lead in jewellery*
  RAC assumed a daily mouthing time of 1 h for jewellery but noted that this is a worst-case estimate. RAC came to the conclusion based on the data from Groot et al. as reported in the RIVM Children's Toys Fact Sheet (Bremmer and van Veen 2002). The categories 'other toys' and 'non-toys' were considered in the default values that were assumed as relevant by RAC to assume a value of 1 hour mouthing of jewellery per day (thus excluding 'pacifiers' and 'toys for mouthing').

  As mouthing of jewellery can be assumed to be far less frequent than mouthing of toys, childcare articles, or other plastic items containing DINP or DIDP, it would be inconsistent to assume a mouthing time lower than 1 h in the exposure assessment of children to DINP and DIDP from such articles.

- *The mouthing time used in the EU Risk Assessment for DINP and DIDP*
  The EU Risk Assessment (2003) for DINP used a mouthing time of 3 h/day based on the maximum total mouthing time (excluding pacifiers but including all other mouthing activity) in the observation study undertaken by RIVM (171.5 min/day in Groot et al. 1998).

- *The mouthing time according to the ECHA guidance*
  Chapter R.17 Estimation of exposure from articles of the ECHA Guidance on information requirements and chemical safety assessment advises to use parameters of mouthing behaviour provided by RIVM (Van Engelen et al. 2006). Section 3.5.3 of Van Engelen et al. (2006) reviewed the available literature comprising the studies from De Groot et al. (1998); Juberg et al. (2001); Smith and Norris (2003); and Babich et al. (2004) . It was observed that the mouthing data was highly skewed and included children that do not mouth at all. The authors concluded that, to safeguard the relatively small group of children that display these longer mouthing times, the value of 3 hours as adopted by the CSTEE opinion of 1998 on phthalates in toys remains the recommended value for risk assessments.

4.6.2.1.5 Conclusion

The following results from the key mouthing studies could be used to inform the derivation of a reasonable worst case mouthing estimate (mean mouthing times inform the lower boundary and maxima the higher boundary to a reasonable worst case estimate for mouthing of articles containing DINP or DIDP):

- Juberg et al. (2001) reported mean mouthing times for non-pacifiers[53] of 70 min/day, and for plastic toys and teethers 48 min/day, for children of 0-18 months old (without zeros, i.e. only taking into account children that mouthed).
- Greene (2002) reported mean mouthing times for non-pacifiers of 70 min/day and excluding mouthing of anatomy a mean of 46 min/day for 3-11 month old children[54].
- Smith and Norris (2002) reported highest mean values for mouthing articles, excluding pacifiers and fingers, of 64 min/day for 6-9 month olds and 43 min/day for 21-24 month old children.

[53] The category 'non-pacifiers' included mouthing of the anatomy.
[54] These bootstrapped results include all children, also possibly children that do not mouth.

- Greene (2002) reported 95th percentiles of 18 min/day for soft plastic items and 134 min/day for non-pacifiers for children of 3-11 months.
- Smith and Norris (2002) reported a maximum value of 227 min/day for mouthing of 'toys' for children aged 6-9 months (with a mean of 39 min/day), and 178 min/day for 'other objects' for children of 2 years old (with a mean of 22 min/day).
- Juberg et al. (2001) reported maximum mouthing times for non-pacifiers of over 200 and 300 min/day for 0-18 and 4-21 month old children respectively.

Based on these estimates, and considering the limitations and discrepancies in the data, as well as the skewness and difficulties to determine appropriate article categories discussed above, it seems that a mouthing time of 2 hour is appropriate for a reasonable worst case scenario for mouthing of articles containing DINP or DIDP by children up till 18 months.

RAC (2013a,b) is of the opinion that the assumption of 2 hour mouthing time per day is appropriate for a reasonable worst case scenario for mouthing of articles containing DINP or DIDP by children. RAC noted that a mouthing time of 3 hours/day was assumed in the EU RARs from 2003 and that 3 hours mouthing time is also the recommended value for risk assessment according to the ECHA guidance.

Average mouthing behaviour in the child population (0-18m)
Deriving an average mouthing time is not crucial for risk assessment purposes, but it can give an estimate of the likely exposure of an average child in the population. This average estimate includes the children that do not mouth or mouth very little, which is the majority of children: Juberg et al. (2001) reported 33% children that did not mouth in the age category 0-18 months, and roughly 50% mouthed less than 20 min/day.

A weighted average (weighted by the number of subjects) of the means in the age categories 1-18 months of Smith and Norris (2002) was calculated for all objects without pacifiers and without fingers. This would give 38 min/day. Similarly, from Greene (2002), a weighted average of means from the age category 3-11 and 12-23 for non-pacifiers and excluding anatomy gives 37 min/day.

Thus, a mean mouthing case of 30 min/day might be assumed for mouthing of the relevant articles. It is estimated that half of those articles would be made of plastic (Smith and Norris 2002). An arbitrary assumption could be made that half of these articles would contain DINP or DIDP if the current restriction on toys and childcare articles would be lifted. This would lead to a rough exposure estimate of 7.5 min/day for the average child of 0-18 months to DINP or DIDP containing articles. As this estimate does not impact the conclusions of the risk assessment, no further attempts were made to refine this estimate.

4.6.2.1.6 Migration rates

The other major source of discrepancy between the various published estimates of children's exposure to DINP and DIDP from mouthing toys is the difference in the estimated migration rates of the phthalates from toys (Table 4.68). Some additional measurements of the content of phthalates in toys and childcare articles on the EU market are available, but as no migration rates were reported, the information is not presented here.

The RIVM (1998) study measured DINP in saliva of 20 adult volunteers biting and sucking four samples with a surface of 10m^2. The samples tested were a control disk (PTFE), a standard PVC sample disk (38% w/w DINP), a finger of a hand shaped teether and a disk punched from the same toy (content unknown). The duration of the experiments was four times 15 minutes for each sample. The average levels of release into saliva of the three samples tested were:

1.4 µg/min, 2.4 µg/min and 1.6 µg/min, respectively (ranging from 0.3 to 8.3 µg/min; from 0.9 to 8.9 µg/min and from 0.9 to 5.7 µg/min, respectively). The EU Risk Assessment for DINP used a migration rate of 8.9 µg/10cm^2/min (53.4 µg/cm2/h) selected from the highest individual estimate from the RIVM study.

Table 4.68 Migration rates used in assessments of children's exposure to DINP in toys

Study	Rate µg/cm^2/h	Comments
RIVM (1998); Meuling et al. (2000)	10.8	Adult volunteer study: chew and spit, 10 cm^2 disc Mean levels of leaching for 3 objects, 8.28, 14.64 and 9.78 µg/cm^2/h respectively (overall mean 10.8 µg/cm^2/h)
EU Risk Assessment (EC 2003a)	53.4	Highest rate of leaching in the RIVM (1998) study
Sugita et al. *(*2003)	9.24 +/- 5.68	Study in adult volunteers asked to suck or lick specimens of PVC toys. Average rates for individual toys ranged from 1.32 to 24.04 µg/cm^2/h
US CPSC (2002)	7.0	*In vitro* rates of 6.0-66.6 µg/cm^2/h with mean of 24.6 µg/cm^2/h determined for 41 children's objects (head over heel method); calibrated against chew and spit in vivo measurements in adult volunteers (mean in vivo: *in vitro* ratio 0.28)
CHAP (2001)	60	95% upper confidence bound from US CPSC data (Chen 1998 as reported in CHAP 2001)

Published data on migration rates in in vivo experiments and in vitro experiments for DINP and DIDP show a very wide range of estimated values (Table 4.69 and Table 4.70). Even where similar methodologies have been used, there was considerable variation in the reported rate of DINP release from PVC.

The available studies assume that all the saliva is swallowed, which seems reasonable. During child mouthing some saliva will remain on the article and some lost from the mouth by drooling. On the other hand, the saliva remaining on the article might be mouthed again, and might thus result in much longer migration times than obtained with mouthing events only.

Migration of phthalates depends on type of contact, time, temperature, plasticiser concentration difference, plasticiser concentration level, molecular weight and molecular structure (ECPI 2011b). Another element that seems important in determining the migration rate is the process conditions for PVC manufacturing (Simoneau 2009[55]; RIVM 1998; ExxonMobil 2011b). A relationship between the plasticiser content of PVC and the migration of plasticiser from PVC cannot be established based on experimental data (as also noted by Babich et al. 2004; Simoneau et al. 2009; Health Canada 1998). The likely reason for this is the multitude of factors influencing the migration from PVC in combination with differences in experimental settings amongst the studies. Niino et al. (2002a) reported high effects of

[55] Simoneau et al. (2009) demonstrated that the release from samples with a systematic manufacturing process and containing different phthalates at different concentrations showed correlations to their concentrations. The authors suggest, since previous studies using commercial toys had no showed such specific trends, these results suggest that the production process of toys may be an important issue with respect to release properties. For DINP, DIDP and DEHP the release of the plasticiser showed non linear tendencies and the study results indicated saturation of release for high formulation contents (Simoneau et al., 2009).

especially rotation speed (a migration rate of ca. 20 µg/cm^2/h at 200 rpm versus ca.150 µg/cm^2/h at 400 rpm) and temperature (a migration rate of ca. 80 µg/cm^2/h at 20 °C versus ca.170 µg/cm^2/h at 40 °C). Within the same experiment higher percentages of DINP in general seemed to have resulted in higher migration rates (Figure 4.10), although it has to be noted that data reported in Chen (1998) did not indicate a clear relation between phthalate content and in vitro migration rates measured by means of impaction from 35 toys and childcare articles.

Migration study methodologies and the effect of the nature and concentration of phthalates on their migration from PVC materials under dynamic simulated conditions of mouthing have been studied by the Joint Research Centre (Simoneau et al. 2001; Simoneau and Rijk 2001; Simoneau et al. 2009). In an interlaboratory comparison, the "head over heels" method showed the better reproducibility amongst the studied in vitro methods. The European standard operating procedure (EUR 19899 EN) for the determination of release of DINP in saliva simulant from toys and childcare articles makes us of a head over heels dynamic agitation device (Simoneau and Rijk 2001). The EUR 19899 EN methodology seems to have been used as the basis for the validated EN 71-10 standard (Part 10 "Organic chemical compounds - Sample preparation and extraction" of the European Standard EN 71 for safety of toys).

There are no standardised or validated in vivo methods for determining the migration rate of DINP and DIDP from mouthing of toys. A lack of standardisation makes comparison of results from in vivo methods difficult. Apart from differences in results from experiments resulting from different experimental protocols, in vivo methods introduce within the individual experiment variabilities as a result of differences in chewing and mouthing activity by volunteers, in pH of the saliva, and in amount of saliva produced. In addition the importance of absorption and adsorption in the mouth cavity, the accidental ingestion of saliva and the impact of the differences in composition of saliva of children and adults is unknown and is not accounted for in the in vivo results. These possibly influencing elements were ignored by the Dutch Consensus group (RIVM 1998). Chen (1998) conducted a study with 10 human volunteers mouthing and chewing one and the same sample (Toy Duck#2, 42.66% w/w DINP). The mean value for the sample was 26 µg/cm^2/h. The protocol was similar to RIVM (1998). The reported variability is striking: the maximum migration rate of the same sample and conditions varied with a maximum migration rate of 80.24 µg/cm^2/h and a minimum of 3.18 µg/cm^2/h, or a ratio of 25.2. As a comparison, the variability in the RIVM (1998) study showed for the three samples a range between minimum and maximum migration rate resulting in ratios of respectively 6.4, 9.9, and 28. Individuals in the Chen (1998) study showed better consistency with (ratios from 1.57 to 3.10 amongst the 10 volunteers). The average migration rates for individual volunteers ranged from 6.14 to 57.93 µg/cm^2/h (ratio of 9.4).

When children chew or bite toys or childcare articles, pieces might chip off from the article which might be ingested subsequently. This might in particular be relevant for children over 7-8 months, the age when teething typically begins (Tulve et al. 2002). In this respect, Van Engelen et al. (2008) concluded that in vitro and in vivo methods both give no clear picture on the effect of chewing on migration and that swallowing of small pieces that chip of will most probably result in higher migration. In case it is plausible that there is ingestion, the authors recommend to use the EN 71-3 standard for oral ingestion testing (a more aggressive method). If not, the standard EN 71-10 (head over heels) is considered sufficient.

As discussed, the lack of standardisation of in vivo methods and the very high variability in measurement results from in vivo studies is problematic. In addition, considering the paucity of in vivo data (data on 14 articles from 5 different studies), the variability of phthalate concentrations in PVC and the variability in its production process, the in vivo data might not give a good representation of the population of toys on the European market. On the other

hand, in vitro data might overestimate average real-life migration rates, and many of the available in vitro data have not been carried out according to the current European standard.

In a recent migration study by TNO (2010), ordered by ExxonMobil, the head over heels in vitro method was used. A risk assessment was submitted to ECHA by ExxonMobil using these TNO data (ExxonMobil 2011b). The risk assessment was reported to be "*conducted using the most robust scientific data, using flexible PVC disks made with known amounts of plasticisers, and carried out according to EU standard migration procedures*", the EU standard procedures were specified as "*EUR report EUR19899 EN, Standard Operation Procedure for the Determination of Release of Di-isononyl phthalate (DINP) in Saliva Simulant from Toys and Childcare Articles Using a Head Over Heels Dynamic Agitation Device (2001)*". In this respect ECPI (2012a) commented that "*The test method developed by JRC ISPRA using a head over heels methodology, was developed specifically to simulate the in vivo migration levels, and is the preferred in vitro methodology. The in vivo migration levels were obtained from healthy adult volunteers with a full set of teeth (RIVM 1998), and as such are very much a worst case for a child mouthing a soft PVC toy.*".

Conclusion on migration rate from mouthing of toys
Considering all of the above, it is not straightforward to give preference to the in vivo data over the in vitro data as an estimate of the real-life migration during mouthing behaviour of children.

It seems reasonable to take the mean of all the mean in vivo estimates in Table 4.69 as a typical case, which results in a **typical migration rate of 14 µg/cm²/h**[56].

As a **reasonable worst case** estimate, the in vitro migration rate of **45 µg/cm²/h** measured for a plate containing 40.7 % w/w DINP data from TNO (2010) could be used. This value is right in between the highest of the means from in vivo studies in Table 4.69 (32.6 µg/cm²/h for Plate A containing 48% w/w DINP from Niino et al. 2002a) and the highest measured value of a single sample in the RIVM (1998) of 53.4 µg/cm²/h that was used in the EU Risk Assessments for DINP and DIDP (EC 2003a,b).

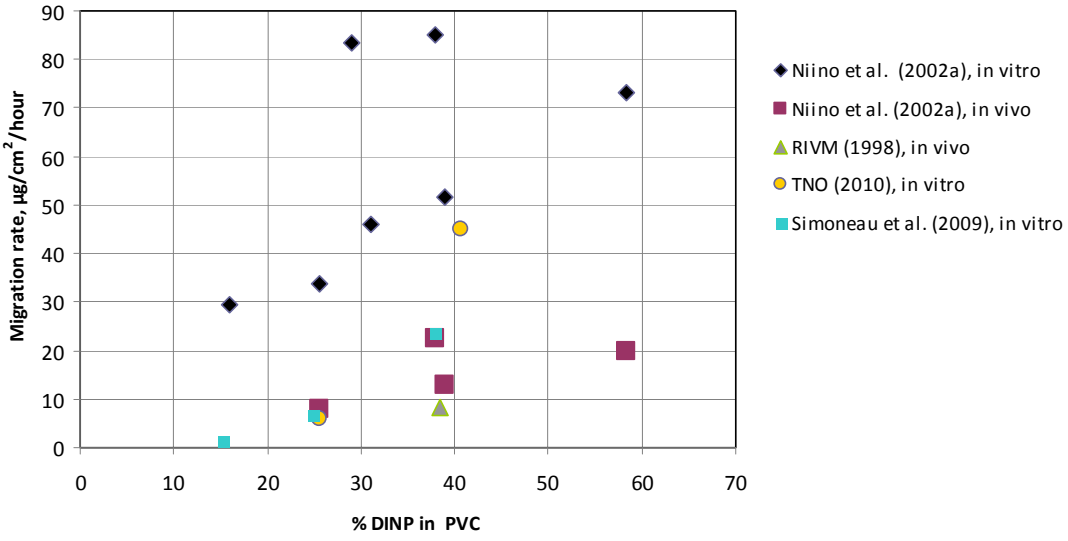


Figure 4.10: Relationship between DINP content and migration rate in selected studies where both are reported

[56] To avoid double counting samples, the Fiala et al. 1998 1h chewing value and the Niino et al. 2002b values were used to calculate the average.

Migration from pacifiers

RIVM (1998) and CHAP (2001) assumed that pacifiers are rarely made of soft PVC. The nipple of pacifiers on the Danish market are typically made of latex or silicone, and the shield of polypropylene or polycarbonate based on a survey by Tonning et al. (2009). A quick survey of the products on offer from Boots and Mothercare, the major baby care stores in the UK, confirmed this seems also to be the case in the UK. Pacifiers might contain PVC in the mouth shield and/or the handle which might be to some extent softened with DINP or DIDP (Tonning et al. 2009 found very low concentrations in one pacifier shield). The shield comes into oral (and dermal) contact while mouthing the pacifier and the shield and handle may be also mouthed as such. Also, it should be noted that DINP was measured in a pacifier in a Japanese study (Nino et al. 2002a,b[57]). CHAP (2001) indicated that oral intake of DINP from pacifiers in the US was not expected due to withdrawal of the substance from these products by voluntary industry agreements.

Thus, nipples of pacifiers in the EU seem rarely to have contained DINP or DIDP. There is some evidence suggesting that DINP or DIDP could occur in pacifiers if the existing restriction would not be in place (in either the nipple, the mouth shield and/or the handle). The presence of DINP or DIDP in the nipples or mouth shield of pacifiers could lead to considerably higher levels of exposure than those associated with toys alone.

Considering the overall evidence, pacifiers were not included in the risk assessment for mouthing of articles containing DINP or DIDP.

[57] Nino et al. (2002a,b) do not specify the part of the pacifier that contains DINP, but considering that the authors selected articles that are mouthed by children and considering the content is 54% w/w of DINP, it seems not reasonable to assume that the authors would have measured this DINP content in any other part than the nipple of the pacifier.

Table 4.69 Data on migration rates for DINP

Material/product	Medium	Concentration in material, %	Migration rate, µg/cm²/h	Method	Country	Data source
PVC standard sample	Saliva	38.5	8.2	*In vivo*, chewing, 4 times 15 min	The Netherlands	RIVM (1998); Meuling et al. (2000)
Finger of PVC teething ring in the form of a hand	-"-	n.i. *[7]	14.6	-"-	-"-	-"-
Flat part of PVC teething ring in the form of a hand	-"-	n.i.	9.8	-"-	-"-	-"-
Pacifier	Saliva simulant	58.3	73.2	*In vitro*, rotary shaking, 15 min	Japan	Niino et al. (2002a)
Teether	-"-	38.9	51.6	-"-	-"-	-"-
Rattle	-"-	38.0	85.2	-"-	-"-	-"-
Toy food	-"-	31.1	46.0	-"-	-"-	-"-
Soft doll B	-"-	29	83.6	-"-	-"-	-"-
Ball C	-"-	25.6	33.6	-"-	-"-	-"-
Soft doll A	-"-	16	29.6	-"-	-"-	-"-
Plate A	-"-	48.8	124.8	-"-	-"-	-"-
Rattle	Saliva	38.0	22.4	*In vivo*, chewing, 4 times 15 min	-"-	-"-
PVC pacifier	-"-	58.3	20.0	-"-	-"-	-"-

PVC teether	-"-	38.9	12.8	-"-	-"-	-"-
Ball C	-"-	25.6	7.8	-"-	-"-	-"-
Plate A	-"-	48.8	32.4	-"-	-"-	-"-
Pacifier	Saliva simulant	53.8	73.3	In vitro, rotary shaking, 15 min	Japan	Niino et al. (2002b)
Teether	-"-	38.9	51.7	-"-	-"-	-"-
Rattle	-"-	38.0	83.5	-"-	-"-	-"-
Plate	-"-	46.2	124.8	-"-	-"-	-"-
Pacifier	Saliva simulant	53.8	117.3	In vitro, vertically shaken, 15 min	-"-	-"-
Teether	-"-	38.9	93.1	-"-	-"-	-"-
Rattle	-"-	38.0	112.5	-"-	-"-	-"-
Plate	-"-	46.2	148.5	-"-	-"-	-"-
Pacifier	Saliva simulant	53.8	68.3	In vitro, horizontally shaken, 15 min	-"-	-"-
Teether	-"-	38.9	22.4	-"-	-"-	-"-
Rattle	-"-	38.0	25.1	-"-	-"-	-"-
Plate	-"-	46.2	88.3	-"-	-"-	-"-
Pacifier	Saliva	53.8	20.0	In vivo, chewing, 4 times 15 min	-"-	-"-
Teether	-"-	38.9	12.5	-"-	-"-	-"-
Rattle	-"-	38.0	21.9	-"-	-"-	-"-
Ball	-"-	25.5	7.8	-"-	-"-	-"-
Soft doll	-"-	16.0	3.8	-"-	-"-	-"-
Plate	-"-	46.2	32.6	-"-	-"-	-"-

Toy A	Saliva	39	9.2 (s.d. 5.7)	In vivo, unknown (Japanese)	-"-	Sugita et al. (2002)
Toy B	-"-	58	10.7 (s.d. 7.2)	-"-	-"-	-"-
Toy C	-"-	38	8.7 (s.d. 8.3)	-"-	-"-	-"-
Yellow teether	Saliva	36	13	*In vivo,* chewing 1 h	Austria	Fiala et al. (1998, 2000)
-"-	Saliva	36	8.7	*In vivo,* chewing 3 h	-"-	-"-
-"-	-"-	-"-	8.3	*In vivo,* sucking 1 h	-"-	-"-
-"-	-"-	-"-	3.0	*In vivo,* sucking 3 h	-"-	Fiala et al. (1998, 2000) *[2]
Teether	Saliva simulant	-"-	0.1	*In vitro*, shaking 3h	-"-	-"-
Teether	Saliva simulant	n.i.	232	In vitro, shaking	Denmark	Rastogi et al. (1997)
Teether	Saliva simulant	n.i.	1.8	*In vitro*, shaking	USA	Earls et al. (1998)
-"-	-"-	n.i.	10.8	In vitro, shaking	-"-	-"-
-"-	-"-	n.i.	9.6	*In vitro*, tumbling	-"-	-"-
Teether, toys, pacifiers, 27 samples	Saliva simulant	4-44	0.03	In vitro, impaction	Canada	Health Canada (1998)
8 teethers, toys, 31 samples	Saliva simulant	15-54	Average: 3.2; 0.07 *[4]	In vitro, impaction	USA	Chen (1998a)
Teether #5 (included in average above)	-"-	54	2.0; 0.07 *[4]	-"-	-"-	-"-
Teether #6	-"-	50	1.6; 0.05 *[4]	-"-	-"-	-"-

(included in average above)						
Teether #3 (included in average above)	-"-	43	2.6; 0.09 *[4]	-"-	-"-	-"-
Teether #1 (included in average above)	-"-	37	4.5; 0.2 *[4]	-"-	-"-	-"-
Teether #4 (included in average above)	-"-	33	1.9; 0.07 *[4]	-"-	-"-	-"-
Teether #2 (included in average above)	-"-	30	1.1; 0.04 *[4]	-"-	-"-	-"-
Teether #7 (included in average above)	-"-	26	0.6; 0.02 *[4]	-"-	-"-	-"-
Teether #8 (included in average above)	-"-	19	1.2; 0.04 *[4]	-"-	-"-	-"-
Toy Duck#2	Saliva	42.66	26	*In vivo*, chewing, 4 times 15 min	-"-	-"-
Teether	Saliva simulant	n.i.	1.1	In vitro, impaction	USA	Chen (1998b)
Toys, teethers,	Saliva simulant	26 – 41.7	24	In vitro, tumbling	EU	Simoneau et al. (2001)
-"-	-"-	-"-	5	*In vitro,* mild shaking	-"-	-"-
-"-	-"-	-"-	28	In vitro, stringent shaking	-"-	-"-

Toys, teethers, 10 samples	Saliva simulant	21.0-46.6	14 (average)	In vitro, tumbling	The Netherlands	Rijk and Ehlert (1999); Rijk et al. (1999)
Toys, 24 samples	Saliva simulant	12.9 – 39.4	24	In vitro, tumbling	USA	Chen (2002)
Toys, teethers, 20 samples	Saliva simulant	n.i.	1	In vitro, static	U.K.	Axford et al. (1999)
	-"-	-"-	3	In vitro, shaking	U.K.	-"-
-"-	-"-	-"-	35	*In vitro,* ultrasound	U.K.	-"-
Plastic discs	Saliva simulant	25.5	6	*In vitro,* agitated *[5]	The Netherlands	TNO (2010)
-"-	-"-	40.7	45	-"-	-"-	-"-
Sex toys	Water	39 (average) 77 (maximum)	52 (average) 224 (maximum)	*In vitro*, agitated *[5]	The Netherlands	VWA (2009)
-"-	Artificial sweat adjusted to pH 4.5	50	< 0.05	CEN final draft prEN-1400-3 (2002)	Denmark	Nilsson et al. (2006)
Plastic disc	Saliva simulant	15.2	1.2 *[6]	*In vitro*, agitated *[5]	EU	Simoneau et al. (2009)
-"-	-"-	24.8	6.6 *[6]	-"-	-"-	-"-
-"-	-"-	38.0	23.4 *[6]	-"-	-"-	-"-
-"-	-"-	45.1	28.8 *[6]	-"-	-"-	-"-

*[2] Selected data, data for more in vitro methods included in the report.
*[3] Selected data, samples for more toy samples in the report.
*[4] Indicates migration rates from impacted and non-impacted surfaces, respectively. Only 8 measurements of the 35 samples are given here as examples.

*[5] Extraction method described standard operation procedure in Simoneau and Rijk (2001).
*[6] Data for GC:MS analysis. Slightly different results reported for HPLC analysis.
*[7] Although RIVM (1998) indicated that the commercial specimen 2 and 3 (teething hands) contained ca. 43% w/w DINP, according to the actual study report by Meuling et al. (2000) it was assumed that the concentration in these samples was almost equal to that of specimen 1 (about 38%). The actual composition of the specimens was not established. It therefore has to be concluded that the concentration of DINP was unknown.

Table 4.70 reports in vitro migration rates of DIDP. The data from the most recent study from TNO (2010) suggests that DINP and DIDP have similar migration rates (see Table 4.69 and Table 4.70).

Table 4.70 Recent data on migration rates for DIDP

Material/ product	Medium	Concentration in material, %	Migration rate, µg/cm²/h	Method	Country	Data source
Plastic discs	Saliva simulant	25.5	3	*In vitro,* agitated *[1]	The Netherlands	TNO (2010)
-"-	-"-	40.7	44	*In vitro,* agitated *[1]	-"-	-"-
Sex toys, 8 samples	Water	27 (average) 55 (maximum)	140 (average) 332 (maximum)	*In vitro,* agitated *[1]	The Netherlands	VWA (2009)
Plastic disc	Saliva simulant	24.2	6 *[2]	*In vitro,* agitated *[1]	EU	Simoneau et al. (2009)
-"-	-"-	38.7	12 *[2]	-"-	-"-	-"-
-"-	-"-	52.5	15.6 *[2]	-"-	-"-	-"-
			0.9-4.6			CSTEE (1997d) in EC (2003b)
			not detected - 0.084 mg/kg/6 h			Artsana in CSTEE (1997e) in EC (2003b)
			5			Gesondheidsbescherming in CSTEE (1997f) in EC (2003b)

*[1] Extraction method described in standard operation procedure in Simoneau and Rijk (2001).
*[2] Data for GC:MS analysis. Slightly different results reported for HPCL analysis.

4.6.2.1.7 Conclusions on exposure from mouthing

There are substantial differences amongst the reported mouthing times and migration rates in the literature.

Table 4.71 gives the estimated exposure to the age categories 0-6 months, 6-12 months and 12-18 months. No distinction could be made for mouthing times of 0-6 months and 6-12 months, but their different body weights result in different exposure estimates.

Table 4.71 Estimated exposure to DINP and DIDP in 0-6 months, 6-12 months and 12-18 months old children associated with mouthing articles

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Child weight (kg)	6.21	6.21	7.62	7.62	9.47	9.47
Mouthable surface (cm^2)	10	10	10	10	10	10
Daytime mouthing duration (min/day)	7.5	120	7.5	120	7.5	120
Migration rate ($\mu g/cm^2$/hour)	14	45	14	45	14	45
Exposure mouthing articles (µg/kg bw/day)	**2.8**	**145**	**2**	**118**	**2**	**95**

[*1] Body weight and surfaces values from Bremmer and van Veen (2002), half of the surface for both hands in the typical case, for the worst case 1/3rd of the total body surface.

4.6.2.2 Dermal exposure

Dermal exposure to DINP or DIDP in PVC articles can arise where such articles are in direct contact with the skin. Exposure levels are determined by the frequency, duration and exposure and the area of exposed skin.

PVC is used in some childcare articles such as changing mats, pushchairs, high chairs, baby diaper covers, cribs, playpens, changing table pillows, carrying slings, breastfeeding pillows and car seats. Also toys such as play maths, inflatable soft plastic aquatic toys, masquerade masks, can result in prolonged dermal contact. Also pacifiers might contain DINP or DIDP in the mouth shield and/or the handle, although it seems from market research that they are usually made of polypropylene or polycarbonate (see section 4.6.2.1.6).

PVC is used in a wide variety of other articles that are not covered by the existing restriction on toys and childcare articles that can be placed in the mouth by children. This could include toys and childcare articles that cannot be mouthed, but also clothing including gloves, footwear (e.g. rain shoes, boots, shoes, shoe insoles, slippers, sandals, 'jelly' sandals), vinyl baby pants, wet weather wear (e.g. pants, coats, ponchos, hats). In addition dermal exposure can also arise from contact with PVC flooring and house dust.

The EU Risk Assessment (EC 2003a,b) calculated a maximum internal[58] dermal exposure of 1 µg/kg/day used the result of a study by Deisinger et al. (1998) giving a dermal absorption rate of 0.24 µg/cm^2/h with rats exposed to a film of 0.51 mm thickness with 40.37% DEHP (see also section 4.4.1), a factor of 10 lower absorption of DINP as compared to DEHP, an exposure time of 3 h, an exposed surface of 100cm^2 and a body weight of 8 kg to come to a. The EU Risk Assessment did not explicitly include childcare articles in the exposure assessment. The EU Risk Assessment did not apply a correction factor to take into account lower absorption in humans as compared to the rat. Rodent skin is up to 10-fold higher permeable than human skin (Wester and Maibach 1983 in US CPSC 2002).

Tonning et al. *(*2009) have estimated the exposure of a 2 year old child to DINP arising from the use of a changing mat as 0.9 µg/kg bw/day (or 22.5 µg/kg bw/day if we would assume an absorption of 4%), for a child of 15.2 kg in contact for 10 minutes/day with 100% of a 2000 cm^2 changing mat that had a migration rate of 6.6 µg/200cm^2/4 hours, and assuming 0.5% absorption.

The advantage of the Deisinger et al. (1998) data is that it might mimic a realistic scenario of dermal exposure, integrating both migration from a PVC surface and dermal absorption as opposed to experiments applying neat DINP on the skin in a dermal rat study by Midwest Research Institute (Midwest Research Institute 1983b in EC 2003a ; McKee et al. 2002). However, the scenario might not reflect slightly less favourable scenarios with mechanical stress (e.g. from stretching of PVC clothing or sandals) and/or moist conditions (e.g. hands of a child covered in saliva or sweaty skin). The study was carried out with DEHP which can be assumed to have a different migration behaviour and a different absorption. Indeed, the EU Risk Assessment assumed a factor of 10 lower skin absorption based on a comparison of absorption kinetics of DEHP and DIDP in a study by Elsisi et al. (Elsisi et al. 1989 in EC 2003a,b). This seems a fair assumption, especially keeping in mind also the lower dermal absorption in humans compared to rats.

The dermal absorption figure of 0.024 µg/cm^2/h can be divided by and absorption factor of 0.04 (see DNEL setting, section 4.4.11) to give an estimated migration rate of 0.6 µg/cm^2/h from a PVC foil of 0.51mm thickness with 40% DINP. The absorption assumption (and thus the migration estimate) does not introduce additional uncertainty since the same absorption factor is also used in the DNEL calculations, and will thus be cancelled out in the risk characterisation ratio.

As a typical case it seems reasonable to assume that a child is holding DINP or DIDP containing articles for 3 hours per day with both hands. As a reasonable worst case estimate of dermal exposure it is assumed that in addition to hand contact from holding articles there is dermal contact with a changing mat. An estimated dermal contact area assuming a child lies naked on its changing mat containing DINP or DIDP for 15 min/day with approximately one third of its total body surface area in contact with the mat. Alternatively, a reasonable worst case could be assumed for a case where in addition to hand contact from holding articles there is dermal contact from the upper arms with a play math during about 3 hours/day, which would result in virtually the same exposure estimate[59]. Note that the estimates presented in Table 4.72 are external exposure estimates and do not yet account for the (low) dermal absorption rates.

[58] Interal dermal exposure takes the absorption through the skin into account. In this report the REACH chemical safety assessment paractices are followed where typically the external exposure is calculated and the absorption factors are taken into account in the DNEL setting.

[59] Assuming that half of the total arm surface is upper arm and one third comes into contact with the play math, a child of 6-12 months with a total arm surface of 0.07 m^2 (US EPA 2011) gives a surface area of 117 cm^2. A reasonable case for the 6-12 months old children would thus result in an estimated exposure of 24 + 28 = 52 µg/kg bw/day.

Both the typical case as the reasonable worst case can be assumed to cover the overall dermal exposure from the many possible article types described above.

Table 4.72 Estimated (external) dermal exposure to DINP and DIDP in 0-6, 6-12 and 12-18 m children

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Body weight (kg) [*1]	6.21	6.21	7.62	7.62	9.47	9.47
Migration rate (µg/cm^2/h)	0.6	0.6	0.6	0.6	0.6	0.6
Surface (cm^2) [*1]	88	88[*2] ; 1153[*3]	103	103 ; 1327	124	124 ; 1557
Duration (min/day)	180	180 ; 15	180	180 ; 15	180	180 ; 15
Exposure (µg/kg bw/day)	26	26 + 28 = 54	24	24+ 26 = 50	24	24 + 25 = 49

[*1] Body weight and surfaces values from Bremmer and van Veen (2002), half of the surface for both hands in the typical case, for the worst case 1/3rd of the total body surface
[*2] Value for contact from holding articles
[*3] Value for hand contact with changing math

To test the realism of the cases under moist conditions the migration rate of 0.6 µg/cm^2/h can be compared with the rates obtained from the mouthing studies (14 - 45 µg/cm^2/h). Thus the migration rate seems rather low for a moist condition migration and/or the assumed dermal absorption is high. If the probably more realistic absorption of 0.5% assumed by Tonning et al. (2009) is used, the migration rate would become 4.8 µg/cm^2/h, which might still be low for more unfavourable moist conditions. Migration of DINP and DIDP from articles to saliva covered hands and from clothing to sweat is likely to be fairly similar to the migration observed in saliva experiments (the mechanical stress from stretching of PVC clothing might be somewhat comparable to mouthing and chewing in vivo and to mechanical agitation in in vitro tests), although can be expected to be lower as there can be assumed to be much less flow of liquid over the PVC surface, thus creating a smaller gradient of phthalate concentration (which is the driving force of migration).

All in all, the calculations are considered reasonable estimates and are well in line with the assumption of 1 µg/kg/day in the EU Risk Assessment for dermal exposure to toys (as a comparison, the figures in Table 4.72 correspond to internal exposures of 1 - 4.5 µg/kg/day) and the estimate of 0.9 µg/kg/day for dermal exposure to a changing mat for a 2-year old child by Tonning et al. (2009).

4.6.3 Dermal exposure for adults

Dermal exposure to DINP or DIDP in PVC articles can arise where such articles are in direct contact with the skin. Exposure levels are determined by the frequency, duration and exposure and the area of exposed skin.

Adults can be dermally exposed through a plethora of garments. PVC is used in gloves, vinyl incontinence pants, wet weather wear (e.g. pants, coats, ponchos, hats) and footwear (e.g. rain shoes, boots, shoes, shoe insoles, slippers, sandals, 'jelly' sandals). It is also used in skin-tight trousers, artificial leather pants, jackets, shirts and underwear that can be marketed as e.g. fashion clothing, rock style clothing, gothic style clothing, or as erotic articles.

Amongst the many other article groups adults can be exposed to are plastic bags, shower curtains, oilcloth, dinner maths, handles of articles (e.g. tools), articles for sports activities (PVC in exercise mats, exercise balls, swimming equipment, etc.), artificial leather on sofa's and chair covering, air matrasses, wires and cables, flooring, etc.

The EU Risk Assessment estimated an internal exposure of 0.7 µg/kg/day for DINP or DIDP for a 60 kg adult wearing PVC gloves for 2 h/day. This was based on a published estimate of dermal exposure for an adult wearing gloves containing DINP assuming a contact surface of 840 cm^2 and a dermal absorption rate of 0.024 µg/cm^2/h derived from the dermal absorption of DEHP in experiments with rats and allowing a factor of 10 for the poorer penetration of skin by DINP than DEHP as determined in an in vitro assay.

Most of the mentioned articles will not result in significant dermal exposure, either because the duration and frequency of contact are very low or because the articles do not contain DINP or DIDP. For the vast majority of the population, the PVC item that is likely to be worn close to the skin is gloves. Wet weather wear is usually worn over other clothing and is usually rather loose. Some individuals may wear PVC sandals or flip flops during warmer weather. There are likely to be temporal variations and regional differences in clothing and patterns of clothing use.

There is insufficient available data to make any assumptions for realistic contact times and probabilities that articles contain DINP or DIDP to come to realistic estimates of a daily dermal exposure. Instead, it seems pragmatic to carry out a first tier dermal exposure assessment for a dermal contact surface and duration that can be expected to result from one or several articles.

A typical case for dermal exposure could thus be assumed from wearing PVC gloves for 30 min/day and contact with for example a steering wheel for 2 h/day to calculate a typical case of exposure. Assuming a total hand surface for both hands (front and back) of 890 cm^2 for adult females (Exposure factors handbook, US EPA 2011) coming in contact 100% for gloves and for 1/3rd for a steering wheel, it can derived that the total hand surface is exposed for 70 min/day.

For a reasonable worst case it can be assumed that PVC trousers are worn close to the skin (e.g. "skinny faux leather pants") for 10 h/day for two weeks per month or 300 min/day. The exposed surface can be assumed to be 5980 cm^2 (the mean surface area for legs of women from the Exposure factors handbook, US EPA 2011).

The calculations of the exposure in Table 4.73 assumed the same migration rate of 0.6 µg/cm^2/h as for the children in the previous section.

Table 4.73 Estimated (external) dermal exposure to DINP and DIDP in adults

	Typical case	Reasonable worst case
Body weight (kg) *1	60	60
Migration rate (µg/cm^2/h)	0.6	0.6
Surface (cm^2) *1	890	5980
Duration (min/day)	70	300
Exposure (µg/kg bw/day)	10	299

*1 Adult body weight for adult females from the default assumptions in the ECHA guidance R 15.

Similarly as discussed in the previous section on dermal exposure for children, the experiment with PVC foil to a rat skin might not be representative for possibly higher exposure as a result of perspiration in PVC clothing and a small minority of the population may wear such pants for much longer periods and/or wear a wide variety of other PVC garments next to the skin, which could lead to exposures that are several orders of magnitude higher[60]. Nevertheless, the reasonable worst case is considered to cover the largest part of the adult population and thus consists of an appropriate estimate to be used for risk assessment.

[60] As a reference, one could calculate an extreme case where PVC garments are worn all day long (16h/day) and in addition to PVC pants also the upper arms would be covered (with a surface area of 2370 cm^2 according to Exposure factors handbook, US EPA 2011), which would in such an extreme case lead to an exposure of 1336 µg/kg bw/day. As a slightly less extreme case, one could assume that PVC trousers are worn close to the skin for 16 h/day every day, which would lead to an estimated exposure of 957 µg/kg bw/day.

4.6.4 School materials

Soft PVC that may contain DINP or DIDP is used in a variety of items used by school age children. The Danish Environmental Protection Agency published a study by Svendsen et al. (2007) of several substances (incl. DEHP and DIBP) in school bags, toy bags, pencil cases and erasers. Svenden et al. (2007) concluded that in general the substances in the tested products did not present any health risk under normal use conditions, but however, highlighted that daily intake of a small amount of eraser or daily sucking of an eraser with DEHP during a longer period may represent a health risk. The Danish EPA (2007a,b) also published two memorandums concerning erasers containing DINP and DEHP respectively concluding that the intake of eraser does not pose a risk until this has occurred over an extended period. The Danish EPA (2007a,b) considers that since exposure over longer periods is unlikely but cannot be ruled out the exposure to phthalates through erasers is unacceptable.

Svendsen et al. (2007) measured DINP in 6 erasers in concentrations between 32 and 70% w/w. Five out of the six phthalates were considered as toys by the Danish Safety Technology Authority and were thus considered cases of incompliance (Danish EPA 2007a). The Danish EPA estimated exposure to DINP for a child sucking an eraser for one hour per day based on a 1 h synthetic saliva migration experiment with 1g eraser containing 44 % w/w DEHP. The eraser was cut into pieces of 2-3 mm. The exposure via swallowed particles was calculated for 8, 50 and 100 mg of particles per day, corresponding to estimated internal oral exposure of 216, 1350 and 2700 µg/kg bw/day for a 20 kg child. It was acknowledged that in general swallowing a large piece of eraser will be a one-time occurrence. An internal oral exposure from sucking ca. 1 x 3.1 x 1 cm of the eraser was calculated to be 230 µg/kg bw/day. Migration was however carried out with small pieces of eraser with an approximate surface area of 211 cm^2. A migration rate of 1.24 mg/g was assumed for DINP.

The studies published by the Danish EPA (Danish EPA 2007a,b and Svenden et al. 2007) were subsequently reviewed by the EU Scientific Committee on Health and Environmental Risks (SCHER, 2008). SCHER (2008) considered that small particles that are bitten off are sharp and not easily swallowed. SCHER considered the particle consumptions of 50 and 100 mg/day as unrealistic and considered the extent to which children indulge in such behaviour as highly uncertain. SCHER derived a worst-case exposure estimate for sucking and licking of 0.1 mg/child and from biting off and swallowing pieces 4 mg DEHP/child, resulting in a total of 4100 µg/child or 200 µg/kg bw/day for a 6 years old child of 20 kg of weight. SCHER considered, however, that the swallowing of a larger number of particles from an eraser containing DEHP was an infrequent event and that sucking and chewing erasers represents a short-time habit of children.

It is here assumed that the biting off and swallowing of pieces of erasers would be a one time or very short-time habit (matter of weeks) of a small population of children and can be disregarded for the current risk assessment. It would be inappropriate to compare such very short-time exposures to an oral DNEL derived from liver effects seen in chronic studies. No typical case is assumed here, since the behaviour is not considered to persist over a longer period and is not considered to be a typical behaviour of children. A reasonable worst case can be assumed from the mouthing of erasers assuming that a 6 year old child of 20 kg daily mouths 1 cm^2 of eraser during one hour. A migration rate of 45 µg/kg bw/day can be assumed for migration of DINP and DIDP from erasers similarly to the worst case migration rate assumed in the scenario for mouthing of PVC articles (see section 4.6.2.1.6). This migration rate seems more realistic, since the Danish EPA used only one eraser sample with DEHP to estimate exposure from mouthing erasers with DINP, and the assumed migration rate was unclear from Danish EPA (2007a,b) and Svenden et al. (2007).

Table 4.74 Estimated reasonable worst case of mouthing an eraser with DINP or DIDP by a 6 y old child

	Reasonable worst case
Body weight (kg) *1	20
Migration rate (µg/cm^2/h)	45
Surface (cm^2) *1	1
Duration (min/day)	60
Exposure (µg/kg bw/day)	2.3

4.6.5 Sex toys

Sex toys are available in many geometries and designs and are mainly made of soft PVC or rubber latex (Nilsson et al. 2006). Sex toys can result in oral, vaginal, rectal and dermal exposure of adults to DINP or DIDP. Nilsson et al. (2006) and VWA (2009) concluded from market surveys that a very large part of sex toys is produced in China. A very common sex toy is the vibrator[61] (Herbenick et al. 2009; Nilsson et al. 2006; VWA 2009).

Phthalate content and migration rate
The Danish EPA published a survey on chemical substances in sex toys. Phthalates were measured in 10 out of 15 tested articles in concentrations up to 70.2 % w/w (Nilsson et al. 2006). Two out of the 10 articles contained DINP in concentrations of 60% and over 50% respectively. Both articles were vibrators. A static migration test was carried out using artificial sweat that was adjusted to pH 4.5, the vaginal pH level of healthy women, for one hour at 40°C (using CEN final draft prEN-1400-3 (2002)). The vibration speed was set to maximum. The experiment was carried out with one vibrator containing over 50% w/w DINP giving a rather low migration rate of <0.05 µg/cm^2/h. Similarly, very low migration was obtained with a vibrator containing 70.2 % DEHP (0.06 µg/cm^2/h). The latter vibrator underwent two other migration experiments, one with a water based lubricant and one with an oil based lubricant, giving migration rates of 0.4 and 54.8 µg/cm^2/h respectively.

Voedsel en Waren Autoriteit (Dutch Food and Consumer Product Safety Authority) in the Netherlands published a study on consumer products in the adult industry (VWA 2009). In an earlier study of 2004, the tested articles (9 vibrators and 1 dildo) were found all to be made out of PVC with plasticiser concentrations between 40 and 70% w/w.

In the newer study, 71 erotic articles were tested comprising sex toys and erotic lingerie. About half of the articles were made of plasticised PVC. Of the 71 articles, 18 articles contained DINP in concentrations ranging from 6 to 77 % w/w and 8 articles contained DIDP in concentrations ranging from 14 to 55 % w/w. The articles consisted of dildo's, but plugs, vibrators, a nipple toy and a Ben Wa ball. Migration testing was carried out on most of these positive tested articles according to the head over heels method as described in Simoneau and Rijk (2001) using water as medium (2 measurements per article). The results are presented in Table 4.75.

Via the public consultation of the ECHA's draft review report, the Bavarian State Ministry of the Environment and Public Health (2012) submitted data on phthalate concentrations measured in articles on the EU market. Out of 7 sex toys analysed, four were made out of PVC. All four samples analysed in 2011-2012 contained phthalates in high concentrations. The two dildo's contained 48% DIDP and 49% DIDP (+0.19% DINP) respectively. An artificial vagina 48.8% DEHP and a sample of Ben Wa balls contained 32% DINP.

[61] "*Vibrators are handheld electrical devices that produce pulses of variable amplitude and frequency, and enhance sexual arousal and latency to orgasm in both women and men.*" (Herbenick et al. 2009).

Table 4.75 Migration data for DINP and DIDP in sex toys (VWA 2009)

	n	Average migration rate µg/cm²/hour	Median migration rate µg/cm²/hour	75th percentile migration rate µg/cm²/hour	Maximum migration rate µg/cm²/hour
DINP	13[*1]	56.2	22.8	86.4	224.4
DIDP	6	140.3	132.0	216.8	332.4
Merged data[*2]	19	82.7	64.8	120.9	332.4

[*1] One value was excluded from the measurements as the sample was reported to contain 60% DINP, but showed 0 µg/cm2/hour migration.
[*2] Data of DINP and DIDP taken together.

From the similar migration behaviour of DINP and DIDP in an in vitro migration test from TNO (2010) it can be assumed in this assessment that the migration rate of DINP and DIDP from sex toys is the same (see also Table 4.69 and Table 4.70).

The migration rates measured for DIDP in the study by VWA (2009) are high in comparison to measurements from studies with other PVC articles (Table 4.69 and Table 4.70). It is unclear what could have caused the discrepancy. It is possible that the PVC matrix of the tested sex toys was of bad quality in comparison with other samples for which migration data is available (mostly from PVC in children's toys). The high concentrations of DIDP seen in 3 out of the 6 articles that were tested for DIDP-migration by VWA (2009) could be an additional explanation (50-55% w/w): an excess of plasticiser might result in low matrix interactions, thus facilitating migration. There could have been experimental factors influencing the results as well.

It is important to consider that the actual driver for migration is determined by thermodynamics, i.e. a reduction of free energy (INEOS ChlorVinyls 2012). Phthalates are highly lipophilic, and therefore fatty simulants can produce significant migration in contrast with non-lipophilic media (See comment reference 9 from INEOS ChlorVinyl). This is an important aspect considering that oil-based personal lubricants are often used with sex toys.

In conclusion, there are other plausible explanations for the high migration rates in the VWA (2009) study than possible experimental flaws, and migration rates to aqueous media might not be representative of a reasonable worst case for use with oil-based personal lubricants. Furthermore, the head over heels method used by VWA (2009) was considered relevant for the migration conditions (vibration and movements, and thus a high liquid flow at the PVC surface, facilitate migration). Thus, it was considered prudent to use the VWA (2009) data.

For the typical case of exposure (typical for the population that uses sex toys), the median migration rate of 65 µg/cm²/h was used from the merged data in and for the reasonable worst case the 75th percentile of 121 µg/cm²/h (see Table 4.75).

Prevalence, frequency and duration of use
There is great uncertainty concerning the typical frequency and duration of vibrator use. Based on a search of the peer reviewed medical literature using PubMed, a wider internet search and enquiries to several organisations and specialists in the field the available published data describing the typical prevalence, frequency and duration of use of sex toys is extremely scarce.

Nilsson et al. (2006) indicated that from the visited shops in the survey (n = 6) the estimated contact periods for dildos/vibrators and artificial vaginas were around 10-15 min with a normal use frequency of once a week.

Herbenick et al. (2009) surveyed in 2008 the prevalence and characteristics of vibrator use of 2338 (from 3800 contacted) women aged 18-60 years considered representative for the US population. The authors found that the prevalence of vibrator use in women was 52.5% ("ever users"), with significant differences according to marital status, sexual orientation, race/ethnicity, education and attending religious services. A total of 41% of the ever users had used a lubricant with a vibrator, and only few users (7.4%) had ever used a condom over the vibrator.

In a similar US study from 2008 (n = 1047), Reece et al. (2009) found that the prevalence of vibrator use in men was 44.8%. Most of the vibrator use in men was partnered use, and 16.6% had used vibrators during solo masturbation.

In a large study from 2009 (n = 25294), Reece et al. (2011) found that half (49,8%) of gay and bisexually identified men had reported having used vibrators[62], mostly during masturbation by insertion into the anus or rectum.

These data indicate that the use of vibrators is at least common in the US, but give very little information concerning the frequency, and no information at all concerning the duration of use. It can be assumed that in the EU the prevalence of vibrator use in women and men aged 18-60 years is high. The best estimate for frequency and duration of use is once a week for 10-15 min as estimated by Nilsson et al. (2006) based on expert opinions from sex shop personnel. In the typical case of exposure it will thus be assumed that the duration of vibrator use is 2.14 min/day. As a reasonable worst case estimate a daily use for a period of 15 minutes seems sensible.

Other risk assessments
The EU Risk Assessments did not assess exposure of DINP or DIDP from sex toys.

Nilsson et al. (2006) assumed as a typical case for vibrator use a exposure duration of 0.0357 h/day and as a worst case 1 h/day. An internal exposure to DEHP of 1.7 µg/kg bw/day was calculated for the 'normal case' and 47 µg/kg bw/day for the worst case for a vibrator containing 70.2 % w/w DEHP, assuming a bw of 70 kg, a 120 cm^2 surface area and a migration of 54.8 µg/cm^2/day from the migration experiment with oil based lubricant.

RIVM (Janssen and Bremmer 2010) conducted a risk assessment for plasticizers in sex toys. RIVM calculated the exposure to individual plasticizers from the private use of erotic objects on the basis that they may be used intravaginally (or anally) for one hour two times per week and that the contact surface with well-perfused tissue is 125 cm^2. The estimated time period was not based on research and was seen as a worst case assumption. It was assumed that absorption was 100% given that there is no available absorption data and considering the fact that vaginal and mucous membranes are well-perfused tissues. Exposure via lesser-perfused tissues was neglected. The head over heels migration experiment was carried out on isolated parts of the articles, therefore a correction factor of 0.5 for one-sided contact in the practical use situation. Exposure was calculated on the basis of the maximum migration rate. The results were intended to be a worst case estimate based on limited data.

Janssen and Bremmer (2010) estimated worst case exposures of 67 and 100 µg/kg bw/day for DINP and DIDP respectively, associated with 2 hours of use per week and based on the

[62] In this study a "vibrator" was defined broadly as "*products such as vibrators, vibrating butt plugs, vibrating dildos, vibrating cock-rings or other sex toys that can vibrate"*.

maximum migration rates of 224 and 332 µg/cm^2/hour respectively. A second scenario of exposure was calculated for a sub-population of heavy users (professional use) that was assumed to be exposed during 10 h/week giving 5 times higher exposures, i.e. 330 and 500 µg/kg/day for DINP and DIDP respectively.

Exposure estimation

The estimated typical and reasonable worst case exposure to DINP and DIDP are shown in Table 4.76. Despite a lack of information on use frequency and duration of sex toys, the exposure duration times are believed to correspond to real-life exposure situations. The corresponding percentages of the population to these exposure scenarios are unknown, however. If a migration rate of 14 µg/cm^2/day were to be assumed in the typical case and a migration rate of 45 µg/cm^2/day for the reasonable worst case, the resulting estimated exposure would be respectively and 1 and 23 µg/kg bw/day.

Table 4.76 Estimated exposure of DINP and DIDP associated with the use of sex toys

	Typical case[*1]	**Reasonable worst case**
Body weight (kg)	60	60
Migration rate (µg/cm^2/h)	65	121
Surface (cm^2)	125	125
Duration (min)	2.14	15
Exposure (µg/kg bw/day)	4.8	63.0

[*1] Typical case for the population of users

4.6.6 Indoor air and house dust

This section describes exposure to DINP and DIDP in a typical room environment. Phthalates are emitted as vapours from vinyl floor coverings, wall coverings and other PVC materials. The phthalate vapours then adsorb to suspended particles in indoor air (see EU Risk Assessments). In addition as PVC materials degrade during use, they will eventually start to release particles of PVC containing phthalate. Exposure to dust in indoor environments occurs through the inhalation of airborne dust, accidental ingestion of settled dust and dermal contact with settled dust. Small quantities of dust are present on most indoor surfaces, that is readily transferred to hands on contact with surfaces leading to a low level of dermal exposure and also accidental ingestion of settled dust via hand-mouth contact (both subconscious hand-face contact and also while eating, drinking or smoking; EA 2009).

Infants and small children are likely to have higher exposures to indoor dust than adults because they play on the floor leading to greater dermal contact with dust and are also more likely to put non-food items into their mouth (EA 2009). EA (2009) has estimated a dust/soil ingestion intake of 25 mg/day for adults and 100 mg/day for small children. In comparison, the US EPA 2011 Exposure Factors Handbook (US EPA 2011) indicates that the typical intake of settled dust for babies of 6 weeks to 1 year, children of 1 to <21 years and adults are 30, 60 and 30 mg/day respectively with an upper percentile (reasonable worst case estimate) of 100 mg/day for children aged between 3 and <6 years. For the purposes of risk assessment, RIVM (Oomen et al. 2008) has indicated that concentrations of airborne dust in indoor air are 60 µg/m^3 in homes and moderately crowded places and 100 µg/m^3 in crowded places. The ECHA guidance R15 refers to Oomen et al. (2008), for a dust intake estimate of 100 mg per day. The latter value has been used in this report. Dust intakes in adults and babies were estimated at 25% of the intake of young children.

4.6.6.1 Risk Assessments

The EU Risk Assessment exposure estimates derived for DINP and DIDP in indoor air are shown in Annex 3. The EU Risk Assessments indicate that the vapour pressures of DINP, DIDP and DEHP at 20°C are $6x10^{-5}$, $2.8x10^{-5}$ and $3.4x10^{-5}$ Pa respectively. The estimated saturated vapour concentrations of DINP, DIDP and DEHP in indoor air calculated from these vapour pressures in the EU Risk Assessments were 10, 5 and 5.3 µg/m^3 respectively.

For phthalates bound to particles the EU Risk Assessments for DINP, DIDP and DEHP all rely on the results of a Norwegian study (Oie 1997) that report the quantity of DEHP bound to particles (less than 2.5 µm in diameter), which was 1-3 times greater than that present in the vapour phase.
The estimated concentrations of DINP and DIDP were 40 (10+30) and 20 (5+15) µg/m^3, respectively, in the EU Risk Assessments (EC 2003a,b).

Inhalation exposure of all age groups to DINP and DIDP in indoor air is likely to have been overestimated in the EU Risk Assessments as it seems unlikely that DINP or DIDP concentrations in indoor air reach the estimated levels when comparisons are made with more recently published data on phthalates in indoor air. The exposure estimates, however, neglect the contribution of inadvertent ingestion and dermal contact with settled dust in the indoor environment (see above).

4.6.6.2 Other assessments

A number of studies have investigated concentrations of phthalates in indoor air and/or samples of settled dust, but only a few of these studies report levels of DINP or DIDP. The other studies, however, are still informative about the potential levels of DINP and DIDP in air, if these plasticizers have replaced other phthalates, particularly DEHP, in applications that

could contribute to dust and vapours present in indoor air. Given that the vapour pressure of DEHP is about half of that of DINP, future concentrations of DINP in indoor could potentially reach higher levels than reported for DEHP, if DINP is used widely as a replacement for DEHP in plastics present in the indoor environment. The vapour pressure of DIDP is similar to that of DEHP and future concentrations of DIDP may be similar to those reported for DEHP, if DIDP were used extensively in applications where DEHP was formerly used.

Most of the studies of phthalates in indoor air have included less than about 40 properties and reported substantial variations in the levels and substances found in different properties. The studies of phthalates in settled dust include some that investigated a much larger number of properties, but also report substantial variability between properties. The composition of house dust is very inhomogeneous and apparent concentrations of phthalates in house dust are strongly dependent on the particle size distribution that has been analysed (Wensing et al. 2005). The results of different studies are therefore not necessarily comparable. Most studies have also been conducted in Western Europe. Given the regional differences in climate and also in interior design preferences across the EU, the available measurement data may not be representative for the EU as a whole. It seems likely, however, that houses in the warmer parts of Europe would be better ventilated than in the cooler climes of Western Europe and indoor concentrations of airborne phthalate in these areas may be generally lower than reported in the available studies. Summaries of the available information on phthalates in settled dust and indoor air are provided below.

4.6.6.3 Reported concentrations of phthalates in settled dust

Relatively few studies have reported levels of DINP in house dust and even fewer have reported levels of DIDP (Abb et al. 2009; Bornehag et al. 2004). There is considerably more information about levels of DEHP in house dust than is available for DINP or DIDP (Abb et al. 2009; Bornehag et al. 2004; Becker et al. 2004; Hwang et al. 2010; studies undertaken for Greenpeace as cited in Hwang et al. 2010; Clausen et al. 2003; Fromme et al. 2004; Kolarik et al. 2008). Table 4.4.77 provides a summary of reported concentrations of DINP, DIDP and DEHP in house dust. More information is provided in the following text.

Table 4.4.77 Summary of reported DINP, DIDP and DEHP concentrations in indoor dust (mg/kg); the central tendency is the median or geometric mean in most studies or the mean (italicised) in two studies, the upper percentile is the 90th or 95th percentile, or if not given, the maximum reported value is shown in italics. More details are provided in the main text.

Study	N	Country	Year of sampling	DINP		DIDP		DEHP	
				Central tendency	Upper percentile	Central tendency	Upper percentile	Central tendency	Upper percentile
Abb et al. (2009)	30	Germany	Not given	129	700	33.6	400	604	1600
Bornehag et al. (2004)	346	Sweden	2001-2	639	1930	-	-	1320	40459
Becker et al. (2004)	254	Germany	2001-2	-	-	-	-	508	1840
Studies for Greenpeace cited Hwang et al. (2008)	29	UK	2003-4	-	-	-	-	192	416
	22	Spain	2003-4	-	-	-	-	317	2151
	5	Italy	2003-4	-	-	-	-	503	933
	31	France	2003-4	-	-	-	-	505	3298
	23	Belgium	2003-4	-	-	-	-	339	841
Clausen et al. (2003)-schools	15	Denmark		-	-	-	-	990	8500
Hwang et al. (2010)	11	USA	2004	-	-	-	-	611	2050
Fromme et al. (2004)	30	Germany	2000	-	-	-	-	703	5122
Kolarik et al. (2008)	177	Bulgaria	2004	-	-	-	-	960	29440

Abb et al. (2009) reported a wide range of phthalate concentrations in 30 samples of dust from apartments with carpet, parquet, laminate, flagging, PVC, and linoleum flooring (204-3,360 mg/kg as total phthalate; median 1160 mg/kg). Median concentrations of DINP and DIDP were 129 and 33.6 mg/kg respectively. The 90th percentile concentrations (read from a figure in the paper) were approximately 700 and 100 mg/kg respectively. In comparison median levels of DEHP and DBP were 604 and 87.4 mg/kg respectively. The 90th percentile concentrations for DEHP, DBP and BBP were approximately 1,600, 250 and 400 mg/kg respectively. BBP was detected in 23/20 samples with a median concentration of 15.2 mg/kg but one sample from an apartment with old PVC flooring under a fitted carpet contained 767 mg BBP/kg. in the sampled apartments. Carpet was the most commonly used flooring material and PVC-flooring was found in only two apartments. Phthalate concentrations in dust were unrelated to the percentage of carpet alone and only weakly correlated with the percentage of plastic materials in the apartment. The estimated prevalence of plastic materials in each property did not differentiate between materials likely to contain phthalates and other plastics. The lowest total amount of sum of phthalates in dust (median 362 mg/kg) was found in houses within a minimum coverage of carpet and minimum plastics.

Bornehag et al. (2004) measured concentrations of phthalates including DINP in 346 samples of settled dust from children's bedrooms in Sweden. DINP was detected in 50% of samples with a mean level of 639 mg/kg and a 95th percentile of 1,930 mg/kg. The maximum reported level was 40,667 mg/kg. In comparison, DEHP was detected in over 99% of samples. The mean, 90th percentile and maximum levels of DEHP were 1,310, 40,459 and 40,690 mg/kg respectively. Median levels of DINP and DEHP in rooms with PVC floors were marginally greater than in rooms with other types of floors. Phthalate levels were not correlated with any particular building type or age but were generally higher in children's bedrooms where vinyl floors were present than for other floor coverings. This difference was only statistically significant however for geometric mean levels of DEHP and BBzP in houses built prior to 1960. The presence of vinyl floors elsewhere in the house did not appear to have an important impact on the concentrations found in children's bedrooms. There appeared to be a high background level of phthalate in dust, even in the absence of vinyl flooring. Only 26 bedrooms had vinyl wallpaper which was associated with elevated levels of DEHP. There was some evidence that dampness was associated with elevated levels of DEHP and BBzP.

In conclusion, concentrations of phthalates in settled dust are highly variable. The limited information suggests that mean levels of DINP in house dust may be greater than those of DIDP, which is consistent with the higher vapour pressure and potentially greater release of DINP into room air. Reported mean concentrations of DEHP in different studies range from 192 to 1,310 mg/kg and maximum reported levels range from 3,300 to 40,700 mg/kg. Given the increasing use of DINP and DIDP and probable in increase in the proportion of homes that have PVC floors or wall coverings containing DINP or DIDP rather than other phthalates, it is perhaps reasonable to assume that current mean levels of these compounds would be greater than those reported in the early 2000s and may approach that of DEHP.

4.6.6.4 Reported concentrations of phthalates in indoor air

There is a paucity of published data describing concentrations of DINP and DIDP in indoor air and only a limited quantity of data about levels of other phthalates. Fromme et al. (2004) analysed samples of room air from 59 apartments and 74 kindergartens for 8 phthalates, not including either DINP or DIDP. DBP had the highest concentrations in room air, with median values of 1.083 µg/m^3 in apartments and 1.188 µg/m^3 in kindergartens. Mean concentrations of DEHP in apartments and kindergartens were 0.191 µg/m^3 and 0.599 µg/m^3 respectively, 95th percentile concentrations were 0.156 and 0.458 µg/m^3 respectively and maximum concentrations were 0.390 and 1.510 µg/m^3 respectively. There was no statistically significant correlation of concentrations in house dust and air. Fromme et al. do not provide information about the room characteristics (e.g. vinyl flooring) that may be associated with a high or low concentrations of phthalates in dust or air.

Berg et al. (2011) reported airborne phthalate levels of up to 11 µg/m^3 in 45 multi-storey apartment buildings in Stockholm, Sweden. The differences between the apartments sampled within individual buildings were relatively greater than the differences between different buildings, suggesting that fittings and furnishings are the dominant influence on airborne phthalate concentrations. Their results indicated that PVC flooring is a major source of BBP in indoor air.

In a study of the phthalate concentrations in different size fractions of airborne dust, Rakkestad et al. (2007) reported a more than 10 fold variation in the mean concentrations of total phthalates in samples of PM_{10} and $PM_{2.5}$ collected in 14 different indoor environments. The phthalate content of PM_{10} and $PM_{2.5}$ was 1.1 +/- 0.3% for both size fractions but the ratio of contained in $PM_{2.5}$ relative to PM_{10} ranged from 23-81% at different locations suggesting a range of indoor sources.

Xu et al. (2009) report the development of model to estimate the emission rate of DEHP from vinyl flooring and the evolving gas-phase and adsorbed surface concentrations in a realistic indoor environment. The predicted indoor air DEHP concentration at steady state is 0.15 µg/m^3 which was reached after about a year in the room containing the vinyl floor and about 3 months later in the adjacent room.

They used the model to demonstrate that ventilation rate has a strong influence on DEHP emission rate while total suspended particle concentration has a substantial impact on gas-phase concentration. If DINP replaced DEHP in vinyl flooring, then the steady state concentration of DINP might be double that estimated for DEHP based on its greater volatility, whereas if DIDP replaced DEHP, concentrations of DIDP in air might be expected to be similar to those predicted for DEHP. Actual measurements of DEHP described above, however, are higher than would be anticipated from the model predictions.

Uhde et al. (2001) undertook an experimental investigation of phthalate emissions from PVC-coated wallcoverings in emission test chambers under standard room conditions. Chamber air concentrations were monitored over a 14 day period. The maximum concentrations of DBP, DPP and DEHP that arose were 5.1, 2.08 and 0.94 µg/m^3 respectively. Had DINP and DIDP been present in the test materials and included in the investigation, the concentrations of DINP and DIDP attained might have been approximately double that and equivalent to that of DEHP based on their relative vapour pressures.

4.6.6.5 Estimated exposure to DINP and DIDP in house dust and indoor air

Estimated intakes of DINP and DIDP for interiors where these substances are present in flooring and/or furnishings and wall coverings and the assumptions underlying the estimates are shown in Table 4.78 and Table 4.79.

Exposure from dust
Exposure from dust has been calculated using the assumed dust ingestion rates of 25 for adults, 100 for 12-18 months old and 6-12 months old, and 25 mg/day for 0-6 months old. It was assumed that children less than 6 months in age would be placed in cots, on playmats or similar and relatively less contact with house dust than children between 6 and 18 months.

Levels of DINP in dust reported by Abb et al. (2009) and Bornehag et al. (2004) were used to derive "typical" and "reasonable worst case" estimates. An adjusted mean value was calculated for the typical case ((30*129)+(346*639)/376)=598)). The 95-percentile level for a reasonable worst estimate was rounded to 1900 from 1930 given by Bornehag as no information on 95-percentile is given in Abb et al. (2009).

Predicted exposures to DIDP are less than for DINP because of the lower volatility of DIDP. Levels of exposure to DIDP were estimated at half of those for DINP.

Exposure from air
There is little published evidence on which to base an estimate of the concentrations of DINP and DIDP in indoor air. Based on the published information available for other phthalates, particularly DEHP, and the saturated vapour concentrations for DINP, DIDP and DEHP estimated in the EU risk assessments (10, 5 and 5.3 µg/m^3 respectively), the reasonable worst case estimates of the indoor concentrations of DINP and DIDP were assumed to be 8 and 4 µg/m^3, respectively. Typical levels are likely to be considerably lower and were estimated at 20% of the reasonable worst case (1.6 and 0.8 µg/m^3 for DINP and DIDP, respectively).

The EU Risk Assessment estimated that adults spent 20 hours/day in the indoor environment and infants and newborns (children 0-3 years) spent 22 hours/day indoors. The same assumptions for time spent have been used here for exposure from air.

Table 4.78 Summary of the assumptions used in the calculated intakes of DINP and DIDP in indoor air and house dust

	Adults and children>18 months	**12-18 months old**	**6-12 months old**	**0-6 months old**
Body weight	60	9.47	7.62	6.21
Hours exposed per day	20	22	22	22
Dust ingestion rate mg/day	25 (0.25*100)	100	100	25 (0.25*100)

Table 4.79 Estimated reasonable worst case and typical case levels of exposure to DINP and DIDP in house dust (µg/kg bw/day) and indoor air (µg/m^3)

Source of exposure	Exposed group	Typical case		Reasonable worst case		EU Risk Assessment External dose	
		DINP	DIDP	DINP	DIDP	DINP	DIDP
Concentration in air µg/m^3		1.6	0.8	8	4	40	20
Concentration in air µg/m^3 adjusted to average exposure during 24 h	Adults and children >18 months, 20 h	1.3	0.67	6.7	3.3	33	17
	12-18 months, 22 h	1.5	0.73	7.3	3.6	37	18
	6-12 months, 22 h	1.5	0.73	7.3	3.6	37	18
	0-6 months, 22 h	1.5	0.73	7.3	3.6	37	18

Concentration in dust mg/kg[63]		600	300	1900	950	-	-
Ingestion intake µg/kg/day	Adults and children >18 months	0.25	0.13	0.79	0.40	-	-
	12-18 months	6.3	3.2	20.1	10.0		
	6-12 months	7.9	3.9	24.9	12.5	-	-
	0-6 months	2.4	1.2	7.6	3.8	-	-

4.6.6.6 Vehicle interiors

Concentrations of airborne phthalates within some cars are likely to be substantially higher than in other indoor environments because of the extensive use of plastics (which may or may not contain phthalates) within car interiors. Where plastics do contain phthalates there is potential for significant volatilisation of phthalates to occur when the car is exposed to sunshine and plastic surfaces and the air inside the car are heated to temperatures that greatly exceed ambient.

Information from the EU Risk Assessments
The EU Risk Assessments based their estimates of exposure to DINP and DIDP inside vehicles on the assumption that saturated vapour pressures of DINP and DIDP are achieved and that the concentrations of these phthalates present in the particulate fraction of in-vehicle air are 3 times higher than the vapour concentrations (see discussion of indoor air above and Annex 3). It seems possible that concentrations of DINP or DIDP in air inside vehicles could approach saturation levels during hot weather and the estimated worst case concentration used in the EU Risk Assessment is likely to be representative of levels that might arise during the hottest weather. Levels of long term inhalation exposure are likely to have been substantially over-estimated, particularly for more northern and western parts of the EU where car interiors are not likely to become overheated on more than half of the days per year. The EU Risk Assessment "in car"-exposure estimates for infants does not include any component arising from mouthing of plastic items such as buckles on seatbelts, plastic on the seats in front and so on.

Available studies
Only one study has been undertaken of the actual level of phthalate present in car interiors which did not report concentrations of neither DINP nor DIDP. Geiss et al. (2009) investigated concentrations of airborne phthalates inside 23 used private cars ranging in age from <1 to 18 years. Temperatures inside the cars during the summer were ≤ 70 °C but concentrations of phthalates were only determined in winter. Phthalates were detected in 10 of 17 cars and the most frequently detected phthalates were DBP and DEHP. Concentrations of DEP, DBP and DEHP ranged from 0.2-1.14, 0.193-1.63 and 0.535-3.656 µg/m^3 respectively. The highest concentration of all three substances combined was 4.476 µg/m^3. It seems probable that higher concentrations would have been reached in the summer, but it is difficult to predict how much higher. Summertime concentrations of some volatile substances measured in the same study (including n-dodecane, formaldehyde, propanal and hexanal) were 3 to 4 times higher than those measured in the winter survey. The vapour pressure of DEHP at 70°C is 0.011 Pa compared with 0.000034 Pa at 20°C implying the concentrations of DEHP inside unoccupied cars sitting in sunshine could be very much higher than the levels reported by Geiss et al. Given however, that windows would be opened during hot weather or air conditioning

[63] Levels in dust estimated from Abb et al (2009) and Bornehag (2011): Adjusted mean value ((30*129)+(346*639)/376)=598)); 95-percentile for reasonable worst estimate rounded to 1900 from 1930 given by Bornehag as no information on 95-percentile is given in Abb et al. (2009).

employed, it seems likely that concentrations of airborne phthalate while the vehicle is in use would be only slightly higher in summer than during the winter.

Assumptions for the present assessment
DINP has a higher vapour pressure and higher molecular weight than DEHP such that higher concentrations of DINP might occur than have been reported for DEHP, if DINP has been used extensively in plastics within a car interior.

Based on the maximum concentration of DEHP measured in the winter by Greiss et al. and applying *a factor of about 2* to allow for the vapour pressure of DINP which is almost double that of DEHP at 20°C ($6x10^{-5}$ versus $3.4x10^{-}5$ Pa) *and a further factor of 2* to allow for higher average temperatures and greater release of DINP in the summer versus the winter, *14 µg/m³* has been chosen as representing a reasonable worst case estimate of DINP.
Based on the difference in volatility of DINP and DIDP, a reasonable worst case estimate of DIDP concentrations for a car containing a relatively substantial quantity of DIDP in plastic might be *7 µg/m³*.

A relatively small factor was allowed for the difference between summertime and wintertime conditions because the difference in temperature and DINP volatility while car was occupied would be much less extreme than when the car reached a maximum temperature while unoccupied.
Intake of DINP and DIDP from dust during time spent in cars has not been included in our assessment as it is assumed that the intake during time spent in cars would already be covered by the intake calculations for dust in the indoor environment which are based on the daily intake of dust of 100 mg/day in children.

For calculations of exposure the time spent in cars used in the EU Risk Assessments of 4 h for adults and 2 h for children are used.

The resulting exposures to DINP and DIDP in vehicles are shown in Table 4.80.

Table 4.80 Estimated air concentrations of DINP and DIDP (µg/m³) in cars based on estimated concentration in air derived as described in text.

Exposure	Population Group	Typical – cars with DINP/DIDP		Reasonable worst case		EU Risk Assessments	
		DINP	DIDP	DINP	DIDP	DINP	DIDP
Concentration in air µg/m³		2.8	1.4	14	7	40	20
Concentration in air µg/m³ adjusted to 24 h mean	Adults and children older than 18 months (4 h)	0.45	0.23	2.3	1.2	6.66	3.33
	Children 0-18 months (2 h)	0.23	0.12	1.2	0.58	3.33	1.67

4.6.7 Food

This section summarises the available data on concentrations of DINP and DIDP in food and provides an assessment of potential exposure to these substances in food in the EU.

4.6.7.1 Data availability

The identified data regarding DINP and DIDP in specific foodstuffs are summarised in Annex 5 and Annex 6. The majority of data are for fatty foods that are believed to be more prone to phthalate contamination. There has been a focus on the potential role of food contact materials in giving rise to phthalate contamination. Published data are available from Germany, Denmark, Italy, Switzerland and Austria. The largest survey of phthalates in food was conducted by Germany, covering in total more than 3400 samples analysed between the years 2000 and 2006. The data are unpublished, but a summary of the data was presented to the European Commission in 2007. An extensive review of phthalate esters in food with a discussion of sources, occurrence and analytical methods has recently been prepared by Cao (2010). The review includes relatively few data on DINP and DIDP in food, which are tabulated in Annex 5 and Annex 6.

Overall the existing data for individual foodstuffs provide relatively little information on which to base an estimate of current total dietary exposure. Some additional information about dietary intakes of DINP and DIDP is available from duplicate diet studies, but these are even more limited in scope than the food surveys. Data specific to DINP and DIDP is restricted to studies of hospital patients in Japan (Tsamura et al. 2001a; Tsamura et al. 2003) and dietary exposure of the general population in Europe to these substances may be very different. The duplicate diet studies do not provide information about the potential intake of babies and toddlers who are anticipated to be the most vulnerable group in the population.

4.6.7.2 Analytical issues

The analysis of phthalates in food is technically demanding and there is no information about the comparability of analysis performed in different laboratories at different times. There are no standardised methods for the extraction of phthalates from food for analysis. Generally samples are treated with solvent to extract the phthalates and the solvent extract is cleaned up prior to analysis using liquid/liquid partitioning or gel permeation chromatography.

DINP and DIDP are mixtures that overlap chemically with each other and cannot be clearly distinguished if present in a mixture. The EU has therefore imposed a group restriction on DINP and DIDP for migration from food contact materials (Directive 2007/19/EC, amending Directive 2002/72/EC). Quantification is based on summation of the area under overlapping peaks in chromatograms. This gives rise to some uncertainties as the detector response to different components of the mix will not be identical to substance used for calibration and also because the quantification may include peaks belonging to unrelated substances (interfering phases) that have a similar retention time.

4.6.7.3 Reported levels of DINP and DIDP in food

For food which is not contaminated by DINP or DINP in the packaging, the levels found in milk products and vegetable oils are generally below 0.005 mg/kg (Annex 5 and Annex 6). For food products contaminated by phthalates in the packaging, e.g. in gaskets of PVC in glass jars, the concentration ranges up to 270 mg/kg. US CPSC quotes Freire et al. as reporting a concentration of 11.6% DIDP in pork, but this was reportedly in the packaging and has not been included here.
The largest survey of phthalates in food analysed more than 3,400 samples of German food between 2000 and 2006 but only 175 samples were analysed for DINP, of which 23.4 % of

contained detectable levels of DINP (referred to in Wenzl 2009). The data are unpublished and the LOD is not reported.

4.6.7.4 Sources of DINP and DIDP in food

There is a low background of DINP and DIDP in food resulting from background levels of contamination of the environment arising from the ubiquitous use of plastics and releases from industrial installations, the relative ease of release of these substances from plastics and the entry of these substances into the human food chain. It seems likely that background environmental levels of DINP and DIDP will increase in the coming years as they replace lower molecular weight phthalates in a variety of applications. In addition to background levels of DINP and DIDP in untreated food, DINP and DIDP may be present in equipment such as milking machines (PVC tubing) and in equipment used in food processing and storage of which a small proportion may pass into food during treatment (Cao et al. 2010). Significant DINP migration rates have been measured for PVC tubing used in milking machines with levels of 46, 73 and 95 mg/L in milk reported after incubation for 8 hours at 38^{o}C after pre-treatment with water for 0, 7 and 14 days (Cao et al. 2010). Cao (2010) reported that solvent extraction of DIDP present in non-stick cookware recovered DIDP at levels of 1.2-2.7 µg/dm^2.

Tsumura et al. (2001a) demonstrated substantial phthalate contamination of retail packed lunches sold in Japan prepared using PVC gloves. The study focussed particularly on DEHP and reported concentrations of DEHP of up to 11,800 mg/kg/ in packed lunches. DINP was detected in 9 of 16 samples at concentrations of up to 598 mg/kg. Duplicate diet studies undertaken in 3 Japanese hospitals by Tsumura et al. (2001b) and Tsumura et al. (2010) examined dietary intakes of phthalate in hospitals before and after the regulation of DEHP containing PVC gloves in Japan.
Prior to the regulation of DEHP-containing gloves in Japan, the average intake of DINP based on one week samples collected at each of the 3 hospitals was estimated as 178.3, 7.9 and 10.7 (average 65.7) µg/day (samples below the LOD were estimated to have DINP contents that were 50% of the LOD). Two of the hospitals used disposable PVC gloves when serving foodstuffs and the lowest DINP contents were reported for the hospital where gloves were not used. The levels of DINP in samples were strongly correlated with those of DEHP (R^2= 0.9433). After regulation of DEHP-containing gloves in Japan, estimated intakes of DINP based on 3 one week samples for the three hospitals in 2001 were 5.7, 6.9 and 4.3 µg/day with an average value of 5.6 µg/day, substantially lower than in the earlier survey. This confirmed the importance of PVC gloves in food handling as a potential source of dietary exposure to DINP and other phthalates.

A French study undertaken for Ansell (Sauvegrain and Guidard 2001) investigated the contamination of foodstuffs with phthalate as a result of the use of PVC gloves for handling foods during processing. Tests on a range of foods in 2002 reported total phthalate levels prior to processing of between 7 and 22 mg/kg in beef, pizza and sausages or which levels of DINP were <1 mg/kg. When the same food types were tested in a ready for sale form, after processing, reported levels of DINP ranged from 0.1 to 3.8 mg/kg.

The German Federal Institute for protection of Consumer Health and Veterinary Medicine (BgVV) has recommended in 2001 that soft PVC gloves should not be used in food preparation because of the high levels of migration of phthalates from gloves into food stuffs.

Nanni et al. (2011) reported marked differences in the phthalate contents of vegetable oils sold in Italy that had been derived from different plant sources – olive, sunflower, peanut, corn or mixed seeds – and in oils that had undergone different degrees of processing. DINP contents were much higher than those of DBP and DIBP and DINP accounted for between 57% (extra virgin olive oil) to 95% (corn oil) of total phthalate content. Although no specific information was presented about the impact of processing on DINP contents of oils, the study established that the phthalate content of oils can decrease during refining (extraction,

neutralisation, decoloration and deodorisation). The particularly high phthalate content of virgin olive oil was attributed to the relatively low degree of processing and the relatively high level of contamination of unprocessed oil derived from a perennial plant (with greater potential for bioaccumulation) in comparison to that derived from annual crops.

DINP and DIDP contamination of food may also arise from the presence of these substances in packaging. Traditionally DBP and DEHP were used in food contact materials and most investigations of phthalate concentrations in food have focussed on these substances. These substances were used in printing inks, paper and board packaging, aluminium foil-paper laminates and food-packaging films (Cao 2010). There is much less information about the presence of DINP and DIDP in food packaging and the impact on the concentrations of DINP and DIDP in food. It is possible that DINP and DIDP have replaced DBP and DEHP in food packaging, potentially giving rise to increased levels of DINP and DIDP in food, but there are insufficient data to establish whether this is happening.

A Swiss market survey conducted in 2005 that investigated levels of DINP in oily food in jars with metal closures sealed by a PVC gasket reported that 5.6% of the 158 jars investigated had lids with gaskets containing DINP and 6.8% contained DIDP (Fankhauser-Noti et al. 2006). The DINP contents of the gaskets ranged from 1.2-33%. Foods in jars with lids containing DINP had DINP contents that ranged from 10 to 270 mg/kg but the concentration in food was not correlated with the concentration in the gaskets. The DIDP contents of gaskets, where present, ranged from 0.1-33% and concentrations in food ranged from <0.5-740 mg/kg. Phthalate contamination of food appeared to be most severe for products contained in small jars. In a study of phthalate contamination of food arising from twist closures to glass jars in Denmark, Pedersen et al. (2008) found DINP in one of the 19 samples and DIDP in 6 of the 19 samples. Concentrations of DINP and DIDP ranged from 6-173 mg/kg with the highest concentrations occurring in products of garlic and tomatoes in oil and in fatty food products such as sauce béarnaise and peanut butter. Five of the products were subsequently withdrawn from the market as overall migration of phthalates into the products from the lids exceeded the legal limit.

There have been a small number of studies that have investigated the release of other phthalates from food storage containers. In a Croatian study, Bosnir et al. (2003) exposed 16 specimens of plastic food containers to distilled water, acetic acid and ethyl alcohol for 10 days. Over a ten day period the average rates of release were 16.3±1.2, 8.8±6.8 and 12.5±12.9 mg/kg implying that significant contamination of foods could occur when stored in soft plastic containers containing phthalates.

In a follow up study, Bosnir et al. (2007) investigated the release of phthalates from plastic bottles containing soft drinks and mineral water. The mean pooled phthalate level was 91.67 mg/L and ranged from 20.22 mg/L for mineral water to 542.63 mg/L for soft drinks a products preserved with K-sorbate. DMP showed the highest rate of migration to soft drinks and DBP and DEHP showed highest rate of migration to the mineral water. Phthalate migration rates were enhanced by acidity.

The results of a small biomonitoring study suggest that phthalates in plastic food storage containers can lead to increased levels of phthalate exposure. Colacino et al. (2011) reported a statistically significant relationship between the storage of food in plastic containers and plastic bags and elevated levels of MiBP in girls' urine, consistent with DBP exposure levels that were 50% higher than in families where these items were not used. No evidence was found to indicate that the use of plastic eating utensils was associated with higher levels of exposure to phthalates. If DINP and DIDP were to be used in soft plastic food storage containers, families who used such containers extensively would be expected to have elevated levels of exposure to these substances in food, possibly of the same order of magnitude as predicted for individuals consuming relatively large quantities of food supplied in packaging containing DINP and/or DIDP.

Soft PVC is not used extensively in cookware and other food-related items in the home. There is no information about the use of DINP and DIDP in these applications and it has not been identified as a potential source of exposure in previous risk assessments.

The use of both DINP and DIDP in food contact materials is restricted under Commission Regulation (EU) no 10/2011. Both substances are only to be used as:

- plasticiser in repeated use materials and articles;
- plasticiser in single-use materials and articles contacting non-fatty foods except for infant formulae and follow-on formulae as defined by Directive 2006/141/EC or processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC;
- technical support agent in concentrations up to 0.1 % in the final product.

This regulation imposes a Specific Migration Limit of 9 mg/kg for the sum of DINP and DIDP in food contact materials.

4.6.7.5 Estimated dietary intakes of DINP and DIDP based on measured levels in food

This section derives typical and reasonable worst case estimates of dietary intakes of DINP and DIDP. This includes an assessment of the relative importance of food contact materials versus indirect exposure via the environment giving rise to DINP and DIDP contamination of the food chain. Published estimates of dietary intakes of phthalates have employed two different approaches:

- Estimates based on the phthalate content of specific foods and typical intakes of different food types by different age groups in the population.
- Estimates based on duplicate diet studies.

Both approaches are subject to a high level of uncertainty arising from the relatively poor detection limits/limits of quantification for DINP and DIDP in food. This can lead to a large number of results being reported as non-detected. The way in which non-detected results are included in statistical summaries of analytical data varies between different studies with some authors treating "nondetects" as half the detection limit and other as the detection limit divided by √2.

Information from the EU Risk Assessments

The EU Risk Assessments (EC 2003a,b) separate the intake of DINP and DIDP via food into a "consumer" intake in food arising from food contact materials (this is not explicit in the EU Risk Assessments) and "indirect exposure via the environment" – exposure arising from entry of these substances into the food chain (Annex 4). The estimated "consumer" intakes derived in the EU Risk Assessments were based on the detection limits in a 1996 UK study (<0.01 mg/kg food or <0.17 µg/kg bw/day assuming a food intake of 1 kg per day by a 60 kg adult) intended to be representative of total diet. These phthalates were not detected in any of surveyed foods. In comparison, the same survey reported a daily intake of DEHP of 5 µg/kg bw/day. The EU Risk Assessment for DINP used the detection limit to derive an estimated intake of 0.2 µg/kg/day in food for adults and children aged 3-15 years and intakes of 2.4 µg/kg bw/day and 2.3 µg/kg/day for newborns (0-6 months) and infants (6 months to 3 years) in formula milk as detailed in Annex 4. The EU Risk Assessment for DIDP assumed equivalent intake levels for DIDP. The Risk Assessment for DINP noted that total phthalate levels reported in another study that included 3 EU countries, but was confined to dairy products, were higher than those reported for the UK.

The estimated exposures associated with indirect exposure via the environment presented in the Risk Assessments were derived using EUSES (Annex 4). These intakes primarily reflect exposure to DINP and DIDP via the food chain. The Risk Assessments do not address the

relationship between the estimated intake in food for consumers versus indirect exposure via the food chain. The exposures were assessed separately and summed, although as the method of estimating consumer exposure to food was based on measurement data, it would have been anticipated to incorporate exposure via the food chain. See also section 4.7.7.

4.6.7.5.1 Estimation of total dietary intake based on analysis of specific foodstuffs

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (EFSA 2005)[64] reviewed exposure to DINP and DIDP in food. They used the limited available data on DINP concentration in foods and diets in the UK (1996, 1998) and Denmark (2003) to provide an estimation of the dietary exposure. In the UK, potential exposure to DINP from dietary sources was estimated following a measurement survey that had failed to detect DINP in the foods selected for analysis. Intakes of DINP were estimated to be less than the method detection limit which equated to be less than 0.17 µg/kg bw/day for adults and children over 3 years in age. For newborns (0-6 months) and for infants (>6 months), the potential exposure to DINP derived from infant formulae consumption corresponded to 2.4 µg/kg bw/day and 1.8 µg/kg bw/day, respectively.

A Danish risk assessment for DINP as cited in EFSA (2005) used the EUSES modelling programme to derive an estimated intake of 5 µg/kg bw/day in food for adults. The EFSA considered that the estimates of exposure derived with EUSES were conservative and were not representative of the possible exposure via food contact materials.

Dietary exposure to phthalates has recently been re-evaluated in the UK. The results of the UK total diet study (2007) have been reviewed by the Department of Health's Committee on Toxicology (COT 2011) but are not yet available as a full report from the Food Standards Agency. Levels of DINP and DIDP were below the detection limit. Neither DINP nor DIDP were detected in any of 20 food groups. Based on the estimated dietary intakes of other phthalates associated with typical eating patterns in the UK and allowing for potentially higher detection limits for DINP and DIDP than for some of the other phthalates, it seems likely that current dietary exposures to DINP or DIDP in the UK are less than about 0.8 µg/kg/day for infants and newborn (children under 3 years) and less than 0.2 µg/kg/day for adults and children aged over 3 years (Annex 4).

Table 4.81 Dietary exposure to phthalates in the UK derived in the 2007 UK total diet study (µg/kg/day)– selected age groups (full table available in COT, 2011): Estimated 97.5th percentile (low estimate based on treating below detection limit levels as 0, high estimate based on treating below detection limit levels as being equal to the detection limit)

Age (years)	DEP	DiBP	DBP	BBP	DCHP	DEHP	Total Phthalate
1-2.5	0.3-0.8	1.4-2.7	0.4-1.0	0.07-1.3	0.04-0.8	6.9-9.9	20.2
2.5-3.5	0.3-0.8	1.3-2.1	0.4-0.8	0.07-1.1	0.04-0.6	6.3-7.9	18.1
3.5-4.5	0.3-0.7	1.2-2.0	0.4-0.8	0.004-0.5	0.04-0.5	5.7-6.8	15.8
Adults	0.15-0.3	0.6-0.9	0.2-0.3	0.03-0.2	0.03-0.2	3.4-4	6.4

The dietary intakes of DINP and DIDP for Europeans of all ages modelled by Wormuth et al. (2006) were about 0.01µg/day, considerably lower than the estimated intakes used in the EFSA (2005) risk assessment and EU Risk Assessments. Given, however, that the total intakes

[64] http://www.efsa.europa.eu/en/efsajournal/pub/244.htm

of DINP and DIDP from all sources of exposure modelled by Wormuth et al. were considerably lower than estimates based on urinary metabolite concentrations (see Table 4.85 and Table 4.86), it seems likely that intakes in food were severely under-estimated. This is probably because Wormuth et al. assumed that the DINP and DIDP content of all foods except fish was zero whereas other investigators have assumed that all food types contain some DINP and DIDP but generally at concentrations below the detection limit. Wormuth et al.'s estimates of adult and teenage intakes of DIDP were dominated by DIDP in food (50-60%). In contrast to the estimates of DINP and DIDP in food, Wormuth et al. (2006) calculated a median human exposure of 2.5 and 2.9 µg DEHP/ kg/day for females and males respectively. They concluded that food had an approximately 98% contribution to total DEHP intake in an adult population.

The results of a Danish investigation suggest that the DINP and DIDP contents of reconstituted formula milk and dairy products are generally <0.005 mg/kg with a maximum measured level of 0.012 mg/kg for DINP (Sørensen, 2006). The results of this study imply that maximum intakes of DINP in formula would be 2.0 µg/kg/day for newborns (0-6 months) and 1.4 µg/kg/day for infants (0.5-3 years) with typical intakes of less than 0.9 and 0.6 µg/kg/day respectively. Similarly, based on the findings of Sørensen (2006), maximum intakes of DIDP in formula would appear to be less than 0.9 and 0.6 µg/kg/day for babies and infants respectively (based on the same assumed body weights and consumption patterns as in the Risk Assessments).

4.6.7.5.2 Duplicate diet studies

Duplicate diet studies provide an alternative approach to estimating dietary exposure to phthalates. In these studies, participants prepare food portions that are double the required size of which half is consumed and the other used as an input for the sample for analysis. The analytical sample is prepared by homogenising the collected samples for each meal of which a representative aliquot is analysed. In principle, such studies should give a better measure of intake over the period of investigation but only for a relatively small number of individuals. This gives rise to uncertainties about extrapolation to the wider population as well as uncertainties as to whether people ate normally during the study or modified their dietary habits because they were under scrutiny.

There has been only one recent duplicate diet investigation of phthalate intake in Europe. Fromme et al. (2007) undertook a duplicate diet study to investigate phthalate intakes in food in Germany but were unable to quantify intakes of DINP and DIDP. DINP was detected in only 4 of 350 samples whereas shorter chain phthalates were detected in ≥ 10% samples. The detection limits for DINP and DIDP were between 100 and 150 µg/kg (higher than in most of the studies of individual foodstuffs).

4.6.7.5.3 Assessment of food intakes

Based on the assumptions described in this section Table 4.81 summarises the estimated intakes of DINP and DIDP in food.

Adults and children above 18 months: Reported concentrations of DINP and DIDP food are typically <0.005 mg/kg indicating that the typical dietary intake of DINP and DIDP in adults and children over 18 months of age is <0.14 µg/kg/day (based on a mean adult intake of all foods and liquids of 29 g/kg/day as indicated in the US EPA Exposure Factors Handbook). Based on estimated past levels of exposure to DEHP and taking account of the restrictions on the use of DINP and DIDP in food contact materials, 4 µg/kg/day is considered a reasonable worst case estimate of adult dietary intake.

0-6 months old: The typical intake of DINP and DIDP in this age group stems from formula milk. The calculations are based on the same assumptions regarding intake and DINP content of formula milk as those in the EU Risk Assessments, but the resulting figures differ slightly

due to the differences in age categories used between the assessments. The calculated exposures to DINP is <2.1 µg/kg/day for this age group. This estimate covers both the typical case and a reasonable worst case.
Intakes of DIDP were set at half of those for DINP.

6-12 months old: The dietary exposure stems from formula milk, which for the typical case contributes to an exposure of < 1.9 µg/kg/day. In addition dietary exposure in this age group will also include a component in adult food which gives an exposure of < 0.39 µg/kg/day for the 6-12 months old based on an assumed consumption of one third of an adult diet. Total exposure is then 2.3 µg/kg/day.

Regarding a reasonable worst case for this age group it could be expected to be lower than that for adult foods because neither DINP nor DIDP should be used in the packaging of infant's food. Infants and toddlers will, however, be exposed to these substances in adult food. If a body weight of 7.42 is assumed for 6-12 months old children and the food intake is estimated to one third of adult levels the worst case estimate of intake of DINP from adult food is 10.8 µg/kg/day (1.9 µg/kg/day from formula milk and 8.9 µg/kg/day from food).

Intakes of DIDP were set at half of those for DINP.

12-18 months old: The same assumptions as for the 6-12 months old children were made, except for the food intake which was estimated at one half of an adult diet. This gives a formula milk intake of < 1.4 µg/kg/day and an exposure from food of < 0.45 µg/kg/day for the typical case. The reasonable worst case gives an exposure of 12.7 µg/kg/day (1.4 µg/kg/day from formula milk and 11.3 µg/kg/day from food).

Table 4.82 Estimated current intakes of DINP and DIDP in food (µg/kg bw/day).

	DINP		DIDP		Assumptions (typical and reasonable worst case)
	Typical	Reasonable worst case	Typical	Reasonable worst case	
Adult and children >18 months	0.14	4	0.1	2	Body weight 60 kg, food intake 14 g/kg/day; food and liquid intake 28 g/kg/day
12 -18 months old	1.9	12.7	0.97	6.3	Body weight 9.47 kg, food intake, half of adult intake, formula milk intake 141 g/day (dry weight)
6 -12 months old	2.3	10.8	1.2	5.4	Body weight 7.42 kg, food intake one third of adult intake, formula milk intake 141 g/day (dry weight)
0-6 months old	<2.1	2.1	1.0	1.0	Body weight 6.21 kg, formula milk intake 131 g/day (dry weight)

Conclusions

The EU Risk assessment estimates of exposure to DINP and DIDP arising from food contact materials and via the food chain seem high in comparison to the estimated current intakes in this report based on measurement data and measurement experience with other phthalates. Published measurement surveys have generally reported that concentrations of DINP and DIDP in food were below the detection limit in most samples.

The results of measurement surveys suggest that the highest levels of DINP and DIDP have been reported in food where there have been issues with food contact materials (e.g. Pedersen et al. 2008; Frankhauser-Noti et al. 2006; Grossgut 2011). Otherwise the concentrations of these substances in food are extremely small. Concentrations of DINP and DIDP in food in the absence of issues with food contact materials appear to be very low.

The increasing use of DINP and DIDP at the expense of DEHP and other phthalates and aging of products containing DINP and DIDP is likely to lead to increased levels of DINP and DIDP in the environment and increased contamination of the food chain leading to increased levels of these substances in food. Neither DINP nor DIDP are chemically bound in PVC so it is unlikely that technological innovations will lead to the development of plastics containing these substances that have a substantially lower potential to release DINP and DIDP. DINP and DIDP have similar physicochemical properties to DEHP and would be anticipated to be associated with a similar risk of food contamination. It is likely, however, that reported levels of DEHP in food are largely due to contamination by food contact materials.

The implementation of Commission Regulation (EU) no 10/2011 will limit (but not prevent) future exposure to DINP and DIDP arising from food contact materials. There are likely to be some regional differences in dietary exposure to DINP and DIDP arising from differences in diet and in food packaging. There are no data to determine whether actual differences in dietary intakes exist. There are also likely to be regional differences in the degree of DINP and DIDP contamination of the food chain arising from differences in environmental levels of these substances reflecting differences in use pattern.

4.6.8 Biomonitoring

4.6.8.1 Background to biomonitoring

4.6.8.1.1 Metabolites

DINP and DIDP are rapidly absorbed orally and rapidly eliminated. The parent phthalates can be detected in blood. However, due to fast cleavage of the first ester bond by serum esterases, half-life is very short and therefore, they are not suitable as biomarker (Hays et al. 2011). Human metabolism studies have shown that the first step in the metabolism of DEHP, DINP, DIDP and DPHP is the formation of simple short-lived monoesters, but the major share of the simple monoester is further metabolized to produce a number of oxidative metabolites (alcohols, ketones and carboxylic acids; Wittassek et al. 2011). The secondary, oxidized metabolites that are formed by ω-, ω-1- and β-oxidation are the main metabolites excreted in human urine and are largely eliminated within 24 hours of exposure (Wittassek et al. 2011). In addition DINP metabolites may include some with two functional groups whereas only metabolites with a single functional group have been quantified in studies of urinary metabolites (Koch et al. 2007). Early investigations of urinary metabolites of phthalates measured concentrations of the simple monoester but for DINP and DIDP, the simple monoesters make up only 2% or less of the dose excreted in urine (Wittassek et al. 2011). In comparison with DEHP, less DINP is excreted as monoester and less DINP is excreted as oxidised metabolites in urine (Anderson et al. 2011).

Oxidative products of the DINP and DIDP monoesters can be detected in nearly 100% of urine samples from the general population (Calafat et al. 2011) indicating that these species are a more sensitive marker of exposure than the monoesters. Oxidative metabolism of DINP is enzyme-mediated and oxidative metabolites cannot result from accidental contamination of samples with DINP during sampling, storage or analysis (Silva 2006). The higher concentrations of these substances in urine reduce the uncertainty in comparison of urinary levels between individuals or the reconstruction of phthalate intakes on the basis of observed levels of metabolites. Secondary oxidative metabolites of DINP and DIDP have been detected in blood and breast milk but only at very low levels and these media are therefore less suitable for biomonitoring than urine.

The different studies use different abbreviations for the studied metabolites, but all identified studies focus on one or more of the metabolites listed in Table 4.83 as biomarkers.

Table 4.83 DINP and DIDP metabolites used as biomarkers

Substance name	Chemical name of metabolite	Abbreviation used in this study	Synonyms
DINP	Mono-isononyl phthalate	MiNP	MNP
	Mono(4-methyl-7-hydroxyoctyl)phthalate	MHiNP	OH-MiNP 7OH-MMeOP mono(hydroxyisononyl) phthalate
	Mono(4-methyl-7-carboxyheptyl)phthalate	MCiOP	cx-MiNP 7cx-MMeHP carboxy-MiNP mono(carboxyisononyl) phthalate MCOP
	Mono(4-methyl-7-oxooctyl)phthalate	MOiNP	oxo-MiNP 7oxo-MMeOP mono(oxoisononyl) phthalate
DIDP	Mono-isodecyl phthalate	MiDP	
	Mono-hydroxyisodecyl phthalate	MHiDP	OH-MiDP
	Mono(2,7-dimethyl-7-carboxyheptyl)phthalate	MCiNP	cx-MiDP MCNP mono(carboxyisooctyl) phthalate
	Monooxoisodecyl phthalate	MOiDP	oxo-MiDP

The advantages and disadvantages for available biomarkers for DINP are summarised in below.

Table 4.84 Advantages and disadvantages for available biomarkers for DINP (after Hays et al. 2011)

Analyte	Medium	Advantages	Disadvantages
DINP	Blood	Specific	Invasive; very short half-life; biomarker unstable in collected samples due to serum esterases
MiNP	Urine	Non-invasive; specific to DINP	Minor metabolite; short half-life, susceptible to contamination during collection and analyses
MHiNP	Urine	Non-invasive; specific to DINP, major metabolite	Short half-life
MOiNP	Urine	Non-invasive; specific to DINP, major metabolite	Short half-life
MCiOP	Urine	Non-invasive; specific to DINP, major metabolite	Short half-life
Sum of oxidative metabolites (OH-, oxo and MCiOP)	Urine	Non-invasive; greater percentage of total DINP-related excretion	Short half-life

4.6.8.1.2 Sampling

Both DINP and DIDP are metabolised relatively rapidly leading to a diurnal and day to day variation in the quantities of DINP and DIDP metabolites excreted in urine in response to the variation in intakes of these compounds over a 24 hour period. The variability of DEHP[65] metabolites in samples from individual volunteers showed a greater within day than between day variability with significantly higher concentrations in the evening compared to the morning (Preau et al. 2010). Similarly the within person variability was greater than between persons (Preau et al. 2010).

There are essentially two protocols for collecting urine samples: studies using spot samples and studies using 24 hour samples. In the latter all urine excreted in one day is collected, thus giving the absolute metabolite amounts excreted in one day. The method is however logistically difficult (Wittassek et al. 2011). As a result of the variabilities in metabolite concentrations, a single spot urine sample may not be representative for the mean daily concentration (Wittassek et al. 2011). The variabilities might be balanced out in larger some spot sample studies where urine samples were collected from different individuals at different times of day rather than at a specific time (Wittassek et al. 2011).

4.6.8.1.3 Analytical measurement

Commercial DINP and DIDP are mixtures of esters of ortho-phthalic acid with variously branched C9/C10 alkyl alcohols and alkyl chains with other lengths (see section 4.1.2). In the body, these mixtures are converted to a large number of oxidised metabolites that appear in chromatograms as a series of overlapping peaks. There is thus not just one monoester, hydroxy, oxo and carboxy metabolite like is for instance the case for DEHP (Wittassek and Angerer 2008). This has limited the ability to accurately measure individual urinary metabolites (Wittassek et al. 2011). Measurement is based on the summed area under all of the peaks and

[65] The metabolites of DEHP have a shorter half time of clearance than those of DINP (2-3 hours versus 3-5 hours, Wittassek and Angerer 2008)

2 well characterised standards representative of the metabolites present are used for calibration, for example, mono(4-methyl-7-hydroyxoctyl) phthalate and mono(4-methyl-7-oxyooctyl) phthalate (Wittassek et al. 2007). Calibration functions are developed for the instrument response for the pure calibration standards and then applied to the summed area of the overlapping peaks in the sample chromatograms. This represents an approximation as not all the metabolite species present will give the same detector response (some will be over-estimated and some under estimated). This means that measurements of slightly different mixes of metabolites are not directly comparable, although as the compounds are similar, the differences in the response are likely to be small. The precision of analysis of urinary metabolites of phthalates analysed in the NHANES study, expressed as the relative standard deviation, was 8-10% for low concentration and 6-10% for high concentration quality control samples (Calafat et al. 2011). Calafat et al. (2011) however reported that they were unable to separate the chromatographic peaks of MHiNP and MOiNP, and were thus not able to estimate their concentrations.

Where information about detection limits has been provided in studies, the detection limit for individual metabolites ranged between 0.25 and 2 µg/L with most studies reporting detection limits of less than 0.8 µg/L (Becker et al. 2009; Koch et al. 2007; Goen et al. 2011). In repeated determinations of the levels of an individual metabolite in a sample spiked with a known level of that metabolite, the standard deviation of the repeat measurements can range to up to 15% of the mean (Becker et al. 2009; Koch et al. 2007). This implies a relatively high level of uncertainty around individual measurements compared with that associated with easier analytes in easier matrices.

4.6.8.1.4 Methodology for back calculation

To calculate daily exposure from spot urine samples the following equation is used (Kransler et al. 2012):

$$DI = [UC \times CE / (F_{UE} \times 1000)] \times [MW_d/MW_m]$$

in which
DI = daily intake (µg/kg bw/day);
UC = creatinine corrected urinary metabolite concentration (µg/kg);
CE = creatinine excretion rate (mg/kg bw/day)
F_{UE} = fractional urinary excretion rate of the metabolites (unitless)
MW_d = molecular weight of DINP
MW_m = molecular weight of metabolites

For 24 hour studies the following equation can be used (Kransler et al. 2012):

$$DI = [UC_{pm} \times UV_{24} /(F_{UE} \times BW)] \times [MW_d]$$

in which
DI = daily intake (µg/kg bw/day);
UC_{pm} = urinary metabolite concentration (µmol/l)
UV_{24} = 24-hour urine volume (l/day)
F_{UE} = fractional urinary excretion rate of the metabolites (unitless)
BW = body weight (kg)
MW_d = molecular weight of DINP

The precision of the fractional urinary excretion values is critical for the results of back calculated exposure estimates departing from urinary metabolites. Fractional urinary excretion values for DINP metabolites have been established from the use of deuterium labelled DINP in studies of human volunteers (Table 4.10). These excretion values are based on the

cumulative integration of all structural isomers with the same function group: hydroxyl, oxo or carboxy.

For both datasets to derive the fractional urinary excretion values for DINP, deuterium labelled DINP-2 has been used (CAS No 28553-12-0). The metabolites MiNP, MHiNP, MCiOP, and MOiNP account for ca. 30-40% of the DINP dose, but it is known that further metabolites (e.g. twice oxidised side-chains or side-chains shortened by ß-oxidation) are formed and excreted, which cannot currently be determined quantitatively (UBA 2011; Koch and Angerer 2007; Anderson et al. 2011). Silva et al. (2006b) found that relative amounts of the metabolites of DINP-1 and DINP-2 differed. It is thus likely that fractional urinary excretion values for metabolites would differ with DINP-2 (or even with another batch of DINP-1).

No experimental data in humans is available to derive F_{UE} values for DIDP. The F_{UE} values can be anticipated to be similar to those for DINP (Exxon 2011).

Given that commercial forms of DINP and DIDP contain a range of C8 to C10 isomers with an overlap between the two substances, there will also be an overlap in the urinary metabolites arising from exposure. This will give rise to some uncertainty in the quantification of exposure to specifically DINP or DIDP as opposed to the two isomers together. The extent of potential inter-individual differences in metabolism of DINP is also unknown which gives rise to a further level of uncertainty in the estimation of intakes from concentrations of urinary metabolites.

Creatine is fairly constantly converted to creatinine at a rate of about 2% of total body creatine per day (Barr et al. 2005). Individuals vary in the rate that they excrete urine and in the quantities of urine excreted. Therefore spot sample studies normalise urinary metabolite concentrations against creatinine or daily urinary volume reference values in order to estimate the amount excreted over a full day. Although normalisation of urinary metabolite levels against creatinine reduces one source of uncertainty in the comparison of levels in different individuals, it does introduce other uncertainties related to the potential variability of creatinine excretion rates. Indeed, creatinine is dependent on muscle mass and activity. There are age, gender, race, BMI, fat-free mass and health related (kidney function, hyperthyroidism, hypertension, and diabetes) variations in rates of creatinine excretion, as well as the time of the day of taking samples (Barr et al. 2005). Studies using 24 h collection do not have this problem since the absolute metabolite amounts are available.

4.6.8.2 Estimation of exposure based on urinary metabolite levels

4.6.8.2.1 Available biomonitoring data

Most biomonitoring studies have investigated levels of DINP and/or DIDP metabolites in urine with a much smaller number of investigations of the levels present in blood and breast milk.

There is good information about urinary metabolite levels in Germany and some information for Denmark, the Netherlands and France. There are also good data for the USA and some data from elsewhere in the world (e.g. Israel, Japan, and Taiwan).

Data are available for children older than 4 years and for adults. There is no biomonitoring data for newborns and infants (except for a small number of Taiwanese infants of 2 years old in Lin et al. 2011). This reflects the practical difficulties in collecting urinary samples from this age group and the absence of effective alternatives to urinary markers of exposure to DINP and DIDP for use in biomonitoring (see below).

Very few data on DINP and DIDP in other biological media than urine have been identified. Besides the results shown in the table, Fromme et al. (2011) found the levels of parent DINP and DIDP in breast milk below the level of detection of 0.1 mg/kg. Metabolites of the two phthalates were however not included in the analysis programme. Högberg et al. (2008) did

not find parent DIDP and DINP in detectable concentrations in blood and milk samples. The study did not include measurements of metabolites.

A summary of identified biomonitoring data for DINP and DIDP metabolites is provided in Table 4.85 and Table 4.86 and in Annex 7 and Annex 8, respectively.

4.6.8.2.2 Exposure estimates based on urinary metabolite data

Table 4.85 and Table 4.86 show estimates of DINP and DIDP exposure based on urinary metabolite data.

There is substantial between-study variability that gives rise to two-fold differences in predicted intakes. The between-study variability reflects differences in the urinary metabolites considered, differences in the sampling method and population, differences in the assumed clearance rate and differences in the approach taken to modelling intakes based on DINP metabolites.

Table 4.85 Estimated DINP intakes (µg/kg bw/day) based on urinary metabolite data

Country	Number of subjects	Age (y)	Year	Intake		Basis of estimated intake
				50th p	95th p (max)	
Germany Wittassek et al. (2007)	60 60 60 60 145 68 60 60 59	20-29 20-29 20-29 20-29 20-29 20-29 20-29 20-29 20-29	1988 1989 1991 1993 1996 1998 1999 2001 2003	0.20 0.24 0.22 0.27 0.33 0.30 0.32 0.34 0.40	1.4 2.2 4.5 1.7 1.6 7.8 1.9 2.3 1.5	24 hour urine concentrations of MHiNP and MOiNP Estimate based on %dose excreted in urine over 24 hours following administration of deuterium labelled DINP to single human volunteer (Koch and Angerer, 2007)
Germany Göen et al. (2011) as cited in Kransler et al. (2012)	60 60 60 60	20-29 20-29 20-29 20-29	2002 2004 2006 2008	1.11[2] 1.09[2] 1.34[2] 1.67[2]	3.78 3.60 8.58 7.36	Estimated using 24 hour urine concentrations of MHiNP, MOiNP and MCiOP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
Germany Koch et al. (2011b)	108	5.6-6.7	2007	2.4	9.5 (31.2)	Spot urine samples Based on urine levels of MHiNP, MOiNP, MCiOP Children specific creatinine based calculation model based on excretion of DINP metabolites in urine in adult volunteers (Koch et al. 2007; Wittassek et al. 2007)
Germany Koch and Calafat (2009)	45	adults	2007	1.21	4.04	Spot urine samples (morning) Based on urine levels of MHiNP, MOiNP and MCiOP Calculation by Kransler et al. (2012)
Germany Mother-child pairs Kasper-Sonnenberg et al. (2012) as cited in Kransler et al. (2012)	96 93	mean 6.8 mean 39.2	2007-9	2.85 1.52	N.R. N.R.	Spot urine samples (morning) Based on urine levels of MHiNP, MOiNP and MCiOP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)

Country	Number of subjects	Age (y)	Year	Intake		Basis of estimated intake
				50th p	95th p (max)	
Germany Wittassek and Angerer (2008)	102	6-80	2001-2	0.6	(36.8)	Sampling regime not stated. Based on urine levels of MHiNP, MOiNP and MCiOP Estimation of intake based on fractions of dose excreted in urine in adult volunteers (Anderson et al. 2001; Koch and Angerer, 2007)
Germany Fromme et al. (2007b) as cited in Kransler et al. (2012)	27 women 23 men	14-60	2005	1.42 1.83	3.07 2.99	Spot samples Based on urine levels of MHiNP and MOiNP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
Germany Children Becker et al. (2009) as cited in Kransler et al. (2012)	137 145 149 168	3-5 6-8 9-11 12-14	2003-6	8.45 7.74 7.24 2.82	38.85 34.69 39.62 12.00	Spot samples (morning urine) MHiNP, MOiNP, MCiOP
Denmark Frederiksen et al. (2011) N = 129	 25 26 14 24 29 11	Boys 6-10 11-16 17-21 Girls 6-10 11-16 17-21	2006-8	 2.04 1.42 1.52 1.93 1.53 1.01	 9.02 (9.88) 5.26 (5.36) N.R. (3.63) 10.4 (11.9) 6.99 (7.96) N.R. (2.49)	24 hour urine samples Based on urine levels of MiNP, MHiNP, MOiNP and MCiOP intake based on fractions of dose excreted in urine in adult volunteer experiment (Anderson et al. 2011) using child specific model (Koch, 2007; Wittassek et al. 2007)
Denmark Frederiksen et al. (2010) as cited in Kransler et al. (2012)	60	18-26	2006	1.26[2]	3.48	Spot samples Based on urine levels of MiNP, MHiNP, MOiNP and MCiOP Calculation by Kransler et al. (2012)

Country	Number of subjects	Age (y)	Year	Intake		Basis of estimated intake
				50th p	95th p (max)	
Denmark Boas et al. (2010) as cited in Kransler et al. (2012)	250 girls 250 boys	4-9 4-9	2006-7	2.13 2.25	3.03 3.41	Spot samples Based on urine levels of MiNP, MHiNP, MOiNP and MCiOP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
Netherlands pregnant women Ye et al. (2008) as cited in Kransler et al. (2012)	99	18-41	2002-6	1.18[2]	13.48	Spot samples Based on urine levels of MHiNP and MOiNP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
Norway pregnant women Ye et al. (2009) as cited in Kransler et al. (2012)	11	15-53	2004-6	1.75[2]	N.R.	Spot samples Based on urine levels of MHiNP and MOiNP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
Outside EU						
USA CDC (2010) as cited in Kransler et al. (2012)	389 401 1814	6-11 12-19 20-59	2007-8	2.56[2] 1.76[2] 1.34[2]	12.32 14.55 10.97	Spot samples Based on urine levels of MiNP and MCiOP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
USA NHANES ExxonMobil (2011a)		6-11 12-40 40+ 6-60	2005-6	1.77[2] 1.03[2] 1.20[2] 1.46[2]	7.64 7.86 7.28 9.32	Spot samples Calculation by ExxonMobil (2011a)
USA NHANES III David (2000)	289	20-60	1988-1994	0.21[1]	1.08	Published estimate: Spot urine samples, metabolites of MiNP estimated based on fractions of dose excreted in urine in adult volunteer experiment later published by Anderson et al. (2001) and assumed creatinine excretion rates over 24 hours

Country	Number of subjects	Age (y)	Year	Intake		Basis of estimated intake
				50th p	95th p (max)	
USA NHANES III Kohn et al. (2000)	289	20-60	1988-1994	<LOD	1.7	Published estimate: Spot urine samples, metabolites of MiNP – fractional urinary excretion of MiNP and total DINP metabolites over 24 hours; 2 compartment model
USA Calafat et al. (2011) as cited in Kransler et al. (2012)	356 702 1040 450	6-11 12-19 20-59 60+	2005-6	2.35 1.58 1.38 1.37	8.16 9.15 9.52 8.55	Spot sampling Based on urine levels of MiNP and MCiOP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
USA Silva et al. (2006a)	129	adults	2003-4	2.49	11.39	24 hour urine samples Based on urine levels of MiNP, MHiNP, MOiNP and MCiOP Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
Taiwan Children and pregnant women Lin et al. (2011) as cited in Kransler et al. (2012)	30 59 100	2-3 5-6 23-35	2003-4 2006-7 2001-2	1.92[2] 0.95[2] 0.05[2]	2.00 3 0.20	MHiNP, MOiNP, MCiOP As calculated in the original publication
Israel Pregnant women Berman et al. (2009)	19	24-41	2006	0.74[2]	N.R.	Spot sampling Based on urine levels of MHiNP and MOiNP (note that from Berman et al. 2009 the only measured metabolite seems to be MCiOP) Fractional urinary excretion values from Anderson et al. (2011) Calculation by Kransler et al. (2012)
Japan Pregnant women Suzuki et al. (2009) as cited in Kransler et al. (2012)	50		2005-6	0.06[2]	4.38	MiNP As calculated in the original publication

[1] geometric mean
[2] mean
N.R. = not reported

Table 4.86 Estimated DIDP intakes (µg/kg bw/day) based on urinary metabolite data

Country	Number of subjects	Age (y)	Year	Intake		Basis of estimated intake
				50th p	95th p (max)	
Germany Koch et al. (2011b)	108	5.6-6.7	2007	0.3	1.20 (2.2)	Spot urine samples Based on urine levels of MHiDP, MOiDP, and MCiNP
USA NHANES ExxonMobil (2011a)		6-11 12-40 40+ 6-60	2005-6	1.27 0.56 0.71 0.75	5.99 2.56 3.03 3.72	Spot samples Calculation by ExxonMobil (2011a)
Israel Pregnant women Berman et al. (2009) as cited in ExxonMobil (2011a)	19	24-41	2006	0.41[1]	N.R.	Spot sampling Based on urine levels of MCiNP Calculation by ExxonMobil (2011a)

[1] mean
N.R. = not reported

Adults

It is apparent from Table 4.85 that most studies have reported **median** adult exposure to DINP of **around 1 µg/kg bw/day** with **95th percentile** intakes being generally **less than 10 µg/kg/day**. There is very few biomonitoring data for DIDP. Exposures to DIDP would appear to be smaller than for DINP.

Food is regarded as an important source of phthalate exposure for the general population (Wittassek et al. 2011). To test this hypothesis an exploratory (not peer reviewed) fasting experiment with two males and one female was carried out (Koch et al. 2006 in Wittassek et al. 2011). Urinary metabolites levels of DEHP and DINP dropped to very low levels within 24 h of fasting and remained low throughout the second day. This fasting effect was however not confirmed with DBP, DiBP and BBP in the same experiment. Fromme et al. (2007) compared results from urinary metabolites and food intakes (duplicate diet) from 50 German adults and concluded that both approaches yielded quite similar exposure estimates for DEHP, indicating that the dominant source of DEHP exposure was food ingestion. No or weak correlations were found for DBP and DIBP respectively, indicating that other exposure sources must contribute considerably. DINP was only detectable in 1% of the diet samples and exposure estimates from both methods could thus not be compared. This observation might actually indicate that the 'background' exposure to DINP might actually not necessarily always be food-related.

The few available biomonitoring data seem to confirm the low exposure to DINP of the mean or median adult population data. These figures correspond well to the typical exposure assumed for the indoor environment and food (see sections 4.6.6 and 4.6.7).

The 95th percentiles and maxima values are much higher than the mean or median estimates. This could be indicative of (occasionally or chronically) higher exposed individuals through food (in particular from food packaging materials), the indoor environment and/or indicative for individuals that have worn PVC clothing or used a sex toy before the urine samples were taken. Based on the data it is not possible to conclude on the origin of these higher exposures.

The available studies for adults (Wittassek et al. 2007; Goen et al. 2011; Wittassek and Angerer 2008; Fromme et al. 2007; Ye et al. 2008 and adolescents in Frederiksen et al. 2011) have typically small sample sizes and/or chose age groups that may not be representative for the average EU population. Small sample sizes in some studies might be insufficient to characterise population exposure, especially for spot urine sample studies. It is unlikely that all relevant populations with potentially higher exposures were included in the test population. Indeed most available European data for adults is for German students who may be atypical in terms of lifestyle and potential exposure. Populations elsewhere in Europe may have higher or lower levels of exposure to DINP depending on the extent to which PVC is used in flooring, wall coverings and interior furnishings and the proportion of this PVC that contains DINP as a plasticizer. In addition dietary exposures to DINP may vary across the EU (see section 4.6.7). Silva et al. (2006) measured six times higher median MHiNP concentrations in 129 US adults compared to the Wittassek et al. (2007) study with students. As the market for DINP and DIDP in the US is roughly half of that in the EU, it does not seem likely that the reason is actual higher exposure in the median US population. As suggested by Hays et al. (2011), it is not known if the discrepancy is due to exposure to different constituents of DINP substances (see section 4.6.8.1.4), a difference in analytical methods, differences in fasting times, or another unknown variable. Another reason could be that the populations are markedly different (students versus general population).

RAC (ECHA 2013b) agreed with this assessment for adults.

Children
From the relevant studies in Table 4.85 (Koch et al. 2011b; Kasper-Sonnenberg et al. 2012; Becker et al. 2009; Frederiksen et al. 2011 and Boas et al. 2010) one could calculate for the **typical case** a weighted average of the medium values of those studies of **4 µg/kg bw/day**, and for the **reasonable worst case a 95th percentile of 14 µg/kg bw/day** for children of 3-11 years old (weighed according to number of subjects).

Becker et al. (2009) reported 95th percentiles of up to 39.62 µg/kg/day for children between 3 and 11 years, which is considerably higher than found in other EU studies for children of that age (up to 10.4 µg/kg/day). How to interpret these findings in terms of exposure of children below 18 months old is, however, difficult.

The predicted exposure data from 600 children between 3 and 14 years old from Becker et al. (2009) and data from 129 children/adolescents between 6-21 years old from Frederiksen et al. (2011) indicate a decrease in exposure with an increase in age. This trend was reported to be statistically significant in Becker et al. (2009). This corresponds to the expected higher dust and food intakes combined with lower body weights of children (see sections 4.6.6 and 4.6.7).

There are no biomonitoring data for children under 3 years in age. In any case, such data would not reflect exposure from toys and childcare articles which can be placed in the mouth by children as there is an existing restriction. Such data could however be indicative of the exposure of children resulting from other sources.

Table 4.87 Estimated (external) exposure to DINP based on biomonitoring data

	Children (3-11 years)		Adults	
	Typical case (median)	**Reasonable worst case (95th percentile)**	**Typical case (median)**	**Reasonable worst case (95th percentile)**
Exposure (µg/kg bw/day)	4	14	1	<10

Future trend
It can be expected that the observed increasing trend in DINP and DIDP use (see section 4.2) would lead to an increase in the observed mean/median exposure figures from biomonitoring data. Indeed Wittassek et al. (2007) observed an increasing trend in the estimated exposure from biomonitoring data in the period 1988-2003. If this trend would continue, it could be assumed that estimates based on actual and future biomonitoring data would result in higher exposure estimates than reported in Table 4.85. On the other hand, food exposure might have decreased as a result of the specific migration limit in food for DINP and DIDP from packaging materials (see section 4.6.7). Assuming that some of the peaks in the measurements are a result of other than food exposure, the 95th percentiles could be expected to increase however. In conclusion, the overall consequences from the opposing trends are not clear, but are not considered of high importance in the current assessment.

4.7 Risk characterisation

4.7.1 Toys and childcare articles

In section 4.4.11, DNELs were set for oral and dermal exposure for children. When combined with the exposure calculated in section 4.6.2, this leads to risk characterisation ratios (RCRs) presented in

Table 4.89. For convenience the main assumptions and results from the exposure assessment are presented here again in Table 4.88.

Table 4.88 Summary of main assumptions and results from exposure to toys and childcare articles

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Daytime mouthing duration (min/day)	7.5	120	7.5	120	7.5	120
Migration rate (µg/cm^2/hour)	14	45	14	45	14	45
Exposure mouthing articles (µg/kg bw/day)	**3**	**145**	**2**	**118**	**2**	**95**

The reasonable worst case mouthing time integrated data from several observational studies. There are uncertainties attached to this estimate as a consequence of the limitations and discrepancies in the data, as well as the skewness and difficulties to determine appropriate article categories. The estimated mouthing time of 120 minutes is considered realistic, considering the maximum value of 227 min/day for mouthing of 'toys' for children aged 6-9 months and 178 min/day for 'other objects' for children of 2 years old in Smith and Norris (2002), and maximum mouthing times for non-pacifiers of over 200 and 300 min/day for 0-18 and 4-21 month old children respectively in Juberg et al. (2001).

Similarly, selecting a reasonable worst case estimate for migration rates was not straightforward as a result of the extreme variability observed between studies and within studies. The selected migration rate of 45 µg/cm^2/h is a value from in vitro data submitted to ECHA by ExxonMobil in the framework of the current review. This value is right in the middle of the highest mean estimate from the available in vivo migration data (32.6 µg/cm^2/h) and 53.4 µg/cm^2/h, the highest measured value of a single sample in the in vivo study by Meuling et al. (2000) which was used in the EU Risk Assessments for DINP and DIDP as a basis for risk assessment. The reasonable worst case migration estimate is considered to be realistic.

Against the above considerations of the uncertainties involved, the calculated RCRs for repeated dose toxicity of 1.3 to 2.0 for DINP and DIDP indicate that there would be an oral risk for liver toxicity from the mouthing of toys and childcare articles for the age groups 0-18 months old. Concerning reproductive effects, RCRs of 0.4 to 0.6 were calculated.

Table 4.89 Risk characterisation for repeated dose toxicity from DINP and DIDP in toys and childcare articles

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Oral exposure, excl. pacifiers (µg/kg bw/day)	3	145	2	118	2	95
$DNEL_{oral}$ (µg/kg bw/day)	75	75	75	75	75	75
RCR_{oral}	**0.04**	**1.93**	**0.03**	**1.57**	**0.02**	**1.27**
Dermal exposure (µg/kg bw/day)	26	54	24	50	24	49
$DNEL_{dermal}$ (µg/kg bw/day)	1880	1880	1880	1880	1880	1880
RCR_{dermal}	**0.01**	**0.03**	**0.01**	**0.03**	**0.01**	**0.03**
$RCR_{total\ toys}$	**0.05**	**1.96**	**0.04**	**1.60**	**0.04**	**1.30**

Table 4.90 Risk characterisation for reproductive toxicity from DINP in toys and childcare articles

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Oral exposure, excl. pacifiers (µg/kg bw/day)	3	145	2	118	2	95
$DNEL_{oral}$ (µg/kg bw/day)	250	250	250	250	250	250
RCR_{oral}	**0.01**	**0.58**	**0.01**	**0.47**	**0.01**	**0.38**
Dermal exposure (µg/kg bw/day)	26	54	24	50	24	49
$DNEL_{dermal}$ (µg/kg bw/day)	6250	6250	6250	6250	6250	6250
RCR_{dermal}	**0.004**	**0.009**	**0.004**	**0.008**	**0.004**	**0.008**
$RCR_{total\ toys}$	**0.02**	**0.59**	**0.01**	**0.48**	**0.01**	**0.39**

Table 4.91 Risk characterisation for reproductive toxicity from DIDP in toys and childcare articles

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Oral exposure, excl. pacifiers (µg/kg bw/day)	3	145	2	118	2	95
$DNEL_{oral}$ (µg/kg bw/day)	260	260	260	260	260	260
RCR_{oral}	**0.01**	**0.56**	**0.01**	**0.45**	**0.01**	**0.37**
Dermal exposure (µg/kg bw/day)	26	54	24	50	24	49
$DNEL_{dermal}$ (µg/kg bw/day)	6500	6500	6500	6500	6500	6500
RCR_{dermal}	**0.004**	**0.008**	**0.004**	**0.008**	**0.004**	**0.008**
$RCR_{total\ toys}$	**0.01**	**0.57**	**0.01**	**0.46**	**0.01**	**0.37**

4.7.2 Dermal exposure for adults

In section 4.4.11, DNELs were set for dermal exposure to DINP and DIDP for adults (repeated dose toxicity) and foetal exposure in pregnant women (reproductive toxicity). When combined with the exposure calculated in section 4.6.3 the RCRs presented in the tables below can be calculated. The highest RCR was 0.2, as calculated for reasonable worst case dermal exposure to DIDP for liver effects.

The reasonable worst case assumed that PVC trousers are worn close to the skin (e.g. "skinny faux leather pants") for 10 h/day for two weeks per month. The reasonable worst case is considered to cover the largest part of the adult population and thus consists of an appropriate estimate to be used for risk assessment.

The experimental migration rate assumed might not be representative for possibly higher exposure as a result of perspiration in PVC clothing. In addition, a small minority of the population may wear PVC garments next to the skin for longer periods per day and not necessarily restricted to trousers only. This could lead to higher exposure, but an RCR of 1 cannot be reached using the current assumptions (exposure duration would need to exceed 24 h/day).

It can be concluded that dermal exposure sources to DINP and DIDP as such are not anticipated to result in a risk for adults nor for the developing foetus in pregnant women. It cannot be excluded that in some individuals frequently wearing PVC clothing close to the a large surface of the skin the body burden would be rather high, especially with PVC clothing containing DIDP.

Table 4.92 Risk characterisation for repeated dose toxicity in adults dermally exposed to DINP and DIDP

	Typical case	Reasonable worst case
Dermal exposure (µg/kg bw/day)	10	299
$DNEL_{dermal}$ (µg/kg bw/day)	1880	1880
RCR_{dermal}	**0.005**	**0.159**

Table 4.93 Risk characterisation for reproductive toxicity in adults dermally exposed to DINP

	Typical case	Reasonable worst case
Dermal exposure (µg/kg bw/day)	10	299
$DNEL_{dermal}$ (µg/kg bw/day)	6250	6250
RCR_{dermal}	**0.0016**	**0.0478**

Table 4.94 Risk characterisation for reproductive toxicity in adults dermally exposed to DIDP

	Typical case	Reasonable worst case
Dermal exposure (µg/kg bw/day)	10	299
$DNEL_{dermal}$ (µg/kg bw/day)	2060	2060
RCR_{dermal}	**0.0049**	**0.145**

4.7.3 School materials

In section 4.4.11, DNELs were set for oral exposure of children to DINP and DIDP. When combined with the exposure calculated in section 4.6.4, this leads to risk characterisation ratios (RCRs) presented in the tables below. No typical exposure case was assumed for mouthing of erasers, since the behaviour is in general not considered as a typical behaviour for children, and for those children that would show such behaviour it is considered typically not to persist over a long period of time. As can be seen from the calculated RCRs, no risk can be expected from a reasonable worst case estimate of mouthing erasers with DINP or DIDP.

Table 4.95 Risk characterisation for repeated dose toxicity in 6 year old children orally exposure to erasers with DINP and DIDP

	Reasonable worst case
Oral exposure (µg/kg bw/day)	2.3
$DNEL_{oral}$ (µg/kg bw/day)	75
RCR_{oral}	**0.031**

4.7.4 Sex toys

In section 4.4.11, DNELs were set for oral exposure to DINP and DIDP for adults and the developing foetus in pregnant women. When combined with the exposure calculated in section 4.6.5this leads to risk characterisation ratios (RCRs) presented in the tables below.

Based on the search of PubMed and wider search of the internet, there are no published data describing the absorption of phthalates through the epithelium of the vagina or anal

canal. The absorption through vaginal and mucous membranes (rectum) is assumed to be 50%. Some substances are better absorbed via the rectum than orally, and others are absorbed more poorly (van Hoogdalem 1991). Orally there might be a first pass mechanism that is circumvented via the vagina or mucous membrane route (Jannsen and Bremmer 2010, RIVM). Thus, the actual vaginal/rectal absorption may also be higher than assumed here.

The typical case exposure assumed that a vibrator was used once a week for 15 min. The reasonable worst case estimate assumed a daily use for a period of 15 minutes. These times were believed to correspond to real-life exposure situations. It is conceivable that some individuals (e.g. sex workers) could be exposed for longer periods.

There is considerable uncertainty related to the migration rate assumed in the reasonable worst case (121 µg/cm2/h). Highly lipophilic substances such as phthalates might show higher migration than assumed here when oil-based personal lubricants are used with sex toys (migration experiments used water-based simulants).

With RCRs below 1, it seems not likely that the use of sex toys with DINP or DIDP would result in a risk. However, this conclusion is subject to the abovementioned uncertainties.

Table 4.96 Risk characterisation for vaginal/rectal exposure to DINP in adults

	Repeated dose toxicity		Reproductive toxicity	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Vaginal/rectal exposure (µg/kg bw/day)	4.8	63	4.8	63
$DNEL_{sex}$ (µg/kg bw/day)	150	150	500	500
RCR_{sex}	**0.03**	**0.42**	**0.01**	**0.13**

Table 4.97 Risk characterisation for vaginal/rectal exposure to DIDP in adults

	Repeated dose toxicity		Reproductive toxicity	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Vaginal/rectal exposure (µg/kg bw/day)	4.8	63	4.8	63
$DNEL_{sex}$ (µg/kg bw/day)	150	150	165	165
RCR_{sex}	**0.03**	**0.42**	**0.03**	**0.38**

4.7.5 Indoor air and house dust

In section 4.4.11, DNELs were set for oral and inhalation exposure to DINP and DIDP for children, adults and the developing foetus in pregnant women. When combined with the exposure calculated in section 4.6.6this leads to risk characterisation ratios (RCRs) presented in the tables below. The estimated RCRs from inhalation are very low and do not constitute a considerable body burden. In the reasonable worst case there is a significant body burden for children of 0-18 months old (RCRs for repeated dose toxicity up to 0.4).

Table 4.98 Risk characterisation for repeated dose toxicity from exposure of children to DINP in indoor and vehicle air and from house dust

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from indoor and vehicle air ($\mu g/m^3$)	1.5 + 0.23 = 1.73	7.3 + 1.2 = 8.5	1.5 + 0.23 = 1.73	7.3 + 1.2 = 8.5	1.5 + 0.23 = 1.73	7.3 + 1.2 = 8.5
$DNEL_{inh}$ ($\mu g/m^3$)	260	260	260	260	260	260
RCR_{air}	**0.007**	**0.033**	**0.007**	**0.033**	**0.007**	**0.033**
Exposure from dust (µg/kg bw/day)	2.41	7.65	7.87	24.9	6.33	20.0
$DNEL_{oral}$ (µg/kg bw/day)	75	75	75	75	75	75
RCR_{dust}	**0.032**	**0.102**	**0.105**	**0.332**	**0.084**	**0.267**
RCR_{total}	**0.039**	**0.135**	**0.112**	**0.365**	**0.091**	**0.300**

Table 4.99 Risk characterisation for reproductive toxicity from exposure of children to DINP in indoor and vehicle air and from house dust

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from indoor and vehicle air ($\mu g/m^3$)	1.5 + 0.23 = 1.73	7.3 + 1.2 = 8.5	1.5 + 0.23 = 1.73	7.3 + 1.2 = 8.5	1.5 + 0.23 = 1.73	7.3 + 1.2 = 8.5
$DNEL_{inh}$ ($\mu g/m^3$)	868	868	868	868	868	868
RCR_{air}	**0.002**	**0.010**	**0.002**	**0.010**	**0.002**	**0.010**
Exposure from dust (µg/kg bw/day)	2.41	7.65	7.87	24.9	6.33	20.0
$DNEL_{oral}$ (µg/kg bw/day)	250	250	250	250	250	250
RCR_{dust}	**0.010**	**0.031**	**0.031**	**0.100**	**0.025**	**0.080**
RCR_{total}	**0.012**	**0.041**	**0.033**	**0.110**	**0.027**	**0.090**

Table 4.100 Risk characterisation for exposure of adults to DINP in indoor and vehicle air and from house dust

	Repeated dose toxicity		Reproductive toxicity	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from air ($\mu g/m^3$)	1.3 + 0.45 = 1.75	6.7 + 2.3 = 9.0	1.3 + 0.45 = 1.75	6.7 + 2.3 = 9.0
$DNEL_{inh}$ ($\mu g/m^3$)	350	350	1160	1160
RCR_{air}	**0.005**	**0.019**	**0.002**	**0.006**
Exposure from dust (µg/kg bw/day)	0.25	0.79	0.25	0.79
$DNEL_{oral}$ (µg/kg bw/day)	75	75	250	250
RCR_{dust}	**0.003**	**0.011**	**0.001**	**0.003**
RCR_{total}	**0.008**	**0.030**	**0.003**	**0.009**

Table 4.101 Risk characterisation for repeated dose toxicity from exposure of children to DIDP in indoor and vehicle air and from house dust

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from indoor and vehicle air ($\mu g/m^3$)	0.73 + 0.12 = 0.85	3.6 + 0.58 = 4.18	0.73 + 0.12 = 0.85	3.6 + 0.58 = 4.18	0.73 + 0.12 = 0.85	3.6 + 0.58 = 4.18
$DNEL_{inh}$ ($\mu g/m^3$)	260	260	260	260	260	260
RCR_{air}	**0.003**	**0.016**	**0.003**	**0.016**	**0.003**	**0.016**
Exposure from dust (µg/kg bw/day)	1.21	3.83	3.94	12.5	3.17	10.0
$DNEL_{oral}$ (µg/kg bw/day)	75	75	75	75	75	75
RCR_{dust}	**0.016**	**0.051**	**0.053**	**0.167**	**0.042**	**0.133**
RCR_{total}	**0.019**	**0.067**	**0.056**	**0.183**	**0.046**	**0.149**

Table 4.102 Risk characterisation for reproductive toxicity from exposure of children to DIDP in indoor and vehicle air and from house dust

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from indoor and vehicle air ($\mu g/m^3$)	0.73 + 0.12 = 0.85	3.6 + 0.58 = 4.18	0.73 + 0.12 = 0.85	3.6 + 0.58 = 4.18	0.73 + 0.12 = 0.85	3.6 + 0.58 = 4.18
$DNEL_{inh}$ ($\mu g/m^3$)	904	904	904	904	904	904
RCR_{air}	**0.0009**	**0.005**	**0.0009**	**0.005**	**0.0009**	**0.005**
Exposure from dust (µg/kg bw/day)	1.21	3.83	3.94	12.5	3.17	10.0
$DNEL_{oral}$ (µg/kg bw/day)	260	260	260	260	260	260
RCR_{dust}	**0.005**	**0.015**	**0.015**	**0.048**	**0.012**	**0.038**
RCR_{total}	**0.006**	**0.020**	**0.016**	**0.053**	**0.013**	**0.043**

Table 4.103 Risk characterisation for exposure of adults to DIDP in indoor and vehicle air and from house dust

	Repeated dose toxicity		Reproductive toxicity	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from air ($\mu g/m^3$)	0.67 + 0.23 = 0.90	3.3 + 1.2 = 4.5	0.67 + 0.23 = 0.90	3.3 + 1.2 = 4.5
$DNEL_{inh}$ ($\mu g/m^3$)	350	350	380	380
RCR_{air}	**0.003**	**0.013**	**0.002**	**0.012**
Exposure from dust (µg/kg bw/day)	0.12	0.40	0.12	0.40
$DNEL_{oral}$ (µg/kg bw/day)	75	75	80	80
RCR_{dust}	**0.002**	**0.005**	**0.002**	**0.005**
RCR_{total}	**0.004**	**0.018**	**0.004**	**0.017**

4.7.6 Food

In section 4.4.11, DNELs were set for oral exposure to DINP and DIDP for children, adults and the developing foetus in pregnant women. When combined with the exposure calculated in section 4.6.7 this leads to risk characterisation ratios (RCRs) presented in the tables below.

The RCRs are all below 0.2, indicating that no risk is expected from exposure to DINP or DIDP via food.

Table 4.104 Risk characterisation for repeated dose toxicity from exposure of children to DINP in food

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from food (µg/kg bw/day)	<2.1	2.1	2.3	10.8	1.9	12.7
$DNEL_{oral}$ (µg/kg bw/day)	75	75	75	75	75	75
RCR_{food}	**<0.028**	**0.028**	**0.031**	**0.144**	**0.025**	**0.169**

Table 4.105 Risk characterisation for reproductive toxicity from exposure of children to DINP in food

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from food (µg/kg bw/day)	<2.1	2.1	2.3	10.8	1.9	12.7
$DNEL_{oral}$ (µg/kg bw/day)	250	250	250	250	250	250
RCR_{food}	**<0.008**	**0.008**	**0.009**	**0.043**	**0.008**	**0.051**

Table 4.106 Risk characterisation for exposure of adults to DINP in food

	Repeated dose toxicity		Reproductive toxicity	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from food (µg/kg bw/day)	0.14	4	0.14	4
$DNEL_{oral}$ (µg/kg bw/day)	75	75	250	250
RCR_{food}	**0.002**	**0.053**	**0.001**	**0.016**

Table 4.107 Risk characterisation for repeated dose toxicity from exposure of children to DIDP in food

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from food (µg/kg bw/day)	1	1	1.2	5.4	0.97	6.3
$DNEL_{oral}$ (µg/kg bw/day)	75	75	75	75	75	75
RCR_{food}	**0.013**	**0.013**	**0.016**	**0.072**	**0.013**	**0.084**

Table 4.108 Risk characterisation for reproductive toxicity from exposure of children to DIDP in food

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from food (µg/kg bw/day)	1	1	1.2	5.4	0.97	6.3
$DNEL_{oral}$ (µg/kg bw/day)	260	260	260	260	260	260
RCR_{food}	**0.004**	**0.004**	**0.005**	**0.021**	**0.004**	**0.024**

Table 4.109 Risk characterisation for exposure of adults to DIDP in food

	Repeated dose toxicity		Reproductive toxicity	
	Typical case	**Reasonable worst case**	**Typical case**	**Reasonable worst case**
Exposure from food (µg/kg bw/day)	0.1	2	0.1	2
$DNEL_{oral}$ (µg/kg bw/day)	75	75	80	80
RCR_{food}	**0.001**	**0.027**	**0.001**	**0.025**

4.7.7 Indirect exposure of humans via the environment

The EU Risk Assessment for DINP has estimated the regional exposure for man via environment to be 6.5 µg/kg bw/day for infants (6-36 months) and 1 µg/kg bw/day for adults (EC 2003a). The EU Risk Assessment used EUSES modelling and remarked that in most of the PEC calculations, the porewater concentrations were higher than the water solubility of DINP, which threw some doubt over the estimations. The calculations and assumptions made in the EU Risk Assessment for the local exposure (156 µg/kg bw/day for infants, 28 µg/kg bw/day for adults) are considered highly conservative and are therefore disregarded in the current assessment.

Similarly, the EU Risk Assessment for DIDP has estimated the regional exposure for man via environment to be 13 µg/kg bw/day for infants (6-36 months) and 2 µg/kg bw/day for adults (EC, 2003b). The EU Risk Assessment used EUSES modelling and remarked that in most of the PEC calculations, the porewater concentrations were higher than the water solubility of DIDP, which threw some doubt over the estimations. The calculations and assumptions made in the EU Risk Assessment for the local exposure (179 µ/kg bw/day for infants, 27 µg/kg bw/day for adults) are considered highly conservative and are therefore disregarded in the current assessment.

The EU Risk Assessments did not calculate local and regional exposures for newborns (0-6 months).

No attempts have been made to update the figures from the EU Risk Assessment. This would have required a full environmental exposure assessment, which was not considered within the scope of this review. On the one hand the validity of the EU Risk Assessment estimates for the regional exposures was somewhat questionable, but on the other hand the use in the EU of DINP and DIDP has roughly doubled compared to the data used in the risk assessment (107000 t/y DINP + 200000 t/y DIDP = 300 000 t/y assumed in the EU Risk Assessments versus 650 000 t/y for DINP, DIDP and DPHP as estimated by COWI (2012), see section 4.2.1).

If the regional exposure values as estimated in the EU Risk Assessments for DINP and DIDP are used, the RCRs for reasonable worst case exposure of man via environment can be calculated as in the tables below. These RCR values are not taken further in order to avoid double counting exposure via food.

Table 4.110 Risk characterisation for repeated dose toxicity from reasonable worst case exposure of children via environment to DINP

	0-6 months	6-12 months	12-18 months	Adults
Exposure from food (µg/kg bw/day)	/	6.5	6.5	1
$DNEL_{oral}$ (µg/kg bw/day)	75	75	75	75
$RCR_{via\ env}$	**/**	**0.087**	**0.087**	**0.013**

Table 4.111 Risk characterisation for reproductive toxicity from reasonable worst case exposure of children via environment to DINP

	0-6 months	6-12 months	12-18 months	Developing foetus in pregnant women
Exposure from food (µg/kg bw/day)	/	6.5	6.5	1
$DNEL_{oral}$ (µg/kg bw/day)	250	250	250	250
$RCR_{via\ env}$	**/**	**0.026**	**0.026**	**0.004**

Table 4.112 Risk characterisation for repeated dose toxicity from reasonable worst case exposure of children via environment to DIDP

	0-6 months	6-12 months	12-18 months	Adults
Exposure via env (µg/kg bw/day)	/	13	13	2
$DNEL_{oral}$ (µg/kg bw/day)	75	75	75	75
$RCR_{via\ env}$	**/**	**0.173**	**0.173**	**0.267**

Table 4.113 Risk characterisation for reproductive toxicity from reasonable worst case exposure of children via environment to DIDP

	0-6 months	6-12 months	12-18 months	Developing foetus in pregnant women
Exposure via env (µg/kg bw/day)	/	13	13	2
$DNEL_{oral}$ (µg/kg bw/day)	260	260	260	80
$RCR_{via\ env}$	**/**	**0.05**	**0.05**	**0.025**

4.7.8 Risk characterisation for aggregated exposure [66]

In the following tables the most relevant combinations of exposure scenarios are made in order to give realistic risk characterisation ratios for exposure from different sources (aggregated exposure). For this purpose, reasonable worst case exposure from articles was combined with typical exposure from other sources ('background' exposure).

With regard to direct exposure from DINP or DIDP in articles, the calculated RCRs indicate a risk related to mouthing of toys and childcare articles in children younger than 18 months (under the hypothesis that the existing restriction on these would not be in place). The RCR for exposure from the mouthing of erasers was 0.03 for 6 year old children. It is clear that no concern is to be expected from this source of exposure. Furthermore, no risk is likely to be associated with the use of DINP- or DIDP-containing sex toys by the adult population with an RCR for repeated dose toxicity of 0.42.

The background exposure from food and the indoor environment leads to RCRs below 0.15 in children, and is not significant in the adult population with RCRs of maximum 0.01 in the typical case. These low exposure estimates for the general adult population are well in line with the RCRs calculated for biomonitoring exposure estimates in the typical case (Table 4.121). Thus, the few available biomonitoring data seem to confirm the low exposure to DINP of the mean or median adult population.

A comparison between the calculated exposure in the reasonable worst case scenarios for exposure to articles with the 90th/95th percentiles of biomonitoring estimates is not straightforward.

[66] "Aggregated exposure" includes all routes, pathways, and sources of exposure to a given chemical (SCHER/SCENIHR/SCCS 2011).

Biomonitoring data for children is scarce and only available for children above 3 years old. The 95th percentiles of the studies suggest that RCRs are below 0.2. A maximum value of 39 µg/kg bw/day was observed in a study with 137 children of 3-5 years old. With this value an RCR of 0.5 can be calculated for liver toxicity of DINP.

From the available biomonitoring data it is reasonable to conclude that the largest part (>95%) of the adult population is not expected to be at risk. A maximum value of 37 µg/kg bw/day was observed in a study with 102 individuals of 6-80 years old. With this value an RCR of 0.5 can be calculated for liver toxicity of DINP. The values between the 95th percentiles and the maxima observed in the biomonitoring studies could be indicative of (occasionally or chronically) higher exposed individuals through food or the indoor environment, or for example through exposure from PVC clothing or use of a sex toy before the urine samples were taken.

Table 4.114 Risk characterisation for repeated dose toxicity from reasonable worst case exposure of children to DINP from toys and childcare articles combined with typical exposure estimates for exposure from the indoor environment and food

	0-6 months	6-12 months	12-18 months
RCR_{toys}	1.96	1.60	1.29
$RCR_{air/dust}$	0.039	0.112	0.091
RCR_{food}	0.028	0.031	0.025
RCR_{total}	**2.03**	**1.74**	**1.41**

Table 4.115 Risk characterisation for repeated dose toxicity from reasonable worst case exposure of children to DIDP from toys and childcare articles combined with typical exposure estimates for exposure from the indoor environment and food

	0-6 months	6-12 months	12-18 months
RCR_{toys}	1.96	1.60	1.29
$RCR_{air/dust}$	0.019	0.056	0.046
RCR_{food}	0.013	0.016	0.013
RCR_{total}	**1.99**	**1.67**	**1.35**

Table 4.116 Risk characterisation for reproductive toxicity from reasonable worst case exposure of children to DINP from toys and childcare articles combined with typical exposure estimates for exposure from the indoor environment and food

	0-6 months	6-12 months	12-18 months
RCR_{toys}	0.59	0.48	0.39
$RCR_{air/dust}$	0.012	0.033	0.027
RCR_{food}	0.008	0.009	0.008
RCR_{total}	**0.61**	**0.52**	**0.42**

Table 4.117 Risk characterisation for reproductive toxicity from reasonable worst case exposure of children to DIDP from toys and childcare articles combined with typical exposure estimates for exposure from the indoor environment and food

	0-6 months	6-12 months	12-18 months
RCR_{toys}	0.57	0.46	0.37
$RCR_{air/dust}$	0.006	0.016	0.013
RCR_{food}	0.004	0.005	0.004
RCR_{total}	**0.58**	**0.48**	**0.39**

Table 4.118 Risk characterisation for reasonable worst case exposure of adults to DINP from sex toys combined with typical exposure estimates for exposure from the indoor environment, food and dermal exposure

	Repeated dose toxicity	Reproductive toxicity
$RCR_{sex\ toys}$	0.420	0.126
RCR_{dermal}	0.005	0.002
$RCR_{air/dust}$	0.008	0.003
RCR_{food}	0.002	0.001
RCR_{total}	**0.44**	**0.13**

Table 4.119 Risk characterisation for reasonable worst case exposure of adults to DIDP from sex toys combined with typical exposure estimates for exposure from the indoor environment, food and dermal exposure

	Repeated dose toxicity	Reproductive toxicity
$RCR_{sex\ toys}$	0.420	0.382
RCR_{dermal}	0.005	0.005
$RCR_{air/dust}$	0.004	0.004
RCR_{food}	0.001	0.001
RCR_{total}	**0.43**	**0.39**

Table 4.120 Risk characterisation for exposure of children of 3-11 years old to DINP based on biomonitoring estimates

	Repeated dose toxicity		Reproductive toxicity	
	Typical case	Reasonable worst case	Typical case	Reasonable worst case
Estimated exposure from biomonitoring (as oral exposure in µg/kg bw/day)	4	14	4	14
$DNEL_{oral}$ (µg/kg bw/day)	75	75	250	250
$RCR_{biom.}$	**0.05**	**0.19**	**0.02**	**0.06**

Table 4.121 Risk characterisation for exposure of adults to DINP based on biomonitoring estimates

	Repeated dose toxicity		Reproductive toxicity	
	Typical case	Reasonable worst case	Typical case	Reasonable worst case
Estimated exposure from biomonitoring (as oral exposure in µg/kg bw/day)	1	<10	1	<10

$DNEL_{oral}$ (µg/kg bw/day)	75	75	250	250
$RCR_{biom.}$	**0.01**	**<0.13**	**0.00**	**<0.04**

4.7.9 Risk characterisation for combined exposure [67]

One of the conditions to combine risks from DINP and DIDP is that there are simultaneous exposures or exposures in short succession (NRC 2008).

Since DINP and DIDP both are used in a wide variety of consumer articles and construction materials, with largely overlapping uses, this condition clearly is satisfied. This is confirmed by biomonitoring data, showing that metabolites of both DINP and DIDP (MCINP and MCIOP)were detected in most of the tested persons (Calafat et al. 2011, data from the US). According to the opinion of SCHER/SCENIHR/SCCS (2011), the dose/concentration addition[68] method should be preferred over the independent action approach if no mode of action information is available.

The liver effects in experimental studies with DINP and DIDP in rats are very similar and occur at similar dose levels. This is supported by the structural similarities between DINP and DIDP. The EU Risk Assessments for DIDP and DINP reported that a study on water solubility of DINP and DIDP confirmed that these phthalates might contain many common constituents (Exxon Biomedical Sciences 1996a as reported in EC 2003a,b). Presence of metabolites of DINP (MCiOP) in a study with single oral dosing of rats with 300 mg/kg DIDP (CAS 68515-49-1) suggests that DINP constituents are present in DIDP (Kato et al. 2007). Also Rastogi (1998) suggested overlap of isomeric peaks for DINP and DIDP. To some extent this blurs the chemical difference between DINP and DIDP.

In fact, it should be reminded in this context that also DINP-1 (CAS 68515-48-0) and DINP-2 (CAS 28553-12-0) are different substances, each requiring separate registrations, and that all the testing and assessments carried out by authorities as well as industry use combined risk assessment for these two substances based on their close structural similarity. The same can be said for the two substances commonly termed DIDP (CAS No 68515-49-1 and 26761-40-0).

In the light of the above considerations, it is considered appropriate to apply dose/concentration addition method for assessing the combined risks from DINP and DIDP. The preferred approach is the hazard index (HI), which is the sum of the hazard quotients (HQ, or RCRs in this case), i.e. the ratios between exposure and the reference value (RV, or DNELs in this case) for each component to be evaluated (SCHER/SCENIHR/SCCS 2011).

Combined risk characterisation can in this case not be applied to direct exposure from articles containing DINP or DIDP. The central assumption in the exposure assessment for direct exposure to articles is that those articles would contain either DINP or DIDP. Thus, dose addition can only be applied to the RCRs for exposure via food and the indoor environment.

Summation of reasonable worst case estimates for several routes and several substances is likely to result in over-conservative estimates. According to Frederiksen et al. (2010), however, it seems that participants with a high exposure to one phthalate was also highly

[67] "Combined exposure" includes all routes, pathways, and sources of exposure to multiple chemicals (SCHER/SCENIHR/SCCS 2011).

[68] Dose/concentration-addition (similar action, similar joint action, relative dose addition) occurs when chemicals in a mixture act in the same way, by the same mechanism/mode of action, and differ only in their potencies. Dose/Concentration-addition implies that the effects of exposure to a mixture of such compounds are equivalent to the effects of the sum of the potency-corrected doses of each component compound.(SCHER/SCENIHR/SCCS 2011).

exposed to other phthalates. Even if it cannot entirely be excluded that lifestyle could result in a higher exposure to both phthalates, summation of reasonable worst case exposure of different sources would most likely not represent a realistic exposure scenario. Therefore the summation of reasonable worst case RCRs are given for illustration purposes only.

Table 4.122 and Table 4.123 present RCRs for combined risk characterisation for repeated dose toxicity in children aged 0-18 months old. The combined assessment indicates that exposure via food and the indoor environment could constitute a considerable body burden, with RCRs up to of 0.2 in the typical case. In a reasonable worst case RCRs could be higher, but as previously indicated it is not necessarily realistic to sum reasonable worst cases for several routes and several substances. As an indication of what a realistic reasonable worst case could mount up to, one could assume the reasonable worst case exposure from the indoor environment for DINP summed with the typical case for DIDP and the typical case RCRs for both phthalates via food, resulting in an RCR of 0.47 in 6-12 months old children.

It can be concluded that no risk is expected from combined exposure to DINP and DIDP for children exposed via food and the indoor environment.

As was clear from the previous section, the background exposure from food and the indoor environment is not very significant in the adult population. The benefit of calculating RCRs based on combined exposure of DINP and DIDP for the adult population could therefore be questioned. Nevertheless, Table 4.124, Table 4.125 andpresent the results for combined repeated dose toxicity effects upon exposure to DINP and DIDP.

Insufficient data was available to calculate combined risk characterisation ratios on the basis of biomonitoring estimates.

Table 4.122 Combined risk characterisation for repeated dose toxicity from combined exposure of children to DINP and DIDP from food and the indoor environment

	0-6 months		6-12 months		12-18 months	
	Typical case	**Reasonable worst case**[*1]	**Typical case**	**Reasonable worst case**[*1]	**Typical case**	**Reasonable worst case**[*1]
$RCR_{food, DINP}$	<0.028	0.028	0.031	0.144	0.025	0.169
$RCR_{food, DIDP}$	0.013	0.013	0.016	0.072	0.013	0.084
$RCR_{food, comb}$	**<0.041**	**0.041**	**0.047**	**0.216**	**0.038**	**0.253**
$RCR_{indoor, DINP}$	0.039	0.135	0.112	0.365	0.091	0.300
$RCR_{indoor, DIDP}$	0.019	0.067	0.056	0.183	0.046	0.149
$RCR_{indoor, comb}$	**0.058**	**0.202**	**0.168**	**0.548**	**0.137**	**0.449**
$RCR_{ind./food, comb}$	**<0.10**	**0.24**	**0.21**	**0.76**	**0.17**	**0.70**

[*1] Values are given for illustration only. Summation of reasonable worst case estimates for several routes and several substances is likely to lead in overestimates. According to Frederiksen et al. (2010), however, it seems that participants with a high exposure to one phthalate was also highly exposed to other phthalates.

Table 4.123 Combined risk characterisation for repeated dose toxicity by combining the reasonable worst case exposure of children to DINP (or DIDP) from toys and childcare articles with combined typical exposure to DINP and DIDP from the indoor environment and food

	0-6 months	6-12 months	12-18 months
RCR_{toys}	1.96	1.60	1.29
$RCR_{ind./food, comb}$	0.10	0.21	0.17
$RCR_{total, comb}$	**2.06**	**1.82**	**1.47**

Table 4.124 Combined risk characterisation for repeated dose toxicity from combined exposure of adults to DINP and DIDP from food and the indoor environment

	Adults	
	Typical case	**Reasonable worst case**[*1]
$RCR_{food, DINP}$	0.002	0.053
$RCR_{food, DIDP}$	0.001	0.027
$RCR_{food, comb}$	**0.003**	**0.080**
$RCR_{indoor, DINP}$	0.008	0.030
$RCR_{indoor, DIDP}$	0.004	0.018
$RCR_{indoor, comb}$	**0.013**	**0.048**
$RCR_{ind/food, comb}$	**0.02**	**0.13**

[*1] Values are given for illustration only. Summation of reasonable worst case estimates for several routes and several substances is likely to lead in overestimates. According to Frederiksen et al. (2010), however, it seems that participants with a high exposure to one phthalate was also highly exposed to other phthalates.

Table 4.125 Combined risk characterisation for repeated dose toxicity by combining the reasonable worst case exposure of adults to sex toys, the typical dermal exposure, and the combined typical exposure to DINP and DIDP from the indoor environment and food

	Adults
$RCR_{sex\ toys}$	0.420
RCR_{dermal}	0.005
$RCR_{ind/food, comb}$	0.016
$RCR_{total, comb}$	**0.44**

4.8 Summary on hazard and risk

Hazard

Repeated dose toxicity - DINP
A NOAEL of 15 mg/kg bw/day with a LOAEL of 152 mg/kg bw/day (Exxon 1986) and a NOAEL of 88 mg/kg/day with a LOAEL of 359 mg/kg bw/day (Aristech 1994) were identified in the two key repeated dose toxicity studies based on statistically significant increases of incidence of spongiosis hepatis together with other signs of hepatotoxicity.

As a result of the methodological difference (amount of examined liver sections), the Exxon (1986) study was considered the most appropriate to use. Thus a NOAEL of 15 mg/kg bw/day was selected for repeated dose toxicity of DINP. This conclusion was supported by RAC (ECHA 2013a). RAC however noted that the NAEL could be higher given the large dose spacing in the Exxon study.

Repeated dose toxicity - DIDP
Subchronic studies in respectively the dog (Hazleton 1968b) and rat (BASF 1969) were available. From the rat study, a NOAEL of 60 mg/kg bw/day can be assumed based on dose-related increase of relative liver weights in females. A NOAEL of 15 mg/kg bw/day can be derived for the study in dog on the basis of hepatic effects. However, the large limitations of the study need to be emphasised.

In a new 2-year rodent carcinogenicity study by Cho et al. (2008, 2010) a LOAEL of 22 mg/kg bw/day based on spongiosis hepatis in a 2-year study in rat could be derived. However, there are some questions related to the reliability of these findings.

In line with the opinion of RAC (ECHA 2013a,b), a weight of evidence approach was used for DNEL calculation on the basis of a LOAEL of 22 mg/kg bw/day (Cho et al. 2008, 2010), a NOAEL of 15 mg/kg bw/day (Hazleton 1968b) and a NOAEL 60 mg/kg bw/day (BASF 1969b).

Reproductive toxicity- DINP
Decreases foetal testicular testosterone concentration during critical time window of masculinisation and increased incidence of multinucleated gonocytes and Leydig cell aggregates were observed with a NOAEL of 50 mg/kg bw/day. In a two-generation reproductive toxicity study the offspring bodyweight was decreased with a LOAEL of 159 mg/kg bw/day (no NOAEL) and increased skeletal variations were observed in a prenatal developmental toxicity study with a NOAEL of 100 mg/kg bw/day. The in vivo findings indicate that DINP has anti-androgenic potency but may also exhibit its effects through other modes of action.

Effects on fertility occur at higher dose levels, with a NOAEL for decreased live birth and survival indices of 622 mg/kg bw/day and a NOAEL of 276 mg/kg bw/day for decreased testicular weights.

Reproductive toxicity - DIDP
The most critical reproductive effect for DIDP is the decreased survival of F2 pups observed in both two-generation reproductive toxicity studies with rats, leading to a NOAEL of 33 mg/kg bw/day. A NOAEL of 40 mg/kg bw/day can be derived for foetal variations from prenatal developmental toxicity studies.

DIDP did not induce substantial anti-androgenic activity in available studies; in particular it did not reduce foetal testicular T levels or affect gene expression levels related to

masculinisation during critical time window during development. DIDP seems to have a partly different spectrum and/or potency of toxicological properties than several other phthalates, such as DINP, DEHP and DBP.

Other effects on fertility occurred at higher doses with a NOAEL of 427 mg/kg bw/day (0.8% dietary level) based on a two-generation reproductive toxicity study.

Sensitisation - DINP and DIDP
In general, phthalates (including DINP and DIDP) lack intrinsic sensitising potential. However, both DINP and DIDP share at least some of the adjuvant properties demonstrated for phthalates and an effect on atopic responses in humans cannot be excluded. An association has been shown between exposure to phthalates and asthma and allergic disease in epidemiological studies. However, a causal relationship remains to be established.

Carcinogenicity – DINP
The renal tumors seen in rats are assumed to stem from an alpha-2u-globulin mode of action which is not considered to be relevant for humans.

Liver neoplasia were seen in rats and mice with a NOAEL of 112 mg/kg bw/day. It is believed that peroxisome proliferation is the underlying mode of action for development of liver tumors with DINP, and that PPARα is involved in hepatic tumour formation. However, the more recent literature indicates that the mechanisms of liver carcinogenicity in rodents with peroxisome proliferators have not entirely been elucidated and that multiple pathways seem to exist. Some of those pathways seem to be PPARα-independent, which might indicate a need for some caution when interpreting the relevance of rodent carcinogenicity with DINP to humans.

The increased incidences in MNCL seen in rats with a NOAEL of 15 mg/kg bw/day might have a human counterpart. The available information does not allow to draw definite conclusions on the relevance of the findings. As MNCL is likely to follow a threshold mode of action with a NOAEL equal to that for repeated dose toxicity, the finding would not be a driver for the risk assessment. Therefore, the endpoint is not taken further to the risk characterisation step.

Carcinogenicity – DIDP
Although no treatment-related tumours were observed in a 2-year carcinogenicity study with rats, DIDP has been shown to induce liver adenomas in a 26-week study in rasH2 mice (NOAEL of 0.33% in feed, estimated to correspond to approximately 500 mg/kg bw/day). It is assumed that the increased incidence of liver adenomas in mice is related to peroxisome proliferation, and that PPARα is involved in hepatic tumour formation. However, the more recent literature indicates that the mechanisms of liver carcinogenicity in rodents with peroxisome proliferators have not entirely been elucidated and that multiple pathways seem to exist. Some of those pathways seem to be PPARα-independent, which might indicate a need for some caution when interpreting the relevance of rodent carcinogenicity with DINP to humans.

The increased incidences in MNCL seen in a 2-year carcinogenicity study with rats (NOAEL of 110 mg/kg bw/day) might have a human counterpart. The available information does not allow to draw definite conclusions on the relevance of the findings. As MNCL is likely to follow a threshold mode of action with a NOAEL well above that for repeated dose toxicity, the finding would not be a driver for the risk assessment. Therefore, the endpoint is not taken further to the risk characterisation step.

Considerations on combined[69] risk assessment of DINP and DIDP (and other phthalates)
Different phthalates seem to exhibit various effects on certain endocrine parameters. Phthalates having the same mode of action or the same adverse outcome are candidates for combined risk assessment.

DINP has anti-androgenic properties and it could be appropriate to include this substance in a combined risk assessments of phthalates with anti-androgenic properties. DIDP, on the other hand, does not have similar properties/potency and it would not be justified to group DIDP with phthalates with anti-androgenic properties for combined risk assessment.

There might be combined liver effects from DINP and DIDP, and potentially other phthalates (DEHP shows spongiosis hepatis albeit the NOAEL of 147 mg/kg bw/day indicates lower potency).

Risk

Children
Reasonable worst case RCRs ranging from 1.3 to 2.0 indicate a risk of liver toxicity for children of 0-18 months old from mouthing toys and childcare articles containing DINP or DIDP. Thus, it is concluded that a risk from the mouthing of toys and childcare articles with DINP and DIDP cannot be excluded if the existing restriction were lifted (i.e. in the scenario where DINP or DIDP would be present in toys and childcare articles). This conclusion was supported by RAC (ECHA 2013a,b).

It is not anticipated that mouthing of erasers containing DINP or DIDP would lead to a considerable risk for children. Furthermore, no risk is expected from combined exposure to DINP and DIDP for children exposed via food and the indoor environment.

Biomonitoring data for children is scarce and only available for children older than 3 years. The 95th percentiles of the studies suggest that RCRs are below 0.2. A maximum value of 39 µg/kg bw/day DINP was observed in a biomonitoring study with 137 children of 3-5 years old. The RCR corresponding to this maximum would be 0.5 for liver toxicity.

The combined exposure to DINP and DIDP via food and the indoor environment could constitute a body burden with RCRs up to of 0.2 in the typical case and could amount to 0.5 in a realistic combination of reasonable worst case and typical exposure situations for the two phthalates[70]. It can be concluded that no risk is expected from combined exposure to DINP and DIDP for children exposed via food and the indoor environment (RCRs <1).

Adults
With RCRs of 0.4 in the reasonable worst case use of sex toys, it seems not likely that the use of sex toys with DINP or DIDP would result in a risk. This conclusion is subject to substantial uncertainties with regard to exposure duration and migration rates of the phthalates from sex toys.

Dermal exposure from for instance PVC garments is not anticipated to result in a risk for the adult population.

Exposure from food and the indoor environment are not very significant in the adult population, which is confirmed by the available biomonitoring data.

[69] "Combined exposure" includes all routes, pathways, and sources of exposure to multiple chemicals (SCHER/SCENIHR/SCCS 2011)
[70] The reasonable worst case exposure from the indoor environment for DINP summed with the typical case for DIDP and the typical case for both phthalates via food, results in an RCR of 0.47 in 6-12 months old children.

5. Consultation

Data on manufacturing and import, migration rates from articles, biomonitoring and food was collected by Amec Environment & Infrastructure UK Limited on behalf of ECHA under Framework contract No ECHA/2008/02 between ECHA and AMEC Environment & Infrastructure UK Limited (AMEC). The work has been led by COWI, supported by IOM, BRE and AMEC. As part of the data collection, the following stakeholders have been contacted and sources of data reviewed:

- 35 institutions which are partners of two projects under the 7th Framework Programme, DEMOCOPHES and COPHES, have been contacted. The projects involve organisations from almost all EU Member States. The overall tasks of the projects is to harmonise and coordinate national and local activities on human biomonitoring to contribute to better data comparability across the EU and to achieve comparable human biomonitoring data across Europe.
- Presenters at the conference "Human Exposure to Phthalates: Relevant Sources, Exposure Paths, and Toxicokinetics – Examples DEHP and DINP" 22 February 2010.
- Main authors of key scientific papers in the field.
- Selected national food authorities (e.g. as referred to by COPHES partners).
- The European Food Safety Authority, EFSA.
- The European Council for Plasticisers and Intermediates, ECPI.
- Members of Chemicals Working Group under Health & Environment Alliance (NGO).
- US Consumer Product Safety Commission and US EPA.
- The Kinsey Institute in the US
- Family Planning Association in the UK
- a major retailer of sex toys in the UK (Anne Summers)

In order to gather information on the frequency and duration of sex toys ECHA consulted the following stakeholders:

- Seksualiteit.nl / Rutgers Nisso Groep.
- Vlaamse vereniging voor Seksuologie vzw (Flemish federation of sexology NGO, member of the Dutch Flemish Federation of Sexology and of the European federation of sexology).
- Section Reproduction, Sexuality & Well-being of the Department of Reproduction, Development & Regeneration, K.U.Leuven.
- Sensoa, Flemish expertise centre for sexual health.

Several meetings were held with the industry trade organisation The European Council for Plasticisers and Intermediates (ECPI) and with ExxonMobil in the course of the review process.

A preliminary draft report was peer reviewed by Kirsi H Vähäkangas. KH Vähäkangas is a Professor of Toxicology at the University of Eastern Finland since 2000. She is Honorary Professor at the School of Biological Sciences of the University of Hong Kong and currently (during 2012) on research sabbatical at the Laboratory of Human Carcinogenesis (Chief Curtis C Harris), NCI, NIH, USA. She leads a research group on environmental carcinogenesis since 1986, is president of the Finnish Society of Toxicology and has been a referee in scientific journals in the fields of toxicology, pharmacology, carcinogenesis, and molecular epidemiology.

A draft of the current report was subject to a 12-week public consultation, from 7 May to 31 July 2012. Moreover, ECHA's Committee for Risk Assessment (RAC) was requested to provide a scientific opinion on the draft review report. Through the opinion making process, there was additional stakeholder consultation. Furthermore, independent of the public consultation and the opinion forming process in RAC, ECHA has received correspondence and documents from ECPI and ExxonMobil. See also section 2.3.

References

Abb M, Heinrich T, Sorkau E and Lorenz W (2009). Phthalates in house dust. *Environ Int* **35(6)**: 965-970.

ACC (American Chemistry Council) Panel (2005). *Comments of the phthalate esters panel of the American Chemistry Council on the revised technical review of diisononyl phthalate.* Docket No TRI- 2005-0004.

Adamsson A, Salonen V, Paranko J and Toppari J (2009). Effects of maternal exposure to di-isononylphthalate (DINP) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p*,*p*_-DDE) onsteroidogenesis in the fetal rat testis and adrenal gland. *Reprod Toxicol* **28**: 66–74.

Adham IM, Emmen JMA and Engel W (2000). The role of the testicular factor INSL3 in establishing the gonadal position. *Mol Cell Endocrinol* **160**: 11-16

Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, Nelson H, Bhat HK, Perera FP, Silva MJ and Hauser R (2008). Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. *Environ Health Perspect* **116(4)**: 467-473.

Akahori Y, Nakai M, Yakabe Y, Takatsuki M, Mizutani M. Matsuo M and Shimohigashi Y (2005). Two-step models to predict binding affinity of chemicals to the human estrogen receptor α by three-dimensional quantitative structure-activity relationships (3D-QSARs) using receptor-ligand docking simulation. *SAR & QSAR in Environmental Research* **16(4)**: 323–337.

Akahori Y, Nakai M, Yamasaki K, Takatsuki M, Shimohigashi Y and Ohtaki M (2008). Relationship between the results of in vitro receptor binding assay to human estrogen receptor alpha and in vivo uterotrophic assay: comparative study with 65 selected chemicals. *Toxicol In vitro* **22(1)**: 225-31.

Anderson WA, Castle L, Hird S, Jeffery J and Scotter MJ (2011). A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate. *Food Chem Toxicol* **49(9)**: 2022-2029.

Andrade AJM, Grande SW, Talsness CE, Grote k, Golombiewski A, Strener – Kock A and Chahoud I (2006). A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. *Toxicology* **225(1)**: 64-74.

Apostolakis EM, Ramamurphy M, Zhou D, Onate Sand O'Malley B (2002). Acute disruption of select steroid receptor coactivators prevents reproductive behavior in rats and unmasks genetic adaptation in knockout mice. *Mol Endocrinol* **16**: 1511–1523.

Aristech Chemical Corporation (1995c). TSCA 8(e) Aristech Submission 8EHQ-0794-13083. Corroborative information in second species (as cited in EC 2003a).

Aristech Chemical Corporation (1994). 2-Year Dietary Oral Toxicity Study in Rats with Diisononyl Phthalate. TSCA 8(e) Submission 8EHQ-0794-13083. CAS Number 68515-48-0. (as cited in EC 2003a).

Aso S, Ehara H, Miyata K, Hosyuyama S, Shiraishi K, Umano T and Minobe Y (2005). A two-generation reproductive toxicity study of butyl benzyl phthalate in rats. *J Toxicol Sci* **30**: 39-58.

Auger AP, Tetel MJ and McCarthy MM (2000). Steroid receptor coactivator-1 (SRC-1) mediates the development of sex-specific brain morphology and behavior. *Proc Natl Acad Sci* **97(13)**: 7551–7555.

Axford IP, Earls AO, Scott RP and Braybrook JH (1999). *Interlaboratory validation of laboratory-based agitation methods for the determination of phthalate plasticizer migration from PVC toys and childcare articles laboratory of the government chemist.* Teddington, Middlesex, UK. (as cited in US CPCS 2010).

Babich MA, Chen SB, Greene MA, Kiss CT, Porter WK, Smith TP, Wind ML and Zamula WW (2004). Risk assessment of oral exposure to diisononyl phthalate from children's products. *Regul Toxicol Pharmacol* **40(2)**: 151-167.

Babich MA and Osterhout A (2010). *Toxicity review of diisononyl phthalates (DINP).* U.S. Consumer Product Safety Commission. Available at: http://www.cpsc.gov/about/cpsia/toxicityDINP.pdf

Babich MA (2013). Personal communication regarding mouthing times in the paper by Babich et al. (2004), 8 January 2013.

Bannasch P (2003). Comments on R Karbe and RL Kerlin (2002). Cystic degeneration/spongiosis hepatis (Toxicol Pathol 30: 216-227). *Toxicol Pathol* **31**: 566-570.

Bankston JR (1992). Supplement: 13-Week Subchronic Dietary Oral Toxicity Study with Di(Isononyl) Phthalate in Mice with Cover Letter Dated 070692 and Attachments. Aristech Chemical Corporation, Pittsburgh, PA 15230. EPA document no. 89-920000303 (as cited in US CPSC 2010a).

Barlow NJ, McIntyre BS and Foster PMD (2004). Male reproductive tract lesions at 6, 12, and 18 months of age following in utero exposure to di(n-butyl) phthalate. *Toxicol Pathol **32**: 79–90.*

Barlow NJ and Foster PMD (2003). Pathogenesis of male reproductive tract lesions from gestation through adulthood following in utero exposure to di(n-butyl) phthalate. *Toxicol Pathol* **31(4)**: 397–410.

Barlow NJ, Phillips SL, Wallace DG, Sar M, Gaido KW and Foster PM (2003). Quantitative changes in gene expression in fetal testes following exposure to Di(n-butyl)phthalate. *Toxicol Sci* **73**: 431-441 (as cited in Foster 2006).

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL and Pirkle JL (2005). Urinary Creatinine Concentrations in the U.S. Population: Implication for Urinary Biologic Monitoring Measurements. *Environ Health Perspect* **113(2)**: 192-200.

BASF AG (1969b). Bericht über den 90-Tage-Ratten-Fütterungsversuch mit PALATINOL Z (as cited in EC 2003b).

BASF AG (1995a). *Study of the Prenatal* Toxicity *of Palatinol DN (test substance N° 92/64) in Rats after Oral Administration (gavage) performed by BASF Aktiengesellschaft Department of Toxicology, FRG.* Project No 10R0126/91088, Report dated 06 September 1995, Study carried out in 1992 (as cited in EC 2003a).

BASF AG (1995b). *Study of the Prenatal Toxicity of Palatinol N (test substance N° 91/126) in Rats after Oral Administration (gavage) performed by BASF Aktiengesellschaft Department of Toxicology, FRG.* Project No 10R0126/91088, Report dated 04 May 1995, Study carried out in 1992 (as cited in EC 2003a).

Bavarian State Ministry of the Environment and Public Health (2012). Submission of information via the public consultation on ECHA's draft review report by the Bavarian State Ministry of the Environment and Public Health (Bayerisches Staatsministerium für Umwelt und Gesundheit). Comment reference number 19. Public comments are available on http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

Beamer P, Key ME, Ferguson AC, Canales RA, Auyeung W and Leckie JO (2008). Quantified activity pattern data from 6 to 27-month-old farmworker children for use in exposure assessment. *Environ Res* **108(2)**: 239-246.

Becker K, Göen T, Seiwert M, Conrad A, Pick-Fuss H, Müller J, Wittassek M, Schulz C and Kolossa-Gehring M (2009). GerES IV: phthalate metabolites and bisphenol A in urine of German children*. Int J Hyg Environ Health* **212(6)**: 685-692.

Becker K, Seiwert M, Angerer J, Heger W, Koch HM, Nagorka R, Rosskamp E, Schlüter C, Seifert B and Ullrich D (2004). DEHP metabolites in urine of children and DEHP in house dust. *Int J Hyg Environ Health* **207(5)**: 409-417.

Berman T, Hochner-Celnikier D, Calafat AM, Needham LL, Amitai Y, Wormser U and Richter E (2009). Phthalate exposure among pregnant women in Jerusalem, Israel: results of a pilot study. *Environ Int* **35(2)**: 353-357.

Bennasroune A, Rojas L, Foucaud L, Goulaouic S, Laval-Gilly P, Fickova M, Couleau N, Durandet C, Henry S, Falla J (2012). Effects of 4-nonylphenol and/or diisononylphthalate on THP-1 cells: Impact of endocrine dusruptors on human immune system parameters. *Int J Immunopathol Pharmacol* **25(2)**: 365-376.

Benson R (2009). Hazard to the developing male reproductive system from cumulative exposure to phthalate esters--dibutyl phthalate, diisobutyl phthalate, butylbenzyl phthalate, diethylhexyl phthalate, dipentyl phthalate, and diisononyl phthalate. *Regul Tocicol Pharmacol* **53(2)**: 90-101.

Berry C (2012). *Expert Review: Evaluation of the significance of changes found in studies on DINP (Di-isononyl phthalate) and DIDP (Di-isodecyl phthalate) in the rat, 22 May 2012*. Sponsor: European Council for Plasticisers and Intermediates. Submission of information via the public consultation on ECHA's draft review report by ECPI. Comment reference number 16, Attachment II. Public comments are available on http://echa.europa.eu/web/guest/addressing chemicals of concern/restriction/consultations-draft-review-report

BfG (2009). Österreichischer Ernährungsbericht 2008. 1. Auflage, März 2009. Institut für Ernährungswissenschaften and Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH for the Bundesministeriums für Gesundheit. [In German]

BIBRA (1986). *Rat Liver and Lipid Effects of Representative Phthalate Esters with EPA Acknowledgement letter*. British Industrial Biological Research Association (BIBRA), Unpublished Laboratory Report (BIBRA Project No 3.0542) submitted to the Chemical Manufacturers Association (CMA) (as cited in EC 2003a).

BIBRA (1985). *A 21-Day Feeding Study of Diisononyl phthalate to Rats: Effects on the Liver and Liver Lipids.* British Industrial Biological Research Association (BIBRA), Unpublished Laboratory Report, Report No 0495/6/85 submitted to the Chemical Manufacturers Association (CMA) (as cited in EC 2003a).

Bisig MD (2009). *Plasticizer Market Update.* SPI Vinyl Products Division 20th Annual Vinyl Compounding Conference, July 19-21, 2009. Presented by Michael D. Bisig, BASF

Corporation. Accessed October 2011 at:
http://www.plasticsindustry.org/files/about/VPD/A_Bisig_BASF.pdf

Bio/dynamics (1982a). *One Week Prechronic Oral Feeding Study*. Test Materials: MRD 8240, MRD 8241. Project number VO 4053, performed by Bio/dynamics, Inc., Unpublished Laboratory Report submitted to Exxon Biomedical Sciences, Inc., November 19, 1982 (as cited in EC 2003a).

Bio/dynamics (1982b). *Thirteen Week Pre-chronic Oral Feeding Study in Fischer 344 Rats*. Test Material: MRD-82-41. Project VO 4154-F, performed by Bio/Dynamics, Inc., Report submitted to Exxon Biomedical Sciences, Inc., December 8, 1982 (as cited in EC 2003a).

Bio/dynamics (1982c). *Thirteen Week Pre-chronic Oral Feeding Study in Sprague-Dawley Rats*. Test Material: MRD-82-41. Project No VO 4154-S, performed by Bio/Dynamics, Inc., Report submitted to Exxon Biomedical, Inc., December 8, 1982 (as cited in EC 2003a).

Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebæk NE, Hegedüs L, Hilsted L, Juul A and Main KM (2010). Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. *Environ Health Perspect* **118(10)**: 1458-1464.

Boberg J, Christiansen S, Axelstad M, Kledal TS, Vinggaard AM, Dalgaard M, Nellemann C and Hass U (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. *Reprod Toxicol* **31(2)**: 200-209.

Borch J, Vinggaard AM and Ladefoged O (2003). The effect of combined exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate on testosterone levels in foetal rat testis. *Reprod Toxicol* **17**: 487-488 (as cited in US CPSC 2010a).

Borch J, Ladefoged O, Hass U and Vinggaard AM (2004). Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. *Reprod Toxicol* **18**: 53–61.

Borch J, Axelstad M, Vinggaard AM and Dalgaard M (2006a). Diisobutyl phthalate has comparable anti-androgenic effects to din- butyl phthalate in fetal rat testis. *Toxicol Lett* **163**: 183–190.

Borch J, Metzdorff SB, Vinggaard AM, Brokken L and Dalgaard M (2006b). Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis. *Tocicology* **223(1-2)**: 144-155.

Bornehag CG, Sundell J, Weschler CJ, Sigsgaard T, Lundgren B, Hasselgren M and Hägerhed-Engman L (2004). The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. *Environ Health Perspect* **112(14)**: 1393-1397.

Bosnir J, Puntaric D, Skes I, Klaric M, Simic S and Zoric I (2003). Migration of phatalates from plastic products to model solutions. *Coll Antrpol* **27(1)**: 23-30.

Bošnir J, Puntarić D, Galić A, Škes I, Dijanić T, Klarić M, Grgić M, Čurković M and Šmit Z (2007). Migration of phthalates from plastic containers into soft drinks and mineral water. *Food Technol Biotechnol* **45(1)**: 91–95.

Bremmer HJ and Van Veen MP (2002). Children's Toys Fact Sheet. To assess the risk for the consumer. RIVM report 612810012/2002. Available at:
http://www.rivm.nl/bibliotheek/rapporten/612810012.pdf

Breous E, Wenzel A and Loos U (2005). The promoter of the human sodium/iodide symporter responds to certain phthalate plasticisers. *Mol Cell Endocrinol* **244**: 75–78.

Brinkmann AO (2001). Molecular basis of androgen insensitivity. *Mol Cell Endocrinol* **179**: 105-109.

Butala JH, Moore MR, Cifone MA, Bankston JR and Astill B (1996). Oncogenicity study of di(isononyl phthalate in rats*. The Toxicologist* **30(A1031)**: 202 (as cited in EC 2003a).

Butala JH, David RM, Gans G, McKee RH, Guo TL, Peachee VL and White KL Jr (2004). Phthalate treatment does not influence levels of IgE or TH2 cytokines in B6C3F1 mice. *Toxicology* **201**: 77–85.

Calafat AM, Silva MJ, Reidy JA, Earl Gray L, Samandar E, Preau JL, Herbert AR and Needham LL (2006). Mono-(3-carboxypropyl) phthalate, a metabolite of di-n-octyl phthalate. *J Toxicol Environ Health A* **69(3-4)**: 215-227.

Calafat AM, Wong LY, Silva MJ, Samandar E, Preau JL Jr, Jia LT and Needham LL (2011). Selecting adequate exposure biomarkers of diisononyl and diisodecyl phthalates: data from the 2005-2006 National Health and Nutrition Examination Survey*. Environ Health Perspect* **119(1)**: 50-55.

Caldwell JD (1999). Review of Mononuclear Cell Leukemia in F-344 Rat Bioassays and Its Significance to Human Cancer Risk: A Case Study Using Alkyl Phthalates. *Regul Toxicol Pharmacol* **30**: 45–53.

Calvin E (2011). *Plasticizer Market Update.* BASF Corporation. Presentation at the 22nd Annual Vinyl Compounding Conference, Burlington, July 10-13, 2011. Available at: http://www.cpsc.gov/about/cpsia/chap/spi.pdf

Cao X-L (2010). Phthalate esters in foods: Sources, occurrence, and analytical methods. *Comprehensive Reviews in Food Science and Food Safety* **9(1)**: 21–43.

Carruthers CM and Foster PMD (2005). Critical Window of Male Reproductive Tract Development in Rats Following Gestational Exposure to Di-n-butyl Phthalate (2005). *Birth Defects Res B Dev Reprod Toxicol* **74**: 277–285.

Casarett LJ, Doull J and Klaassen CD (2008). *Casarett and Doull's toxicology: the basic science of poisons.* McGraw-Hill Prof Med/Tech.

CDC (Centers for Disease Control and Prevention)(2005). *Third national report on human exposure to environmental chemicals.* Available at: http://www.jhsph.edu/ephtcenter/Third_Report.pdf

CDC (2011). *Fourth National Report on Human Exposure to Environmental Chemicals 2009 and the Updated Tables, February 2011.* Centers for Disease Control and Prevention, Atlanta. Available at: http://www.cdc.gov/ExposureReport/index.html

CEFIC (2012). *CEFIC ECPI (European Council of Plasticisers and Intermediates) comments on the draft RAC 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)".* European Chemical Industry Council (Cefic), November 6, 2012.

CHAP (2001). US Chronic Hazards Advisory Panel. *Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on Diisononyl Phthalate (DINP).* U.S. Consumer Product Safety Commission, Bethesda, MD. Available at: www.cpsc.gov/library/foia/foia01/os/dinp.pdf

Chen SB (1998a). *Laboratory Sciences Report on the migration of diisononyl phthalate from polyvinyl chloride children's products.* U.S. Consumer Product Safety Commission, Washington, DC. November, 1998.

Chen SB (1998b). *Migration of diisononyl phthalate from a Danish polyvinyl chloride teether.* U.S. Consumer Product Safety Commission, Bethesda, MD. July 24, 1998 (as cited in US CPCS 2010a).

Chen SB (2002). *Screening of toys for PVC and phthalates migration.* U.S. Consumer Product Safety Commission, Bethesda, MD. June 20, 2002 (as cited in US CPCS 2010c).

Cho SC, Han BS, Ahn B, Nam KT, Choi M, Oh SY, Kim SH, Jeong J, Jang DD (2008). Peroxisome proliferator di-isodecyl phthalate has no carcinogenic potential in Fischer 344 rats. *Toxicol Lett* **178 (2)**: 110-116.

Cho SC, Han BS, Ahn B, Nam KT, Choi M, Oh SY, Kim SH, Jeong J, Jang DD (2010). Corrigendum to "Peroxisome proliferator di-isodecyl phthalate has no carcinogenic potential in Fischer 344 rats" [Toxicol. Lett. 178 (2008) 110–116]. *Toxicol Lett* **197**: 156.

Cho WS, Jeong J, Choi M, Park SN, Han BS and Son WC (2011). 26-Week carcinogenicity study of di-isodecyl phthalate by dietary administration to CB6F1-rasH2 transgenic mice. *Arch Toxicol* **85(1)**: 59-66.

Clausen PA, Lindeberg Bille RL, Nilsson T, Hansen V, Svensmark B and Bøwadt S (2003). Simultaneous extraction of di(2-ethylhexyl) phthalate and nonionic surfactants from house dust. oncentrations in floor dust from 15 Danish schools. *J Chromatogr A* **986(2)**: 179-90.

Clewell R, Andersen M and Sochaski M (2011a). *Pharmacokinetics and fetal testes effects after diisononyl phthalate administration in rat gestation.* The Hamner Protocol #09016 Final Report, DiNP Phase I Study. The Hamner Institutes for Health Sciences, Research Triangle Park, NC 27709-2137. Sponsored by ExxonMobil Biomedical Sciences Inc. [Published as: Clewell RA, Sochanski M, Edwards K, Creasy DM, Willson G, Andersen ME (2012). Disposition of diisononyl phthalate and its effects on sexual development of the male fetus following repeated dosing in pregnant rats. *Reproductive Toxicology* **35**: 56-69.]

Clewell R, Andersen M and Sochaski M (2011b). *A dose response study of the effects on male rat sexual development after adminstration of diisononyl phthalate to the pregnant and lactating dam.* The Hamner Protocol #10003 Final Report, DiNP Phase II Study. The Hamner Institutes for Health Sciences, Research Triangle Park, NC 27709-2137. Sponsored by ExxonMobil Biomedical Sciences Inc. [Published as: Clewell RA, Thomas A, Willson G, Creasy DM, Andersen ME (2012). A dose response study to assess effects after dietary administration of diidononyl phthalate (DINP) in gestation and lactation on male rat sexual development. *Reproductive toxicology* **35**: 70-80].

Colacino JA, Soliman AS, Calafat AM, Nahar MS, Van Zomeren-Dohm A, Hablas A, Seifeldin IA, Rozek LS and Dolinoy DC (2011). Exposure to phthalates among premenstrual girls from rural and urban Gharbiah, Egypt: a pilot exposure assessment study. *Environ Health* **16**: 10-40.

Committee on Toxicity Of Chemicals In Food, Consumer Products And The Environment (2011). *COT Statement On Dietary Exposure To Phthalates – Data from The Total Diet Study (TDS).* Department of Health for England. COT Statement 2011/04.

Covance (1998). Oncogenicity Study in Rats with DINP Including Ancillary Hepatocellular Proliferation and Biochemical Analyses. Unpublished Report; Study number 2598-104, Final Report, May, 1998, 1-82 (as cited in EC 2003a).

COWI (2012). *Evaluation of new scientific evidence concerning the restrictions on DINP and DIDP contained in entry 52 of Annex XVII to Regulation (EC) No 1907/2006 (REACH). Volumes of DINP and DIDP.* Final Report. Contract number ECHA/2011/96 (SR32) implementing framework contract no ECHA/2008/2. Prepared by COWI, IOM Consulting and AMEC, 30 March 2012.

CSTEE (1998a). *Opinion on Phthalate migration from soft PVC toys and child-care articles.* Opinion expressed at the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) third plenary meeting in Brussels, 24 April 1998.

CSTEE (1998b). *Opinion on Phthalate migration from soft PVC toys and child-care articles.* Opinion expressed at the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) 4th plenary meeting in Brussels, 16 June 1998.

CSTEE (1998c). *Opinion on Phthalate migration from soft PVC toys and child-care articles - Data made available since the 16th of June 1998*. Opinion expressed at the 6th CSTEE plenary meeting, Brussels, 26/27 November 1998.

CSTEE (2001a). Scientific Committee on Toxicity, Ecotoxicity and the Environment. *Opinion on the results of the Risk Assessment of: 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate.* Report version Human Health Effects: Final report, May 2001. Opinion expressed at the 27th CSTEE plenary meeting Brussels, 30 October 2001

CSTEE (2001b). Scientific Committee on Toxicity, Ecotoxicity and the Environment. *Opinion on the results of the Risk Assessment of: 1,2 – Benzenedicarboxylic acid di-C9-11-branched alkyl esters, C10-rich and di-"isodecyl" phthalate.* Report version Human health effects: Final report, May 2001. Opinion expressed at the 24th CSTEE plenary meeting, Brussels, 12 June 2001.

Cullen JM (2012). Expert Review of Revised Technical Review of Diisononyl Phthalate, 30 July 2012. Sponsor: Exxon Mobil Company. Submission of information via the public consultation on ECHA's draft review report. Comment reference number 18. Public comments are available on http://echa.europa.eu/web/guest/addressing chemicals of concern/restriction/consultations-draft-review-report

David RM, Moore MR, Finney DC and Guest D (2000). Chronic toxicity of di(2-ethylhexyl) phthalate in rats. *Toxicol Sci* **55**: 433-443.

David RM (2000). Exposure to phthalate esters. *Environ Health Perspect* **108**: A440. Correspondence.

Deisinger PJ, Perry LG and Guest D (1998). In Vivo Percutaneous Absorption of [^{14}C]DEHP from [^{14}C]DEHP-Plasticised Polyvinyl Chloride Film in Male Fisher 344 Rats. *Food Chem Toxicol* **36(6)**: 521-527.

DeKeyser JG, Laurenzana EM, Peterson EC, Chen T and Omiecinski CJ (2011). Selective Phthalate Activation of Naturally Occurring Human Constitutive Androstane Receptor Splice Variants and the Pregnane X Receptor. *Toxic Scien* **120(2)**: 381-391.

Ding L, Kuhne WW, Hinton DE, Song J and Dynan WS (2010). Quantifiable biomarkers of normal aging in the Japanese medaka fish (Oryziaslatipes). *PLoS One* **5(10)**: e13287.

Dostal LA, Jenkins WL and Schwetz BA (1987). Hepatic peroxisome proliferation and hypolipidemic effects of di(2-ethylhexyl)phthalate in neonatal and adult rats. *Toxicol Appl Pharmacol* **87(1)**: 81 -90.

DVFA (2010). *Phthalater i fødevarekontaktmaterialer, Projekt J. nr.: 2010-20-64-00230 2010.* Danish Veterinary and Food Administration, Copenhagen. [in Danish]

Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen Z, Herrick RF, Christiani DC and Hauser R (2003). Phthalate exposure and human semen parameters. *Epidemiology* **14(3)**: 269-77.

EA (2009). Updated technical background to the CLEA model. Using science to create a better place. Science report SC050021/SR3. Authors: Jeffries J and Martin I. Environment Agency, Bristol, January 2009. http://www.environment-agency.gov.uk/static/documents/Research/CLEA_Report_-_final.pdf

Earls AO, Clay CA, Axford IP, Scott RP and Braybrook JH (1998). *Laboratory-based agitation methods for the determination of phthalate plasticiser migration from PVC toys and childcare articles.* Laboratory of the Government Chemist. Consumer Safety Research Report, LGC Technical Report Number LGC/1998/DTI/009.

EC (2003a). *Risk Assessment Report for DINP*. Final report, European Commission, EUR 20784EN, European Union Risk Assessment Report, Volume 35, Luxembourg: Office for Official Publications of the European Communities.

EC (2003b). *Risk Assessment Report for DIDP*. Final report, European Commission, EUR 20785EN, European Union Risk Assessment Report, Volume 36, Luxembourg: Office for Official Publications of the European Communities.

EC (2006). *Guidance Document on the interpretation of the concept "which can be placed in the mouth" as laid down in the Annex to the 22nd amendment of Council Directive 76/769/EEC*. Available at: http://ec.europa.eu/enterprise/sectors/chemicals/documents/reach/archives/market-restrictions/amendments_en.htm.

EC (2008). *Risk Assessment Report for DEHP*. Final report, European Commission, EUR 23384EN, European Union Risk Assessment Report, Volume 80, Luxembourg: Office for Official Publications of the European Communities.

EC (2011). *Questions and agreed answers concerning the implementation of Annex XVII to REACH on the restrictions on the manufacturing, placing on the market, and use of certain dangerous substances, mixtures and articles.* Version 4 –25 May 2011. Available at: http://ec.europa.eu/enterprise/sectors/chemicals/files/reach/restr-faq-may-2011_en.pdf.

ECB (2000). *Summary record. Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances*. ECBI/51/00 - Rev. 2, 23 November 2000. Arona, 9 May 2000 and ECB Ispra 10 - 12 May 2000.

ECHA (2008). *Code of conduct for observers at ECHA meetings. Decision by the Executive Director.* ED/62/2008, Helsinki, 9 October 2008. Available at: http://echa.europa.eu/documents/10162/13559/conduct_code_stakeholder_observers_en.pdf

ECHA (2009a). *Background document for bis(2-ethylhexyl) phthalate (DEHP)*. Document developed in the Context of ECHA's first Recommendation for the inclusion of substances in Annex XIV. Available at: http://echa.europa.eu/doc/authorisation/annex_xiv_rec/subs_spec_background_docs/dehp.pdf.

ECHA (2009b). *Data on manufacture, import, export, uses and releases of bis(2-ethylhexyl)phthalate (DEHP) as well as information on potential alternative to its use*. Developed under framework contract ECHA/2008/2 by COWI A/S, ECHA, 2009. Available at: http://echa.europa.eu/doc/consultations/recommendations/tech_reports/tech_rep_dehp.pdf

ECHA (2010a). *Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No 1907/2006 (REACH). Review of new available information for di-'isononyl' phthalate (DINP). CAS No 28553-12-0 and 68515-48-0, EINECS No 249-079-5 and 271-090-9.* Review report, July 2010.

ECHA (2010b). *Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No 1907/2006 (REACH). Review of new available information for di-'isodecyl' phthalate (DIDP). CAS NO 26761-40-0 and 68515-49-1, EINECS NO 247-977-1 and 271-091-4.* Review report, July 2010.

ECHA (2010c). *Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No 1907/2006 (REACH). Review of new available information for di-n-octyl phthalate (DNOP). CAS NO 117-84-0, EINECS NO 204-214-7.* Review report, July 2010.

ECHA (2010d). *Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No 1907/2006 (REACH). Review of new available information for bis (2-ethylhexyl) phthalate (DEHP). CAS NO 117-81-7, EINECS NO 204-211-0.* Review report, July 2010.

ECHA (2010e). *Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No 1907/2006 (REACH). Review of new available information for dibutyl phthalate (DBP). CAS NO 84-74-2, EINECS NO 201-557-4*. Review report, July 2010.

ECHA (2010f). *Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (EC) No 1907/2006 (REACH). Review of new available information for benzyl butyl phthalate (BBP). CAS NO 85-68-7, EINECS NO 201-622-7.* Review report, July 2010.

ECHA (2011). Restrictions under consideration. Website of the European Chemicals Agency (ECHA). Accessed October 2011 at: http://echa.europa.eu/reach/restriction/restrictions_under_consideration_en.asp

ECHA (2012a). *Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to Regulation (EC) No 1907/2006 (REACH).* Draft review report, 7 May 2012. Available at: http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

ECHA (2012b). *Committee for Risk Assessment (RAC) Opinion on an Annex XV dossier proposing restrictions on four phthalates.* ECHA/RAC/RES-O-0000001412-86-07/F. Adopted 15 June 2012. Available at: http://echa.europa.eu/documents/10162/77cf7d29-ba63-4901-aded-59cf75536e06

ECHA (2012c). *Rules of procedure for the Committee for Risk Assessment.* MB/40/2012 final (1) final, Helsinki, 28 September 2012. Available at: http://echa.europa.eu/documents/10162/13579/rac_rops_en.pdf

ECHA (2013a). *Opinion of the Committee for Risk Assessment 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)".* ECHA/RAC/A77-O-0000001412-86-10/F, 8 March 2013. Available at:

http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

ECHA (2013b). *Supporting document to the Opinion of the Committee for Risk Assessment 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)".* ECHA/RAC/A77-O-0000001412-86-10/F, 8 March 2013. Available at: http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

ECPI (2009). *Review of Recent Scientific Data on Di-isononyl Phthalate (DINP) and Risk Characterisation for its use in Toys and Childcare articles*. Technical report 2009-0601-DINP, European Council for Plasticisers and Intermediates (ECPI), June 2009.

ECPI (2010). Information provided by the European Council for Plasticisers and Intermediates (ECPI) on 12 March 2010.

ECPI (2011a). Endocrine Data Evaluation Report For selected high molecular weight (HMW) phthalates (DINP, DIDP) and a low molecular weight (LMW) phthalate (DBP) Using the OECD Conceptual Framework. Volume I – Mammalian Data. Scientific Report 110301. European Council for Plasticisers and Intermediates, March 2011.

ECPI (2011b). *PVC – Plasticiser Interactions: binding of high molecular weight (HMW) phthalates within the PVC matrix*. ECPI Technical Report, July 26, 2011.

ECPI (2011c). *Plasticisers*. Plasticisers and Flexible PVC Information Centre. European Council for Plasticisers and Intermediates (ECPI). Accessed 19 September 2011 at: http://www.plasticisers.org/plasticisers

ECPI (2011d). DIDP Information Centre, DINP Information Centre and DPHP Information Centre. The European Council for Plasticisers and Intermediates (ECPI). Accessed 19 September 2011 at: http://www.didp-facts.com/ , http://www.dinp-facts.com/ and http://www.dphp-facts.com/

ECPI (2011e). *Plasticisers*. Website of the European Council for Plasticisers and Intermediates (ECPI). Accessed 19 September 2011 at: http://www.ecpi.org/default.aspx?page=5

ECPI (2011f). Pesonal communication with Maggie Saykali, the European Council for Plasticisers and Intermediates (ECPI), November 2011.

ECPI (2012a). European Council for Plasticisers and Intermediates (ECPI) comments on ECHA presentations of 12 December 2011. DINP and DIDP Exposure data – Final, 16 January 2012.

ECPI (2012b). *Review of Exposure Data from "EVALUATION OF NEW SCIENTIFIC EVIDENCE CONCERNING DINP AND DIDP IN RELATION TO ENTRY 52 OF ANNEX XVII TO REGULATION (EC) NO 1907/2006 (REACH)", 7 May 2012*. Submission of information via the public consultation on ECHA's draft review report by the European Council for Plasticisers and Intermediates (ECPI). Comment reference number 16, Attachment IX. Public comments are available on http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

ECPI (2012c). *ECPI comments on ECHA draft Restrictions Re-evaluation Report – elaborating on comments submitted during the public consultation.* European Council for Plasticisers and Intermediates (ECPI), 10 October 2012.

ECPI (2012d). *Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to Regulation (EC) No 1907/2006 (REACH – Draft Review Report – Version 3 – 7 May 2012.* ECPI comments dated 25 July 2012. Submission of information via the public consultation on ECHA's draft review report by the European Council for Plasticisers and Intermediates (ECPI). Comment reference number 16, Attachment 0. Public comments are available on http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

EFSA (2005a). *Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Di-isononylphthalate (DINP) for use in food contact materials.* doi:10.2903/j.efsa.2005.244. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/244.htm

EFSA (2005b). *Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Di-isodecylphthalate (DIDP) for use in food contact materials.* Available at: http://www.efsa.europa.eu/en/efsajournal/doc/245.pdf

EFSA (2005c). Minutes' Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on the withdrawal of the requirement for peroxisome proliferation studies for alkyl esters used in food contact materials (expressed on 28 June 2005 at its 12th Plenary meeting, corresponding to item 10 of the agenda).

EFSA (2008). *Opinion of the Scientific Panel on Plant Protection products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005.* Available at: http://www.efsa.europa.eu/en/efsajournal/doc/705.pdf

Elsisi A, Carter DE, Sipes IG (1989). Dermal absorption of phthalate diesters in rats. *Fund Appl Toxicol* **12**: 70-77. (as cited in EC 2003a).

Ema M, Miyawaki E and Kawashima K (2000). Effects of dibutyl phthalate on reproductive function in pregnant and pseudopregnant rats. *Reprod Toxicol* **14**: 13–19.

EPL (1999). *Experimental Pathology Laboratories. Histology peer review and pathology working group review of selected lesions of the liver and spleen in male and female F344 rats exposed to DINP.* EPL Project number 303-013, Pathology Report. Submitted to Chemical Manufacturers Association, Arlington, VA.

Ettlin RA, Kuroda J, Plasmann S and Prentice DE (2010). Successful Drug Development Despite Adverse Preclinical Findings Part 1: Processes to Address Issues and Most Important Findings. *J Toxicol Pathol* **4**: 189.

Exxon Biomedical Sciences (1986). *Chronic Toxicity/Oncogenicity Study in F-344 Rats.* Test Material: MRD-83-260. Project No 326075 performed at Exxon Biomedical Sciences, Inc. Unpublished Laboratory Report (as cited in EC 2003a).

Exxon Biomedical Sciences (1994). *Developmental Toxicity Study in Rats with Diisononyl Phthalate (DINP; MRD-92-455).* Project No 145534 performed for Exxon Chemical Company and Exxon Chemical International, Inc. Unpublished Laboratory Report from Exxon Biomedical Sciences, Inc. (as cited in EC 2003a).

Exxon Biomedical Sciences (1995b). *Developmental Toxicity Study in Rats with DIDP (MRD-94-775).* Project No 177534 performed for Exxon Chemical Europe (as cited in EC 2003b).

Exxon (1996a). *Reproduction Toxicity Study in Rats with Diisononyl Phthalate (DINP; MRD-92-455).* Project Number 145535 from Exxon Biomedical Sciences, Inc. submitted to Exxon

Chemical company and Exxon Chemical Europe, Unpublished Laboratory Report (as cited in EC 2003a).

Exxon (1996b). *Two Generation Reproduction Toxicity Study in Rats with Diisononyl Phthalate (DINP; MRD-92-455).* Project from Exxon Biomedical Sciences Inc submitted to Exxon Chemical Company and Exxon Chemical Europe, Unpublished Laboratory Report (as cited in EC 2003a).

Exxon Biomedical Sciences (1997c). *Draft: An Assessment of the Microbial Toxicity of Di-iso-nonyl and Di-iso decyl Phthalate Ester Plasticiser* (as cited in EC 2003b).

Exxon Biomedical Sciences (1997d). *Two Generations Reproduction Toxicity Study in Rats with Di-isodecyl Phthalate (DIDP; MRD-94-775).* Project No 177535 performed for Exxon Chemical Company and Exxon Chemical Europe (as cited in EC 2003b).

Exxon Biomedical Sciences (2000). *Two Generations Reproduction Toxicity Study in Rats with Di-isodecyl Phthalate (DIDP; MRD-94-775)*. Project No 177535A performed for Exxon Chemical Company and Exxon Chemical Europe (as cited in 2003b).

ExxonMobil (2009). REACH registration dossier for 1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich. ExxonMobil Chemical Holland B.V., 2009.

ExxonMobil (2011a). *Comments to the Consumer Product Safety Commission Chronic Hazard Advisory Panel on Phthalates by ExxonMobil Chemical Company*. Technical Contributors: Ammie N. Bachman and Kevin M. Kransler. ExxonMobil Chemical Company (ExxonMobil), 29 March 2011. Available at: www.cpsc.gov/about/cpsia/chap/exxonmobil.pdf

ExxonMobil (2011b). *Risk Assessments for four plasticisers used in toys and childcare articles using migration data from PVC disks made with known quantities of plasticisers*. ExxonMobil Technical Report, 15 July 2011.

ExxonMobil (2011c). *Attachment to DIDP REACH Registration Dossier. Statement relevant to the re-evaluation of DIDP in toys and childcare articles as required by Directive 2005/84/EC*. ExxonMobil Biomedical Sciences Inc., 9 October 2009 – updated 18 July 2011.

ExxonMobil (2011d). *Review of the effects of DINP on male reproductive tract development: reference to two new studies conducted at the Hamner Institutes for Health Sciences.* Technical Contributors: Ammie N. Bachman and Kevin M. Kransler. ExxonMobil Biomedical Sciences Inc., 14 November 2011.

Fankhauser-Noti A, Biedermann-Brem S and Grob K (2006). PVC plasticizers/additives migrating from the gaskets of metal closures into oily food: Swiss market survey June 2005. *Eur Food Res Technol* **223(4)**: 447-453.

Fennell TR, Krol WL, Sumner SCJ and Snyder RW (2004). Pharmacokinetics of dibutylphthalate in pregnant rats. *Toxicol Sci* **82**: 407-418.

Ferrara D, Hallmark N, Scott H, Brown R, McKinnell C, Mahood IK and Sharpe RM (2006). Acute and long-term effects of in utero exposure of rats to di(n-butyl) phthalate on testicular germ cell development and proliferation. *Endocrinology* **147(11)**: 5352-5362.

Fiala F, Steiner I and Kubesch K (1998). *Migration of di-(2-ethylhexyl)phthalate (DEHP) and diisononyl phthalate (DINP) from PVC articles.* Institute of Food Chemistry and Food Technology of Vienna University of Technology and Consumer Council of Austrian Standards Institute, Vienna. Own publication. Available at: http://www.verbraucherrat.at/download/phthalates2.pdf

Fiala F, Steiner I and Kubesch K (2000). Migration of di-(2-ethylhexyl)phthalate (DEHP) and diisononyl phthalate (DINP) from PVC articles. *Deutsche Lebensmittel-Rundschau* **96(2)**: 51-57.

Fisher JS, Macpherson S, Marchetti N and Sharpe RM (2003). Human`testicular dysgenesis syndrome': a possible model using in-utero exposure of the rat to dibutyl phthalate.

Foster PMD (2006). Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. *Int J Androl* **29**: 140–147.

Frederiksen H, Aksglæde L, Sørensen K, Skakkebæk NE, Juul A and Andersson AM (2011). Urinary excretion of phthalate metabolites in 129 healthy Danish children and adolescents: estimation of daily phthalate intake. *Environ Res* **111(5)**: 656-663.

Frederiksen H, Jørgensen N and Andersson AM (2010). Correlations between phthalate metabolites in urine, serum, and seminal plasma from young Danish men determined by isotope dilution liquid chromatography tandem mass spectrometry. *J Anal Toxicol* **34(7)**: 400-410.

Fromme H, Bolte G, Koch HM, Angerer J, Boehmer S, Drexler H, Mayer R, Liebl B. (2007a). Occurrence and daily variation of phthalate metabolites in the urine of an adult population. *Int J Hyg Environ Health* **210(1)**: 21-33.

Fromme H, Gruber L, Schlummer M, Wolz G, Böhmer S, Angerer J, Mayer R, Liebl B and Bolte G. (2007b). Intake of phthalates and di(2-ethylhexyl)adipate: results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. *Environ Int* **33(8)**: 1012-1020.

Fromme H, Gruber L, Seckin E, Raab U, Zimmermann S, Kiranoglu M, Schlummer M, Schwegler U, Smolic S and Völkel W (2011). Phthalates and their metabolites in breast milk--results from the Bavarian Monitoring of Breast Milk (BAMBI). *Environ Int* **37(4)**: 715-722.

Fromme H, Lahrz T, Piloty M, Gebhart H, Oddoy A and Rüden H (2004). Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin (Germany). *Indoor Air* **14(3)**: 188-195.

Gaido KW, Hensley JB, Liu D, Wallace DG, Borghoff S, Johnson KJ, Hall SJ and Boekelheide K (2007). Fetal mouse phthalate exposure shows that gonocyte multinucleation is not associated with decreased testicular testosterone. *Toxicol Sci* **97**: 491-503.

Geiss O, Tirendi S, Barrero-Moreno J and Kotzias D (2009). Investigation of volatile organic compounds and phthalates present in the cabin air of used private cars. *Environ Int* **35(8)**: 1188-1195.

Ghisari M and Bonefeld-Jorgensen EC (2009). Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. *Toxicol Lett* **189**: 67–77.

Glue C, Platzer MH, Larsen ST, Nielsen GD, Skov PS and Poulsen LK (2005). Phthalates potentiate the response of allergic effector cells. *Basic Clin Pharmacol Toxicol* **96**: 140-142.

Göen T, Dobler L, Koschorreck J, Müller J, Wiesmüller GA, Drexler H and Kolossa-Gehring M (2011). Trends of the internal phthalate exposure of young adults in Germany-Follow-up of a retrospective human biomonitoring study. *Int J Hyg Environ Health* **215(1)**: 36-45.

Gray LE Jr, Ostby J, Furr J, Price M, Rao Veeramachaneni DN and Parks L (2000). Perinatal Exposure to the Phthalates DEHP, BBP, and DINP, but No DEP, DMP, or DOTP, Alters Sexual Differentiation on the Male Rat. *Toxicol Sci* **58**: 350-365.

Gray LE Jr, Barlow NJ, Howdeshell KL, Ostby JS, Furr JR and Gray CL (2009). Transgenerational Effects of Di (2-Ethylhexyl) Phthalate in the Male CRL:CD(SD) Rat: Added Value of Assessing Multiple Offspring per Litter. *Toxicol Sci* **110(2)**: 411–425.

Gray LE and Foster P (2003). Significance of experimental studies for assessing adverse effects of endocrine-disrupting chemicals. *Pure Appl Chem* **75**: 2125-2141.

Gray TJB, Rowland IR, Foster PMD and Gangolli SD (1982). Species differences in the testicular toxicity of phthalate esters. *Toxicol Lett* **11**: 141-147.

Gray LE Jr, Laskey J, Ostby J (2006). Chronic di-n-butyl phthalate exposure in rats reduces fertility and alters ovarian function during pregnancy in female Long Evans hooded rats. *Toxicol Sci* **93**: 189–195.

Greene M (2002). *Mouthing times among young children from observational data*. US CPSC, Bethesda, MD 20814, June 17, 2002 (as cited in Babich et al. 2004).

Grosse Y, Baan R, Secretan-Lauby B, Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Islami F, Galichet L, Straif K (2011). Carcinogenicity of chemicals in industrial and consumer products, food contaminants and flavourings, and water chlorination byproducts. WHO International Agency for Research on Cancer Monograph Working Group, International Agency for Research on Cancer (IARC), Lyon, France. *Lancet Oncol* **12**: 328-329.

Grossgut (2011). Personal conversation with R. Grossgut, Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH.

Grumbach M (2005). A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *J Clin Endocrinol Metab* **90**: 3122–3127.

Habert R and Picon R (1982). Control of testicular steroidogenesis in fetal rat: effect of decapitation on testosterone and plasma luteinizing hormone-like activity. *Acta Endocrinol* **99**: 466–473 (as cited in Lambrot et al. 2007).

Habert R and Picon R (1984). Testosterone, dihydrotestosterone and estradiol-17β levels in maternal and fetal plasma and in fetal testes in the rat. *J Steroid Biochem* **21**: 193–198 (as cited in Scott et al. 2009).

Hallmark N, Walker M, McKinnell C, Mahood IK, Scott H, Bayne R, Coutts S, Anderson RA, Greig I, Morris K and Sharpe RM (2007). Effects of Monobutyl and Di(n-butyl) Phthalate in Vitro on Steroidogenesis and Leydig Cell Aggregation in Fetal Testis Explants from the Rat: Comparison with Effects in Vivo in the Fetal Rat and Neonatal Marmoset and in Vitro in the Human. *Environ Health Perspect* **115 (3)**: 390- 396.

Hannas BR, Lambright CS, Furr J, Howdeshell KL, Wilson VS and Gray LE Jr (2011a). Dipentyl phthalate dosing during sexual differentiation disrupts fetal testis function and postnatal development of the male Sprague-Dawley rat with greater relative potency than other phthalates. *Toxicol Sci* **120(1)**: 184-93.

Hannas BR, Lambright CS, Furr J, Howdeshell KL, Wilson VS and Gray LE Jr (2011b). Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate and diisononyl phthalate. *Toxicol Sci* **123(1)**: 206-216.

Hannas BR, Lambright CS, Furr J, Evans N, Foster P, Gray LE and Wilson V (2012). Genomic biomarkers of phthalate-induced male reproductive developmental toxicity: A targeted rtPRC array approach for defining relative potency. *Toxicol Sci* **125(2)**: 544-57.

Harding BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ and Smith KN(1987). Evaluation of 60 chemicals in a preliminary development toxicity test. *Terato Carcin And Mutagenesis* **7**: 29-48 (as cited in EC 2003b).

Harris CA, Henttu P, Parker MG and Sumper JP (1997). The estrogenic activity of phthalate esters In Vitro. *Environ*. *Health Perspectives* **105**: 802-811.

Harris R, Turan N, Kirk C, Ramsden D and Waring R (2007). Effects of endocrine disruptors on dehydroepiandrosterone sulfotransferase and enzymes involved in PAPS synthesis: genomic and ongenomic pathways. *Environ Health Perspect* **115**: 51-54.

Hass U (2003). Abstracts. 31st Conference of the European Teratology Society Elsinore, Denmark, 7th–10th September 2003. *Reprod Toxicol* **17**: 475–508.

Hays SM, Aylward LL, Kirman CR, Krishnan K and Nong A (2011). Biomonitoring Equivalents for di-isononyl phthalate (DINP). *Regul Toxicol Pharmacol* **60**: 181-188.

Hazleton Laboratories (1968a). Three-Month Dietary Administration – Albino Rats DIDP – FDA Grade (Plasticiser) submitted to Dewey and Almy Chemical Division, WR Grace and Company.

Hazleton Laboratories (1968b). 13-Week Dietary Administration - Dogs Plasticiser (DIDP) submitted to WR Grace and Company (as cited in EC 2003b).

Hazleton (1972). Metabolism Study of 14C Phthalate Ester in Rats. Unpublished Laboratory Report from Hazleton Laboratories submitted to Esso Research and Engineering Company,. Final Report, September 25, 1972 (as cited in EC 2003a).

Hazleton (1981a). *Thirteen-Week Toxicity Study in Rats, DINP.* Final Report submitted to Nissan Chemical Industries, Ltd., Tokyo, Japan. Unpublished Results (as cited in EC 2003a).

Hazleton (1981b). *Teratology Study in Rats DINP.* Project No 2096-103 from Hazleton Laboratories America, Inc. submitted to Nissan Chemical Industries, Ltd., Tokyo, Japan, Final Report (as cited in EC 2003a).

Hazleton (1991a). *A Subchronic (13-week) Dietary Oral Toxicity Study of Di(isononyl)Phthalate in Fischer 344 Rats with attachments and cover letter dated 082291.* Unpublished Laboratory Report from Hazleton Laboratories submitted to Aristech Chem. Corporation (as cited in EC 2003a).

Hazleton (1991b). *A Subchronic (4-week) Dietary Oral Toxicity Study of Di(isononyl)Phthalate in B6C3F1 Mice (final report) with cover sheet dated 052991.* Unpublished Laboratory Report from Hazleton Laboratories submitted to Aristech Chemical Corporation. HWA study No 2598-100 (as cited in EC 2003a).

Hazleton (1992). *A 13-Week Subchronic Dietary Oral Toxicity Study in Mice with Di(isononyl)Phthalate Including Ancillary Hepatocellular Proliferation and Biochemical Analyses.* Hazleton Project HWA 2598-103 (as cited in EC 2003a).

Health Canada (1998). *Updated risk assessment on Diisononyl Phthalate in vinyl children's products.* Consumer Products Division, Product Safety Bureau, Environmental Health Directorate, Health Protection Branch. Ottawa, Ontario. November 14, 1998.

Health Council of the Netherlands (2011). Health-based recommended occupational exposure limit on 1,4-Dioxane Dutch Expert Committee on Occupational Safety, a Committee of the Health Council of the Netherlands. Available at: www.gezondheidsraad.nl/sites/default/files/201109.pdf.

Heger N, Hall S, Sandrof M, Hensley J, Johnson K J, Houseman E, Gaido KW and Boekelheide K (2010). Interspecies approach to the assessment of human susceptibility to phthalate-induced endocrine disruption. *The Toxicologist* **114**: 1973 (as cited in ExxonMObil 2011).

Heger N, Hall S, Sandrof M, Hensley J, Johnson KJ, Houseman E, Gaido KW and Boekelheide K (2011). Interspecies approach to the assessment of human susceptibility to phthalate-induced endocrine disruption. *The Toxicologist* **120**: 2191 (as cited in ExxonMObil 2011).

Hellwig J, Freudenberg H and Jäckh R (1997). Differential prenatal toxicity of branched phthalate esters in rats. *Food Chem Toxicol* **35**: 501-512.

Higuchi T, Kane C, Palmer J and Veeramachaneni DNR (1999). Developmental effects of DBP in frogs and rabbits. *Biol Reprod* **60(suppl 1)**: 153 (as cited in Gray et al 2000).

Higuchi TT, Palmer JS, Gray LE Jr and Veeramachaneni DN (2003). Effects of dibutyl phthalate in male rabbits following in utero, adolescent, or postpubertal exposure. *Toxicol Sci* **72**: 301–313 (as cited in Howdeshell et al 2008b).

Hobbie KR, Deangelo AB, George MH and Law JM (2011). Neoplastic and Nonneoplastic Liver Lesions Induced by Dimethylnitrosamine in Japanese Medaka Fish. *Vet Pathol.* Available at: vet.sagepub.com/content/early/2011/07/01/0300985811409443.full.pdf

Holm M, Rajpert-De Meyts E, Andersson AM and Skakkebæk NE (2003). Leydig cell micronodules are a common finding in testicular biopsies from men with impared spermatogenesis and are associated with decreased testosterone/LH ratio. *Journal of Pathology* **199**: 378-386 (as cited in Foster 2006).

Hotchkiss AK, Furr J, Makynen EA, Ankley GT, Gray LE Jr (2007). In utero exposure to the environmental androgen trenbolone masculinizes female Sprague-Dawley rats. *Toxicol Lett* **174(1-3)**: 31-41.

Howdeshell KL, Wilson VS, Furr J, Lambright CR, Rider CV, Blystone CR, Hotchkiss AK and Gray LE Jr (2008a). A Mixture of Five Phthalate Esters Inhibits Fetal Testicular Testosterone Production in the Sprague-Dawley Rat in a Cumulative, Dose-Additive Manner. *Toxicol Sci* **105(1)**: 153-165.

Howdeshell KL, Rider CV, Wilson VS and Gray LE Jr (2008b). Mechanisms of action of phthalate esters, individually and in combination, to induce abnormal reproductive development in male laboratory rats. *Environmental Research* **108**: 168–176.

Högberg J, Hanberg A, Berglund M, Skerfving S, Remberger M, Calafat AM, Filipsson AF, Jansson B, Johansson N, Appelgren M and Håkansson H (2008). Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. *Environ Health Perspect* **116(3)**: 334-339.

Høibye L, Maag J and Hansen E (2011). *Background data for Annex XV dossier - DEHP, BBP, DBP and DIBP.* Environmental Projevt No. 1362. Danish Environmental protection Agency, Copenhagen.

Huhtaniemi I and Toppari J (1995). *Endocrine, paracrine and autocrine regulation of testicular steroidogenesis. Advances in Experimental Medicine and Biology* **377**: 33–54.

Hushka LJ, Waterman SJ, Keller LH, Trimmer GW, Freeman JJ, Ambroso JL, Nicolich M and McKee RH (2001). Two-generation reproduction studies in rats fed di-isodecyl phthalate. *Reproductive Toxicology* **15**: 153-169.

Hwang HM, Park EK, Young TM and Hammock BD (2008). Occurrence of endocrine-disrupting chemicals in indoor dust. *Sci Total Environ* **404(1)**: 26-35.

IARC (2012). *Some chemicals present in industrial and consumer products, food and drinking-water.* IARC monographs on the evaluation of carcinogenic risks to humans, vol 101. International Agency for Research on Cancer (IARC), Lyon.

Imai Y, Kondo A, Iizuka H, Maruyama T, Kurohane K (2006). Effects of phthalate esters on the sensitization phase of contact hypersensitivity induced by fluorescein isothiocyanate. *Clin Exp Allergy* **36**: 1462–1468.

Imperato-McGinley J, Binienda Z, Arthur A, Mininberg DT, Vaughan ED and Quimby FW (1985). The development of a male pseudohermaphroditic rat using an inhibitor of the enzyme 5a-reductase. *Endocrinology* **116**: 807–811(as cited in Foster 2006).

Imperato-McGinley J, Binienda Z, Gedney J and Vaughan ED Jr (1986). Nipple differentiation in fetal male rats treated with an inhibitor of the enzyme 5 alpha-reductase:definition of a selective role for dihydrotestosterone. *Endocrinology* **118**: 132–137 (as cited in Foster 2006).

INEOS ChlorVinyls (2012). Submission of information via the public consultation on ECHA's draft review report by INEOS ChlorVinyls UK Ltd. Comment reference number 9. Public comments are available on http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

Ito Y, Yamanoshita O, Asaeda N, Tagawa Y, Lee C-H, Aoyama T, Ichihara G, Furuhashi K, Kamijima M, Gonzalez FJ and Nakajima T (2007). Di(2-ethylhexyl)phthalate Induces Hepatic Tumorigenesis through a Peroxisome Proliferator-activated Receptor α-independent Pathway. *J Occup Health* **49**: 172-182.

Ito Y and Nakajima T (2008). PPARα- and DEHP-Induced Cancers. *Hindawi Publishing Corporation, PPAR Research.* Article ID 759716.

Ivell R and Hartung S (2003). The molecular basis of cryptorchidism. *Mol Hum Reprod* **9**: 175–181.

Jaakkola JJK and Knight TL (2008). The Role of Exposure to Phthalates from Polyvinyl Chloride Products in the Development of Asthma and Allergies: A Systematic Review and Meta-analysis. *Environ Health Perspect* **116** (7): 845-853.

Jaakkola JJK, Ieromnimon A and Jaakkola MS (2006). Interior surface materials and asthma in adults: a population-based incident case-control study. *Am J Epidemiol* **164**: 742–749.

Janssen and Bremmer (2010). Risk assessment plasticizers from erotic objects. National Institute for Public Health and the Environment (RIVM), 29 September 2010. Available at: www.vwa.nl/txmpub/files/?p_file_id=2201039

Jirtle RL (editor) (1995). *Liver regeneration and carcinogenesis: molecular and cellular mechanisms.* Academic Press.

Johnson S, Saikia N and Sahu R (2010). *Phthalates in toys.* Centre for Science and Environment, New Delhi.

Johnson KJ, McDowell EN, Viereck MP and Xia JQ (2011). Species-Specific Dibutyl Phthalate Fetal Testis Endocrine Disruption Correlates with Inhibition of SREBP2-Dependent Gene Expression Pathways. *Toxicol Sci* **120 (2)**: 460-474.

Jost A, Vigier B, Prépin J and Perchellet JP (1973). Studies on sex differentiation in mammals. *Recent Prog Horm Res* **29**: 1–41 (as cited in Lambrot et al. 2009).

Juberg DR, Alfano K, Coughlin RJ and Thompson KM (2001). An observational study of object mouthing behavior by young children. *Pediatrics* **107(1)**:135–142.

Juberg DR (2013). Personal communication regarding the paper by Juberg et al. (2001), 18 January 2013.

Kaiserling E and Müller H (2005). Neoplasm of hepatic stellate cells (spongiotic pericytoma): a new tumor entity in human liver. (Case Reports). *Pathol Res Pract* **201(11)**: 733-43.

Kanno H, Tanakamaru Z, Ishimura Y, Kandori H, Yamasaki H and Sasaki S (2003). Historical Background Data in CB6F1-Tg-rasH2 Mice and CB6F1-nonTg-rasH2 Mice over a 26 –Week Experimental Period. *J Toxicol Pathol* **16**: 267-274.

Karbe E and Kerlin RL (2002). Cystic degeneration/spongiosis hepatis in rats. *Toxicol Pathol* **30**: 216-227.

McKee RH, El-Hawari M, Stolz M, Pallas F and Lington AW (2002). Absorption, Disposition and Metabolism of Di-isononyl Phthalate (DINP) in F-344 Rats. *J Appl Toxicol* **22**: 293-302.

Keenan C, Elmore S, Francke-Carroll S, Kemp R, Kerlin R, Peddada S, Pletcher J, Rinke M, Schmidt SP, Taylor I and Wolf DC (2009). Best Practices for Use of Historical Control Data of Proliferative Rodent Lesions. *Toxicol Pathol* **37**: 679.

Kerlin RL and Karbe E (2004). Response to comments on E. Karbe and R. L. Kerlin (2002). Cystic degeneration/spongiosis hepatis (Toxicol. Pathol. 30:216-227). *Toxicol Pathol* **32**: 271.

Kato K, Silva MJ, Wolf C, Gray LE, Needham LL and Calafat AM (2007). Urinary metabolites of diisodecyl pthtalate in rats. *Toxicology* **236**: 114-122.

Kaufmann WK, Deckardt RH, McKee JH, Butala and Bahnemann R (2002). Tumor Induction in Mouse Liver: Di-isononyl Phthalate Acts via Peroxisome Proliferation. *Regulatory Toxicology and Pharmacology* **36**: 175–183.

Kasper-Sonnenberga M, Koch HM, Wittsiepea J and Wilhelma M (2012). Levels of phthalate metabolites in urine among mother–child-pairs – Results from the Duisburg birth cohort study, Germany. *International Journal of Hygiene and Environmental Health* **215**: 373–382.

Kimber I and Dearman RJ (2010). An assessment of the ability of phthalates to influence immune and allergic responses. *Toxicology* **271(3)**: 73-82.

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, DeLuca JG, Lai DY, McKee RH, Peters JM, Roberts RA, Fenner-Crisp PA (2003). PPARα Agonist-Induced Rodent Tumors: Modes of Action and Human Relevance. *Critical Reviews in Toxicology* **33(6)**: 655–780.

Kleymenova E, Swanson C, Boekelheide K and Gaido KW (2005). Exposure in utero to di(n-butyl)phthalate alters the vimentin cytoskeleton of fetal rat Sertoli cells and disrupts Sertoli cell-gonocyte contact. *Biol Reprod* **73**: 482–90.

Kohn MC, Parham F, Masten SA, Portier CJ, Shelby MD, Brock JW and Needham LL (2000). Human exposure estimates for phthalates. *Environ Health Perspect* **108**: A440-A442.

Koch HM, Drexler H and Angerer J (2004). Internal exposure of nursery-school children and their parents and teachers to di(2-ethylhexyl)phthalate (DEHP). *Int J Hyg Eviron Health* **207(1)**: 15-22.

Koch HM, Bolt HM, Preuss R and Angerer (2005). New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. *Arch Toxicol* **79**: 367–376.

Koch HM, Müller J and Angerer J (2006). Determination of secondary, oxidised di-iso-nonylphthalate (DINP) metabolites in human urine representative for the exposure to commercial DINP plasticizers. *J Chromatogr B Analyt Technol Biomed Life Sci* **847(2)**: 114-125.

Koch HM, Müller J and Angerer J (2007). Determination of secondary, oxidised di-iso-nonylphthalate (DINP) metabolites in human urine representative for the exposure to commercial DINP plasticizers. *J Chromatogr B* **847**: 114-125.

Koch HM and Angerer J (2007). Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. *Int J Hyg Environ Health* **210(1)**: 9-19.

Koch HM and Calafat AM (2009). Human body burdens of chemicals used in plastic manufacture. *Phil Trans R Soc B* **364**: 2063–2078.

Koch HM, Haller A, Weiß T, Käfferlein HU, Stork J and Brüning T (2011a). Phthalate exposure during cold plastisol application-A human biomonitoring study. *Toxicol Lett.*, doi:10.1016/j.toxlet.2011.06.010 [Epub ahead of print]

Koch HM, Wittassek M, Brüning T, Angerer J and Heudorf U (2011b). Exposure to phthalates in 5-6 years old primary school starters in Germany-A human biomonitoring study and a cumulative risk assessment. *Int J Hyg Environ* Health **214(3)**: 188-195.

Koike E, Yanagisawa R, Sadakane K, Inoue K, Ichinose T, Takano H (2010). Effects of Diisononyl Phthalate on Atopic Dermatitis in Vivo and Immunologic Responses in Vitro.

Kolarik B, Naydenov K, Larsson M, Bornehag CG and Sundell J (2008). The association between phthalates in dust and allergic diseases among Bulgarian children. *Environ Health Perspect* **116(1)**: 98-103.

Kortenkamp A, Martin O, Faust M, Evans R, McKinlay R, Orton F and Rosivatz E (2011). State of the art assessment of endocrine disrupters. Final Report. Project Contract Number 070307/2009/550687/SER/D3. 23.12.2011.

Kransler KM, Bachman AN and McKee RH (2012). A comprehensive review of intake estimates of di-isononyl phthalate (DINP) based on indirect exposure models and urinary biomonitoring data. *Regul Toxicol Pharmacol* **62**: 248-256.

Kratochwil K (1971). In vitro analysis of the hormonal basis for the sexual dimorphism in the embryonic development of the mouse mammary gland. *J Embry Exp Morph* **25**: 141–153.

Kratochwil K (1986). Tissue combination and organ culture studies in the development of the embryonic mammary gland. *Dev Biol* **4**: 315–333 (as cited in ExxonMobil 2011a).

Kratochwil K and Schwartz P (1976). Tissue interaction in androgen response of embryonic mammary rudiment of mouse: Identification of target tissue for testosterone. *Proc Natl Acad Sci* **73**: 4041–4044.

Kratochwil K (1977). Development and loss of androgen responsiveness in the embryonic rudiment of the mouse mammary gland. *Dev Biol* **61**: 358–365 (as cited in ExxonMobil 2011a).

Kubota Y, Temelcos C, Bathgate RA, Smith KJ, Scott D, Zhao C and Hutson JM (2002). The role of insulin 3, testosterone, Mullerian inhibiting substance and relaxin in rat gubernacular growth. *Mol Hum Reprod* **8 (10)**: 900–905.

Krüger T, Long M and Bonefeld-Jørgensen EC (2008). Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. *Toxicology* **246**: 112–123.

Kurata Y, Kidachi F, Yokoyama M, Toyota N, Tsuchitani M and Katoh M (1998). Subchronic Toxicity of Di(2-ethylhexyl)phthalate in Common Marmosets: Lack of Hepatic Peroxisome Proliferation, Testicular Atrophy, or Pancreatic Acinar Cell Hyperplasia. *Toxicol Sci* **42**: 49-56.

Kurata Y, Makinodan F, Shimamura N and Katoh M (2012). Metabolism of di (2-ethylhexyl) phthalate (DEHP): comparative study in juvenile and fetal marmosets and rats. *J Toxicol Sci* **34(1)**: 33-49.

Kwack SJ, Kim KB, Kim HS and Lee BM (2009). Comparative Toxicological Evaluation of Phthalate Diesters and Metabolites in Sprague-Dawley Male Rats for Risk Assessment. *J Toxicol Environ Health A* **72(21-22)**: 1446-1454.

Lake BG, Brantom PG, Gangolli SD, Butterworth KR and Grasso P (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. *Toxicology* **6**: 341–356 (as cited in NRC 2008).

Lake BG (1991). Dose-response relationships for induction of hepatic peroxisome proliferation and testicular atrophy by phthalate esters in the rat. *Human Exp Toxicol* **10**: 67-68 (as cited in EC 2003b).

Lambright C, Furr J, Cardon M, Hannas B, Bermudez D, Wrench N, Wilson V, Foster P and Gray Jr E (2011). Fetal phthalate screen: assessment of several phthalate esters (PE) on fetal rodent testosterone (T) production and gene expression following in utero exposure. Abstract 1022. *The Toxicologist*. *Supplement to Toxicological Sciences* **120**: 218.

Lambrot R, Muczynski V, Lécureuil C, Angenard G, Coffi H, Pairault C, Moison D, Frydman R, Habert R and Rouiller-Fabre V (2009). Phthalates Impair Germ Cell Development in the Human Fetal Testis in Vitro without Change in Testosterone Production. *Environ Health Perspect* **117 (1)**: 32-37.

Larsen ST, Lund RM, Nielsen GD, Thygesen P and Poulsen OM (2002). Adjuvant effect of di-n-butyl-, di-n-octyl-, di-iso-nonyl- and di-iso-decyl phthalate in a subcutaneous injection model using BALB/c mice. *Pharmacol Toxicol* **91(5)**: 264-72.

Lee HY, Kalmus GW and Levin MA (1974). Effects of phthalate esters (plasticisers) on chick embryos and chick embryonic cells. *Growth* **38**: 301-312 (as cited by EC 2003b).

Lee HC, Ko YG, Im GS, Chung HJ, Seong HH, Chang WK, Yamanouchi K and Nishihara M (2006a). Effects of phthalate/adipate esters exposure during perinatal period on reproductive function after maturation in rats. *J Anim Sci & Technol (Kor)* **48(5)**: 651-662.

Lee HC, Yamanouchi K and Nishihara M (2006b). Effects of perinatal exposure to phthalate/adipate esters on hypothalamic gene expression and sexual behavior in rats. *J Reprod Dev* **52(3)**: 343-352.

Lee BM and Koo HJ (2007). Hershberger assay for antiandrogenic effects of phthalates. *J Toxicol Environ Health A* **70(15-16)**: 1365-70.

Lehmann KP, Phillips S, Sa M, Foster PMD and Gaido KW (2004). Dose-dependent alterations in gene expression and testosterone synthesis in the fetal testes of male rats exposed to di (n-butyl) phthalate. *Toxicol Sci* **81**: 60–68.

Li L-H, Donald JM and Golub MS (2005). Review on Testicular Development, Structure, Function, and Regulation in Common Marmoset. *Birth Defects Research (Part B)* **74**: 450–469.

Lin S, Ku HY, Su PH, Chen JW, Huang PC, Angerer J and Wang SL (2011). Phthalate exposure in pregnant women and their children in central Taiwan. *Chemosphere* **82(7)**: 947-955.

Lington AW, Bird MG, Plutnick RT, Stubblefield WA and Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Fund Appl Toxicol* **36**: 79-89.

Livera G, Delbes G, Pairault C, Rouiller-Fabre V and Habert R (2006). Organotypic culture, a powerful model for studying rat and mouse fetal testis development. *Cell Tissue Res* **324(3)**: 507–521.

Lottrup G , Andersson AM, Leffers H, Mortensen GK, Toppari J, Skakkebæk NE and Main KM (2006). Possible impact of phthalates on infant reproductive health. *Int J Androl* **29**: 172–180.

Lovekamp-Swan T and Davis BJ (2003). Mechanisms of phthalate ester toxicity in the female reproductive system. *Environ Health Perspect* **111(2):** 139–145.

Lovekamp TN and Davis BJ (2001). Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. *Toxicol Appl Pharmacol* **172**: 217 - 224.

Lu B and Bishop CE (2003). Late onset of spermatogenesis and gain of fertility in POG-deficient mice indicate that POG is not necessary for the proliferation of spermatogonia. *Biol Reprod* **69(1)**: 161–168.

MacLeod DJ, Sharpe RM, Welsh M, Fisken M, Scott HM, Hutchison GR, Drake AJ and van den Driesche S (2010). Androgen action in the masculinization programming window and development of male reproductive organs. *Int J Androl* **33**: 279–287.

Mahood IK, Hallmark N, McKinnell C, Walker M, Fisher JS and Sharpe RM 2005. Abnormal Leydig cell aggregation in the fetal testis of rats exposed to di (n-butyl) phthalate and its possible role in testicular dysgenesis. *Endocrinology* **146**: 613–623.

Main KM, Mortensen GK, Kaleva M, Boisen K, Damgaard I, Chellakooty M, Schmidt IM, Suomi A-M, Virtanen HE, Petersen JH, Andersson A-M, Toppari J and Skakkebæk NE (2006). Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in three months old infants. *Environ Health Perspect* **114 (2)**: 270-276.

Makris SL, Euling SY, Gray LE Jr, Benson R and Foster PM (2010). Use of genomic data in risk assessment case study: I. Evaluation of the dibutyl phthalate male reproductive development toxicity data set. *Toxicol Appl Pharmacol* [Epub ahead of print].

Mann DR and Fraser HM (1996). The neonatal period: a critical interval in primate development. *J Endocrinol* **149**: 191–197 (as cited in Hallmark et al. 2007).

Maronpot RR, Yoshizawa K, Nyska A, Harada T, Flake G, Mueller G, Singh B and Ward JM (2010). Hepatic enzyme induction: histopathology. *Toxicol Pathol* **38(5)**: 776-95.

Masutomi N, Takagi H, Uneyama C, Takahashi N and Hirose M (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. *Toxicology* **192**: 149–170.

Masutomi N, Shibutani M, Takagi H, Uneyama C, Lee KY and Hirose M (2004). Alteration of pituitary hormone-immunoreactive cell populations in rat offspring after maternal dietary exposure to endocrine-active chemicals. *Arch Toxicol* **78**: 232–240.

Mastelic B, Ahmed S, Egan W, Del Giudice G, Golding H, Gust I, Neels P, Reed S, Sheets R, Siegrist C-A and Lambert P-H (2010). Mode of action of adjuvants: Implications for vaccine safety and design. *Biologicals* **38**: 594-601.

Matsumoto M, Hirata – Koizumi M and Ema M (2008). Potential adverse effects of phthalic acid esters on human health: A review of recent studies on reproduction. *Regulatory Toxicology and Pharmacology* **50**: 37-49.

McKee RH, El-Hawari M, Stoltz M, Pallas F and Lington AW (2002). Absorption, Disposition and Metabolism of Di-isononyl Phthalate (DINP) in F-344 Rats. *J Appl Toxicol* **22**: 293–302.

McKee RH, Pavkov KL, Trimmer GW, Keller LH and Stump DG (2006). An assessment of the potential developmental and reproductive toxicity of di-isoheptyl phthalate in rodents. *Reprod Toxicol* **21**: 241–252.

McKinnell C, Saunders PT, Fraser HM, Kelnar CJ, Kivlin C, Morris KD and Sharpe (2001). Comparison of androgen receptor and oestrogen receptor beta immunoexpression in the testes of the common marmoset (*Callithrix jacchus*) from birth to adulthood: low androgen receptor immunoexpression in Sertoli cells during the neonatal increase in testosterone concentrations. *Reproduction* **122**: 419–429.

Meeker JD, Hu H, Cantonwine DE, Lamadrid-Figueroa H, Calafat AM, Ettinger AS, Hernandez-Avila M, Loch-Caruso R and Téllez-Rojo MM (2009). Urinary Phthalate Metabolites in Relation to Preterm Birth in Mexico City. *Environ Health Perspect* **117(10)**: 1587-1592.

Midwest Research Institute (1983a). Single and Repeated Oral Dose Pharmacokinetics of 14 C-Labeled Diisononyl Phthalate, with cover letter. Unpublished Laboratory Report from Midwest Research Institute prepared for Exxon Corporation, MRI Project No 7282-B, December 19, 1983 (as cited in EC 2003a).

Midwest Research Institute (1983b). Dermal Disposition of 14C-Di-isononyl Phthalate in Rats. Unpublished Laboratory Report from Midwest Res. Inst. prepared for Exxon Corporation, MRI Project No 7572-E, Final Report August 4, 1983 (as cited in EC 2003a)

Moore MR (1998a). *Oncogenicity Study in Rats with Di(isononyl)phthalate Including Ancillary Hepatocellular Proliferation and Biochemical Analyses.* Covance Laboratories, Inc., Vienna, VA 22182. For Aristech Chemical Corporation, Pittsburgh, PA 15230. Covance 2598-104 (as cited in US CPSC 2010a).

Moore MR (1998b). *Oncogenicity Study in Mice with Di(isononyl)phthalate Including Ancillary Hepatocellular Proliferation and Biochemical Analyses.* Covance Laboratories 144 Inc., Vienna, VA 22182. For Aristech Chemical Corporation, Pittsburgh, PA 15230. Covance 2598-105 (as cited in US CPSC 2010a).

Moore N (2000). The oestrogenic potential of the phthalate esters. *Reprod Toxicol* **14**: 183–192.

Moore RW, Rudy TA, Lin TM, Ko K and Peterson RE (2001). Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. *Environ Health Perspect* **109(3)**: 229–237.

Meuling WJA, Rijk MAH and Vink AA (2000). *Study to investigate the phthalate release into saliva from plasticized PVC during sucking and biting by human volunteers*. TNO report V98.680, Final Report, Project number 40393. TNO Voeding, 3700 AJ Zeist, The Netherlands. Sponsored by CEFOC/ECPI.

Mukherjee B, Ghosh S, Das T and Doloi M (2005). Characterization of insulin-like-growth factor II (IGF II) mRNA positive hepatic altered foci and IGF II expression in hepatocellular carcinoma during diethylnitrosamine-induced hepatocarcinogenesis in rats. *J Carcinog* **4**: 12.

Murature DA, Tang SY, Steinhardt G and Dougherty RC (1987). Phthalate esters and semen quality parameters. *Biomedical and Environmental Mass Spectrometry* **14**: 473–477 (as cited in Lottrup et al. 2006).

Mlynarciková A, Ficková M and Scsuková S (2007). The effects of selected phenol and phthalate derivatives on steroid hormone production by cultured porcine granulosa cells. *Altern Lab Anim* **35(1)**: 71-77.

Myers BA (1991). A Subchronic (13-Week) Dietary Oral Toxicity Study of Di(Isononyl)Phthalate in Fischer 344 Rats. Hazleton Washington, Inc. Unpublished Laboratory Report. For Aristech Chemical Corporation, Pittsburgh, PA 15230. August 12, 1991. EPA document no. 89-920000224 (as cited in US CPSC 2010a).

Mylchreest E, Cattley RC and Foster PMD (1998). Male reproductive tract malformations in rats following gestational and lactational exposure to Di(*n*-butyl) phthalate: an anti-androgenic mechanism? *Toxicol Sci* **43**:47–60

Mylchreest E, Sar M, Cattley RC and Foster PM (1999). Disruption of androgen-regulated male reproductive development by di(n-butyl) phthalate during late gestation in rats is different from flutamide. *Toxicol Appl Pharmacol* **156(2)**: 81-95.

Mylchreest E, Wallace DG, Cattley RC and Foster PM (2000). Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl) phthalate during late gestation. *Toxicol Sci* **55**: 143–151.

Mylchreest E, Sar M, Wallace DG and Foster PM (2002). Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(n-butyl) phthalate. *Reprod Toxicol* **16(1)**: 19-28.

Nagao T, Ohta R, Marumo H, Shindo T, Yoshimura S and Ono H (2000). Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: a two-generation reproductive study. *Reprod Toxicol* **14**: 513–532.

Nanni N, Fiselier K, Grob K, Di Pasquale M, Fabrizi L, Aureli P and Coni E (2011). Contamination of vegetable oils marketed in Italy by phthalic acid esters. *Food Control* **22**: 209-214.

Nef S and Parada LF (1999). Cryptorchidism in mice mutant for Insl3. *Nature Genetics* **22**: 295–299 (as cited in Foster 2006).

Nef S, Shipman T and Parada LF (2000). A molecular basis for estrogen-induced cryptorchidism. *Developmental Biology* **224**: 354–361.

Newton PE, Wooding WL, Bolte HF, Derelanko MJ, Hardisty JF and Rinehart WE (2001). A chronic inhalation toxicity/oncogenicity study of methylethylketoxime in rats and mice. *Inhal Toxicol* **13(12)**: 1093-1116.

NICNAS (2007). *Dibutyl Phthlate (DBP), CAS No. 84-74-2. Human Health Hazard Assessment. Draft for Comment*. Available at: http://www.nicnas.gov.au/industry/existing_chemicals/phthalate_hazard_assessments/dbp%20hazard%20assessment%2030-4-07.pdf

Niino T, Asakura T, Ishibashi T, Itoh T, Sakai S, Ishiwata H, Yamada T and Onodera S (2002a). A simple and reproducible testing method for dialkyl phthalate migration from polyvinyl chloride products into saliva simulant. *J Food Hyg Soc Japan* **44(1)**: 13-18.

Niino T, Ishibashi T, Itoh T, Sakai S, Ishiwata H, Yamada T and Onodera S (2002b). Comparison of diisononyl phthalate migration from polyvinyl chloride products into human saliva in vivo and into saliva simulant in vitro. *Journal of Health Science* **48(3)**: 277-281.

Nikiforov AI, Keller LH and Harris SB (1995). Two generation reproduction study in rats with diisononyl phthalate (DINP). *Book of Abstracts. Eurotox'95. Toxicol Lett. Supplement1/78* (as cited in EC 2003a).

Nikiforov AI and Koehler GD (1994). Developmental toxicity studies on diisononyl phthalate (DINP) and di-isodecyl phthalate (DIDP). *Book of Abstracts - Eurotox'94* (as cited in EC 2003a)

Nilsson NH, Malmgren-Hansen B, Bernth N, Pedersen E and Pommer K (2006). *Survey and health assessment of chemicals substances in sex toys.* Survey of Chemical Substances in Consumer Products, No. 77. Danish Environmental Protection Agency, Copenhagen.

Norbäck D, Wieslander G, Nordström K and Wålinder R (2000). Asthma symptoms in relation to measured building dampness in upper concrete floor construction, and 2-ethyl-1-hexanol in indoor air. *Int J Tuberc Lung Dis* **4(11)**: 1016-1025.

NTP-CERHR (2003a). National Toxicology Program. Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR). *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-isononyl Phthalate (DINP).* NIH Publication No 03-4484.

NTP-CERHR (2003b). National Toxicology Program. Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR). *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-isononyl Phthalate (DIDP).* NIH Publication No 03-4485.

Noriega NC, Howdeshell KL, Furr J, Lambright CR, Wilson VS and Gray LE Jr (2009). Pubertal Administration of DEHP Delays Puberty, Suppresses Testosterone Production, and Inhibits Reproductive Tract Development in Male Sprague-Dawley and Long-Evans Rats. *Toxicol Sci* **111(1)**: 163-178.

NRC (2008). Phthalates and cumulative risk assessment – The task ahead, Committee on the Health Risks of Phthalates, National Research Council (USA).

OECD (2008). *Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment.* ENV/JM/MONO(2008)16. Series on Testing and Assessment, Number 43. Organisation for Economic Co-operation and Development (OECD), Paris 2008.

OECD (2011). *Draft Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (No 150)*. Version 11 (May 2011). Organisation for Economic Cooperation and Development, Paris.

Oie L, Hersoug LG and Madsen JO (1997). Residential exposure to plasticizers and its possible role in the pathogenesis of asthma. *Environ Health Perspectives* **105(9)**: 972-978 (as cited in EC 2003a).

Oomen AG, Janssen PJCM, Dusseldorp A and Noorlander CW (2008). Exposure to chemicals via house dust, RIVM rapport 609021064.

Ostby JS, Hotchkiss AK, Furr JR and Gray LE Jr (2001). Investigation of the ability ofdiisononyl phthalate (DINP) to alter androgen-dependent tissue development in Sprague-Dawley rats. *The Toxicologist* **60**: 225 (as cited in US CPSC 2010a).

Parker RM (2006). Testing for Reproductive Toxicity. *In Developmental and Reproductive Toxicology* (Hood RD (ed.), CRC Press, Boca Raton, FL (as cited in ExxonMobil 2011).

Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ and Gray LE Jr. (2000). The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicol Sci* **58**: 339–349.

Parmar D, Srivastava SP, Srivastava SP and Seth PK (1985). Hepatic mixed function oxidases and cytochrome P-450 contents in rat pups exposed to di-(2-ethylhexyl) phthalate through mother's milk. *Drug Metab Dispos* **13(3)**: 368-370 (as cited in NRC 2008).

Patyna PJ, Parkerton TF, Davi RA, Thomas PE and Cooper KR (1999). Evaluation of two phthalate ester mixtures in a three generation reproduction study using Japanese medaka (Oryziaslatipes). *Toxicol Sci* **42**: (1-S).

Paust HJ, Wessels J, Ivell R and Mukhopadhyay AK (2002). The expression of the RLF/INSL3 gene is reduced in Leydig cells of the aging rat testis. *Experimental Gerontology* **37**: 1461–1467.

Pedersen GA, Jensen LK, Fankhauser A, Biedermann S, Petersen JH and Fabech B (2008). Migration of epoxidized soybean oil (ESBO) and phthalates from twist closures into food and enforcement of the overall migration limit. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **25(4)**: 503-510.

Pierik FH, Deddens JA, Burdorf A, de Muinck Keizer-Schrama SMPF, de Jong FH and Weber RFA (2009). The hypothalamus-pituitary-testis axis in boys during the first six months of life: a comparison of cryptorchidism and hypospadias cases with controls. *Int J Androl* **32**: 453-461.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, Silva MJ, Brambilla C, Pin I, Charles MA, Cordier S and Slama R (2011). Exposure to Phthalates and Phenols during Pregnancy and Offspring Size at Birth. *Environ Health Perspect* **120(3)**: 464-70.

Picard K, Lhuguenot J-C, Lavier-Canivenc M-C and Chagnon M-C (2001). Estrogenic activity and metabolism of N-butyl benzyl phthalate in vitro: Identification of the active molecules. *Toxicol Appl Pharmacol* **72**: 108–111.

Plummer S, Sharpe RM, Hallmark N, Mahood IK and Elcombe C (2007). Time-dependent and compartment-specific effects of in utero exposure to di(*n*-butyl) phthalate on gene/protein expression in the fetal rat testis as revealed by transcription profiling and laser capture microdissection. *Toxicol Sci* **97**: 520–532.

Preau JL Jr, Wong LY, Silva MJ, Needham LL and Calafat AM (2010). Variability over 1 week in the urinary concentrations of metabolites of diethyl phthalate and di(2-ethylhexyl) phthalate among eight adults: an observational study. *Environ Health Perspect* **118(12)**: 1748-1754.

Rastogi SC (1998). Gas chromatographic analysis of phthalate esters in plastic toys. *Chromatographia* **47**: 724-726.

Rakkestad KE, Dye CJ, Yttri KE, Holme JA, Hongslo JK, Schwarze PE and Becher R (2007). Phthalate levels in Norwegian indoor air related to particle size fraction. *J Environ Monit* **9(12)**: 1419-1425.

Reddy AP, Spitsbergen JM, Mathews C, Hendricks JD and Bailey GS (1999). Experimental hepatic tumorigenicity by environmental hydrocarbondibenzo[a,l]pyrene. *J Environ Pathol Toxicol Oncol* **18(4)**: 261-269.

Reddy BS, Rozati R, Reddy BVR and Raman NVVSS (2006). Association of phthalate esters with endometriosis in Indian women. *An International Journal of Obstetrics and Gynaecology* **113**: 515 520.

Rijk MAH, Telman J and Ehrlert K (1999). *Addendum to TNO Report V99.598. "Validation of the method 'determination of diisononylphthalate in saliva stimulant.'"* TNO Nutrition and Food Research Institute, Ultrechtseweg, The Netherlands. Report

RIVM (1998). *Phthalate release from soft PVC baby toys.* National Institute of Public Health and Environmental Protection (RIVM). Report from the Dutch Consensus Group. RIVM Report 31 3320 002, Könemann WH (ed), Bilthoven, The Netherlands. Available at: http://www.rivm.nl/bibliotheek/rapporten/613320002.pdf

Roy P, Salminen H, Koskimies P, Simola J, Smeds A, Saukko R and Huhtaniemi IT (2004). Screening of some anti-androgenci endocrine disruptors using a recombinant cell-based in vitro bioassay. *J Steroid Biochem & Mol Biol* **88**: 157-166.

RPA (2000). The availability of substitutes for soft PVC containing phthalates in certain toys and childcare articles. RPA for the European Commission.

Rusyn I and Corton JC (2012). Mechanistic considerations for human relevance of cancer hazard of di(2-ethylhexyl) phthalate. *Mutation Research* **750**: 141-158.

Saillenfait AM, Sabaté JP and Gallissot F (2006). Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butyl phthalate administered by gavage to rats. *Toxicol Lett* **165**: 39–46.

Saillenfait AM, Sabaté JP and Gallissot F (2008). Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat. *Reprod Toxicol* **26**: 107–115.

Sauvegrain P and Guinard I (2001). *Physico-chemical contamination of foodstuffs by food handlers wearing disposable vinyl gloves.* Prepared by Laboratoire National d'Essais Centre Logistique et Emballage at the request of Ansell Healthcare Europe N.V.

SCCP (2007). *Opinion on Phthalates in cosmetic products.* Scientific Committee on Consumer Products, SCCP/1016/06, 21 March 2007.

SCENIHR (2008). *SCENIHR opinion on the Safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk*. Health &

Consumer Protection DG, Directorate C: Public Health and Risk Assessment, European Commission, Brussels, 2008.

SCHER (2008). *Opinion on phthalates in school supplies.* Scientific Committee on Health and Environmental Risks. Opinion adapted by written procedure on 17 October 2008.

SCHER/SCENIHR/SCCS (2011). *Toxicity and Assessment of Chemical Mixtures*. Opinion by the Scientific Committee on Health and Environmental Risks (SCHER), the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), and the Scientific Committee on Consumer Safety (SCCS). Approved by SCHER on 22 November 2011, by SCENIHR on 30 November 2011, and by SCSS on 14 December 2011.

Schumacher V, Gueler B, Looijenga LHJ, Becker JU, Amann K, Engers R, Dotsch J, Stoop H, Schulz W and Royer-Pokora B (2008). Characteristics of Testicular Dysgenesis Syndrome and Decreased Expression of SRY and SOX9 in Frasier Syndrome. *Mol Reprod Dev* **75(9)**: 1484-94.

Scott HM, Hutchison GR, Jobling MS, McKinnell C, Drake AJ and Sharper RM (2008). Relationship between Androgen Action in the "Male Programming Window," Fetal Sertoli Cell Number, and Adult Testis Size in the Rat. *Endocrinology* **149**: 5280-5287.

Scott HM, Mason JI and Sharpe RM (2009). Steroidogenesis in the Fetal Testis and Its Susceptibility to Disruption by Exogenous Compounds. *Endocrine Reviews* **30(7)**: 883–925.

Sharpe RM (2008). ''Additional'' Effects of Phthalate Mixtures on Fetal Testosterone Production. *Toxicol Sci* **105(1)**: 1-4.

Sharpe RM and Skakkebæk NE (2008). Testicular dysgenesis syndrome: Mechanistic insights and potential new downstream effects. *Fertil Steril* **89**: e33-e38.

Shin-Kang S, Ramsauer VP, Lightner J, Chakraborty K, Stone W, Campbell S, Reddy SA and Krishnan K (2011). Tocotrienols inhibit AKT and ERK activation and suppress pancreatic cancer cell proliferation by suppressing the ErbB2 pathway. *Free Radic Biol Med* **51(6)**: 1164-1174.

Shultz VD, Phillips S, Sar M, Foster PM and Gaido KW (2001). Altered gene profiles in fetal rat testes after in utero exposure to di(n-butyl) phthalate. *Toxicol Sci* **64**: 233–242.

Silva MJ, Reidy JA, Herbert AR, Preau JL, Needham LL and Calafat AM (2004). Detection of Phthalate Metabolites in Human Amniotic Fluid. *Bull Environ Contam Toxicol* **72**: 1226–1231.

Silva MJ, Kato K, Wolf C, Samandar E, Silva SS, Gray EL, Needham LL and Calafat AM (2006b). Urinary biomarkers of di-isononyl phthalate in rats. *Toxicology* **223(1-2)**: 101-112.

Silva MJ, Reidy JA, Preau JL Jr, Needham LL and Calafat AM (2006a). Oxidative metabolites of diisononyl phthalate as biomarkers for human exposure assessment. *Environ Health Perspect* **114(8)**: 1158-1161.

Silva MJ, Reidy A, Kato K, Preau L, Needham L and Calafat AM (2007a). Assessment of human exposure to di-isodecyl pthtalate using oxidative metabolites as biomarkers. *Biomarkers* **12(2)**: 122-144.

Silva MJ, Samandar E, Reidy JA, Hauser R, Needham LL and Calafat AM (2007b). Metabolite profiles of di-n-butyl phthalate in humans and rats. *Environ Sci Technol* **41(21)**: 7576-7580.

Simoneau C, Geiss H, Roncari A, Zocchi P and Hannaert P (2001). *Validation of methodologies for the release of di-isononylphthalate (DINP) in saliva simulant from toys.* European Commission, DG-Joint Research Center, Food Products Unit, Institute for health and Consumer Protection, Ispra, Italy. 2001 EUR 19826 EN (as cited in US CPCS 2010).

Simoneau C and Rijk C (2001). *Standard Operation Procedure for the determination of release of di-isononyl phthalate (DINP) in saliva simulant from toys and childcare articles using a head over heels dynamic agitation device"*. EUR technical report EUR 19899 EN.

Simoneau C, Hannaert P and Sarigiannis D (editor) (2009). *Effect of the nature and concentration of phthalates on their migration from PVC materials under dynamic simulated conditions of mouthing.* European Commission. Joint Research Centre. Institute for Health and Consumer Protection.

Sjoberg P, Bondesson U, Kjellen L, Lindquist NG and Plöen L (1985). Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. *Acta Pharmacol Toxicol* **56**: 30-37.

Skakkebæk NE, Rajpert-De Meyts E and Main KM (2001). Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* **16**: 972–978.

Smith S and Norris B (2002). *Research into the mouthing behaviour of children up to 5 years old*. Consumer and Competition Policy Directorate, Department of Trade and Industry (DTI), London.

Smith SA and Norris B (2003). Reducing the risk of choking hazards: mouthing behavior of children aged 1 month to 5 years. *Injury Contr Safety Promo* **10(3)**:145–154.

Sørensen LK (2006). Determination of phthalates in milk and milk products by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* **20(7)**: 1135-1143.

Stengel KR, Thangavel C, Solomon DA, Angus SP, Zheng Y and Knudsen ES (2009). Retinoblastoma/p107/p130 Pocket Proteins. Protein dynamics and interactions with target gene promoters. *Journal of biological chemistry* **284**: 19265-19271.

Stroebel P, Mayer F, Zerban H and Bannasch P (1995). Spongiotic pericytoma: a benign neoplasm deriving from the perisinusoidal cells in rat liver. *Am J Pathol* **146**: 903-913.

Sugita T, Kawamura Y, Tanimura M, Matsuda R, Niino T, Ishibashi T, Hirabahashi N, Matsuki Y, Yamada T and Maitani T (2003). [Estimation of daily oral exposure to phthalates derived from soft polyvinyl chloride baby toys]. Shokuhin Eiseigaku Zasshi. 44(2): 96-102. [Article in Japanese] Source National Institute of Health Sciences: 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Reviewed by CPSC number V99.849 (as cited in US CPCS 2010).

Sultan C, Lumbroso S, Paris F, Jeandel C, Terouanne B, Belon C, Audran F, Poujan N, Georger V, Gobinet J, Jalaguier S, Auzou G and Nicolas JC (2002). Disorders of androgen action. *Semin Reprod Med* **20(3)**: 217-28 (as cited in NRC 2008).

Su Q, Benner A, Hofmann WJ, Otto G, Pichlmayr R and Bannasch P (1997). Human hepatic preneoplasia: phenotypes and proliferation kinetics of foci and nodules of altered hepatocytes and their relationship to liver cell dysplasia. *Virchows Arch* **431**: 391-406.

Su Q, Schröder CH, Hofmann WJ, Otto G, Pichlmayr R and Bannasch P (1998). Expression of hepatitis B virus X protein in HBV-infected human livers and hepatocellular carcinomas. *Hepatology* **27**: 1109-1120.

Suzuki Y, Niwa M, Yoshinaga J, Watanabe C, Mizumoto Y, Serizawa S and Shiraishi H (2009). Exposure assessment of phthalate esters in Japanese pregnant women by using urinary metabolite analysis. *Environ Health Prev Med* **14(3)**: 180-187.

Svendsen N, Bjarnov E and Poulsen PB (2007). *Survey as well as health assessment of chemical substances in school bags, toy bags, pencil cases and erasers.* Survey of Chemical Substances in Consumer Products, No. 84. Danish Environmental Protection Agency, Copenhagen.

Swan SH (2008). Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. *Environmental Research* **108**: 177–184.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL and the Study for Future Families Research Team (2005). Decrease in Anogenital Distance among Male Infants with Prenatal Phthalate Exposure. *Environ Health Perspect* **113 (8)**: 1056-1061.

Swan SH (2006). Prenatal Phthalate Exposure and Anogenital Distance in Male Infants. *Environ Health Perspect* **114**: A88-A89.

Takagi H, Shibutani M, Lee K-Y, MasutomiN, Fujita H, Inoue K, Mitsumori K and Hirose M (2005). Impact of maternal dietary exposure to endocrine-acting chemicals on progesterone receptor expression in microdissected hypothalamic medial preoptic areas of rat offspring. *Toxicol Appl Pharmacol* **208**:127 – 136.

Takeuchi S, Iida M, Kobayashi S, Jin K, Matsuda T and Kojima H (2005). Differential effects of phthalate esters on transcriptional activities via human estrogen receptors alpha and beta, and androgen receptor. *Toxicology* **210**: 223-233.

Tasaki M, Umemura T, Inoue T, Okamura T, Kuroiwa Y, Ishii Y, Maeda M, Hirose M, Nishikawa A (2008). Induction of characteristic hepatocyte proliferative lesion with dietary exposure of Wistar Hannover rats to tocotrienol for 1 year. *Toxicology* **250**: 143–150.

Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, Malarkey DE, Kaufmann W, Küttler K, Deschl U, Nakae D, Gregson R, Vinlove MP, Brix AE, Singh B, Belpoggi F and Ward JM (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol Pathol* **38**: 5S-81S.

Thomas J, Haseman JK, Goodman JI, Ward JM, Loughran TP Jr and Spencer PJ (2007). A review of large granular lymphocytic leukemia in Fischer 344 rats as an initial step toward evaluating the implication of the endpoint to human cancer risk assessment. *Toxicol Scien* **99**: 3-19.

Thompson CJ, Ross SM and Gaido KW (2004). Di(n-butyl) phthalate impairs cholesterol transport and steroidogenesis in the fetal rat testis through a rapid and reversible mechanism. *Endocrinology* **145**: 1227–1237.

TNO (2010). Analytical report prepared for ExxonMobil Petroleum & Chemicals, Machelen, Belgium.

Tønning K, Jacobsen E, Pedersen E, Strange M, Poulsen PM, Møller L and Boyd PB (2009). *Survey and health assessment of the exposure of 2 year-olds to chemical substances in consumer products.* Survey of Chemical Substances in Consumer Products, No. 102. Danish Environmental Protection Agency, Copenhagen.

Tsumura Y, Ishimitsu S, Saito I, Sakai H, Kobayashi Y and Tonogai Y (2001a). Eleven phthalate esters and di(2-ethylhexyl) adipate in one-week duplicate diet samples obtained from hospitals and their estimated daily intake. *Food Addit Contam* **18(5)**: 449-460.

Tsumura Y, Ishimitsu S, Kaihara A, Yoshii K, Nakamura Y and Tonogai Y (2001b). Di(2-ethylhexyl) phthalate contamination of retail packed lunches caused by PVC gloves used in the preparation of foods. *Food Addit Contam* **18(6)**: 569-579.

Tulve NS, Suggs JC, McCurdy T, Cohen Hubal EA and Moya J (2002). Frequency of mouthing behavior in young children. *J Expo Anal Environ Epidemiol* **12(4)**: 259-264.

Tyl RW, Myers CB, MarrMC, Fail PA, Seely JC, Brine DR, Barter RA and Butala JH (2004). Reproductive toxicity evaluation of dietary butyl benzyl phthalate (BBP) in rats. *Reproductive Toxicology* **18**: 241–264.

Turan N, Waring RH and Ramsden DB (2005). The effect of plasticisers on "sulphate supply" enzymes. *Mol Cell Endocrinol* **244**: 15–19.

TURI (2006). Five chemicals study. Toxics Use Reduction Institute (TURI), University of Massachusetts Lowell, for the Commonwealth of Massachusetts. Chapter on alternatives to DEHP. Available at: http://www.turi.org/library/turi_publications/five_chemicals_study/final_report/chapter_7_dehp#7.3

UBA (2011). Substance monograph: Phthalates – New and updated reference values for monoesters and oxidised metabolites in urine of adults and children. Opinion of the Human Biomonitoring Commission of the German Federal Environment Agency, Umweltbundesamt. English translation of German article in *Bundesgesundheitsbl* **54**: 770–785.

Uhde E, Bednarek M, Fuhrmann F and Salthammer T (2001). Phthalic esters in the indoor environment--test chamber studies on PVC-coated wallcoverings. *Indoor Air* **11(3)**: 150-5.

US CPSC (2002). *Updated risk assessment of oral exposure to diisononyl phthalate (DINP) in children's products*. US CPSC, Bethesda, MD 20814, June 2002. Available at: http://www.cpsc.gov/library/foia/foia02/brief/briefing.html

US CPSC (2010a). *Toxicity Review of Diisononyl Phthalate (DINP)*. Memorandum: United States Consumer Product Safety Commission (US CPSC) staff assessment of the potential toxicity associated with diisononyl phthalate (DINP). Available at: http://www.cpsc.gov/about/cpsia/toxicityDINP.pdf

US CPSC (2010b). *Toxicity Review of Di(isodecyl) Phthalate*. Memorandum: United States Consumer Product Safety Commission (US CPSC) staff assessment of the potential toxicity associated with di(isodecyl) phthalate. Available at: http://www.cpsc.gov/about/cpsia/toxicityDIDP.pdf

US CPCS (2010c). *Review of exposure data and assessments for selected dialkyl ortho-phthalates.* Versar Inc. for U.S. Consumer Product Safety Commission, Bethesda.

US EPA (2005a). Addition of Diisononyl Phthalate Category. Community Right-to-Know Toxic Chemical Release Reporting. Notice of Data Availability. Environmental Protection Agency (EPA). Federal Register /Vol. 70, No. 113 /Tuesday, June 14, 2005 / Proposed Rules.

US EPA (2005b). *Revised Technical Review of Diisononyl Phthalate Office of Environmental Information Environmental Analysis Division Analytical Support Branch*. March 4, 2005

US EPA (2009). *US Environmental Protection Agency's Action Plan.* Available at: http://www.epa.gov/oppt/existingchemicals/pubs/phthalates_ap_2009_1230_final.pdf

US EPA (2011). *Exposure factors handbook: 2011 edition*. EPA/600/R-090/052F. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency (US EPA), Washington, DC 20460, September 2011. Available at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=236252

Valles EG, Laughter AR, Dunn CS, Cannelle S, Swanson CL, Cattley RC and Corton JC (2003). Role of the peroxisome proliferator-activated receptor alpha in responses to diisononyl phthalate. *Toxicology* **191(2-3)**: 211-25.

Van Engelen JGM, Park MVDZ, Janssen PJCM, Oomen AG, Brandon EFA, Bouma K, Sips AJAM, Van Raaij MTM (2006). *Chemicals in Toys A general methodology for assessment of chemical safety of toys with a focus on elements.* RIVM report 320003001/2008. National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

Van Hoogdalem E, De Boer AG and Breimer DD (1991). Pharmacokinetics of rectal drug administration, Part I. General considerations and clinical applications of centrally acting drugs. *Clin Pharmacokinet* **21(1)**: 11-26.

Virtanen HE, Rajpert-De Meyts E, Main KM, Skakkebæk NE and Toppari J (2005). Testicular dysgenesis syndrome and the development andoccurrence of male reproductive disorders. *Toxicol Appl Pharmacol* **207**: S501 – S505.

VITO (2011). Guido Vanermen, VITO-MANT. Personal communication, September 2011.

VWA (2009). Consumentenproducten in de eroticabranche. Fact sheet. Voedsel en Waren Autoriteit (VWA), Afdeling Signalering en Ontwikkeling, Regio Noord, Juni 2009.

Ward J, Peters JM, Perella CM and Gonzalez FJ (1998). Receptor and Nonreceptor-Mediated Organ-Specific Toxicity ofDi(2-ethylhexy1) phthalate (DEHP) in Peroxisome Proliferator-Activated Receptor a-Null Mice. *Toxicol Pathol* **26**: 240-246.

Warren DW, Haltmeyer GC and Eik-Nes KB (1972). Synthesis and metabolism of testosterone in the fetal rat testis. *Biol Reprod* **7**: 94–99 (as cited in Scott et al. 2009).

Waterman SJ, Ambroso JL, Keller LH, Trimmer GW, Nikiforov AI and Harris SB (1999). Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. *Reprod Toxicol* **13(2)**: 131-136.

Waterman SJ, Keller LH, Trimmer GW, Freeman JJ, Nikiforov AI, Harris SB, Nicolich MJ and McKee RH (2000). Two generation reproduction study in rats given diisononyl phthalate in the diet. *Reprod Toxicol* **14(1)**: 21-36.

Welsh M, Saunders PTK, Fisken M, Scott HM, Hutchison GR, Smith LB and Sharpe RM (2008). Identification in rats of a programming window for reproductive tractmasculinization, disruption of which leads to hypospadias and cryptorchidism. *J Clin Invest* **118(4)**: 1479 – 1490.

Wenzel A, Franz C, Breous E and Loos U (2005). Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. *Mol Cell Endocrinol* **244**: 63–71.

Wensing M, Uhde E and Salthammer T (2005). Plastics additives in the indoor environment--flame retardants and plasticizers. *Sci Total Environ* **339(1-3)**: 19-40.

Wenzl T (2009). *Methods for the determination of phthalates in food.* Outcome of a survey conducted among European food control laboratories. Joint Research Centre. Institute for Reference Materials and Measurements.

Wilson VS, Lambright C, Furr J, Ostby J, Wood C, Held G and Gray LE Jr (2004). Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. *Toxicol Lett* **146**: 207–215.

Wilson VS, Blystone CR, Hotchkiss AK, Rider CV and Gray LE Jr (2007). Diverse mechanisms of anti-androgen action: impact on male rat reproductive tract development. *Int J Androl* **31**: 178–187.

Wittassek M and Angerer J (2008). Phthalates: metabolism and exposure. *Int J Androl* **31(2)**: 131-138.

Wittassek M, Koch HM, Angerer J and Brüning T (2011). Assessing exposure to phthalates – The human biomonitoring approach. *Mol Nutr Food Res* **55**: 7–31.

Wittassek M, Wiesmüller GA, Koch HM, Eckard R, Dobler L, Müller J, Angerer J and Schlüter C (2007). Internal phthalate exposure over the last two decades--a retrospective human biomonitoring study. *Int J Hyg Environ Health* **210(3-4)**: 319-333.

Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J and Calafat AM. (2008). Prenatal phenol and phthalate exposures and birth outcomes. *Environ Health Perspect* **116(8)**: 1092-1097.

Wormuth M, Scheringer M, Vollenweider M and Hungerbühler K (2006). What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Analysis* **26**: 803-824.

Xu Y, Hubal EA, Clausen PA and Little JC (2009). Predicting residential exposure to phthalate plasticizer emitted from vinyl flooring: a mechanistic analysis. *Environ Sci Technol* **43(7)**: 2374-80.

Xue J, Zartarian V, Tulve N, Moya J, Freeman N, Auyeung W and Beamer P (2010). A meta-analysis of children's object-to-mouth frequency data for estimating non-dietary ingestion exposure. *J Expo Sci Environ Epidemiol* **20(6)**: 536-545.

Ye CW, Gao J, Yang C, Liu XJ, Li XJ and Pan SY (2009). Development and application of an SPME/GC method for the determination of trace phthalates in beer using a calix[6]arene fiber. *Anal Chim Acta* **641(1-2)**: 64-74.

Ye X, Pierik FH, Hauser R, Duty S, Angerer J, Park MM, Burdorf A, Hofman A, Jaddoe VW, Mackenbach JP, Steegers EA, Tiemeier H and Longnecker MP (2008). Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: the Generation R study. *Environ Res* **108(2)**: 260-267.

Zacharewski TR, Meek MD, Clemons JH, Wu ZF, MR Fielden and JB Matthews (1998). Examination of the *in Vitro* and *in Vitro*Estrogenic Activities of Eight Commercial Phthalate Esters. *Toxicol Sci* **46 (2)**: 282-293.

Zhang YH, Lin L, Cao Y, Chen BH, Zheng LX and Ge RS (2009). Phthalate levels and low birth weight: a nested case-control study of Chinese newborns. *J Pediatr* **155(4)**: 500–504.

Zimmermann S, Steding G, Emmen JM, Brinkmann AO, Nayernia K, Holstein AF, Engel W and Adham IM (1999). Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. *Mol Endocrinol* **13**: 681–691.

List of acronyms and abbreviations

ACC	American Chemistry Council
AF	assessment factor
AGD	anogenital distance
AhR	aryl hydrocarbon receptor
ALP	alkaline phosphatase (also AP is used as acronym)
ALT	alanine transaminase
AR	androgen receptor
AS	allometric scaling factor
AST	aspartate transaminase
AUC	area under curve
BBP	benzyl butyl phthalate (CAS No 85-68-7)
BMD	benchmark dose
BMDC	bone marrow derived cells
BSP	Bromsulphalein (BSP) is a dye used to study the liver function
CALB	calbinding-D
CAR	constitutive androstane receptor
CARACAL	Competent Authorities for REACH and CLP
CERHR	Centre For The Evaluation Of Risks To Human Reproduction of the US National Toxicology Program (NTP-CERHR)
CHAP	Chronic Hazard Advisory Panel (CHAP) of the United States Consumer Product Safety Commission (US CPSC)
CHO	Chinese Hamster Ovary
Cmax	maximum concentration
CSR	Chemical Safety Report
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
Cyp	The cytochrome P450 enzyme superfamily (officially abbreviated as Cyp or CYP). They contain a heme cofactor and, therefore, are hemoproteins.
Cyp11	Family of cytochrome P450 enzymes involved in steroid biosynthesis (e.g. Cyp11a1; Cyp17a1; Cyp11b1; Cyp11b2; Cyp2b6 ; Cyp3a4)
Cyp11a1	Cytochrome P450, family 11, subfamily A, polypeptide 1 (P45011a1), often referred to as P450scc (or 20,22-desmolase), is a mitochondrial enzyme associated with the conversion of cholesterol to pregnenolone. The gene name is Cyp11a1
DBP	dibutyl phthalate (CAS No 84-74-2)
DCHP	dicyclohexyl phthalate (CAS No 84-61-7)
DPP	di-n-propyl phthalate (possibly CAS No 131-16-8)
DEHA	di(2-ethylhexyl)adipate (CAS No 103-23-1)
DEHP	bis (2-ethylhexyl) phthalate (CAS No 117-81-7)
DEP	diethyl phthalate (CAS No 84-66-2)
Dhcr7	7-dehydrocholesterol reductase, enzyme mediating the final step in cholesterol production
DHeP	diheptyl phthalate (CAS 3648-21-3)
DHP	dihexyl phthalate (CAS 84-75-3)
DIBP	diisobutyl phthalate (CAS No 201-553-2)
DIDP	di-"isodecyl" phthalate (CAS No 68515-49-1) as well as 1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich (CAS No 26761-40-0)
DIHP	1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7-rich (also called diisoheptyl phthalate) (CAS No 71888-89-6)

DINCH	di-isononyl-cyclohexane-1,2-dicarboxylate (CAS No 166412-78-8)
DINP	di-"isononyl" phthalate (CAS No 68515-48-0) as well as 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich (CAS No 28553-12-0)
DNOP	di-n-octyl phthalate (CAS No 117-84-0)
DNEL	derived no-effect level
DPHP	bis(2-propylheptyl) phthalate (CAS No 53306-54-0)
DOP	dioctyl phthalate (CAS No 117-84-0), according to Ghisari and Bonefeld-Jorgensen (2009)
DOTP	bis(2-ethylhexyl) terephthalate (also called dioctyl terephthalate) (CAS No 6422-86-2)
DPeP	dipentyl phthalate (CAS 131-18-0)
E2	estradiol
ECPI	European Council for Plasticisers and Intermediates
ED	embryonic day
ED50	effective dose which cases 50% effect
EFSA	European Food Safety Authority
ER	oestrogen receptor
F	female
FAI	free androgen index
FITC	fluorescein isothiocyanate
FRTL-5	rat thyroid cell line
FSH	follicle stimulating hormone
GAPDH	glyseraldehyde-3-phophate dehydrogenase
GATA-4	Transcription factor GATA-4 is a protein that in humans is encoded by the *GATA4* gene
GD	gestation day
GH3	rat pituitary cell line
GIT	gastrointestinal tract
GLP	good laboratory practice
GnRH	gonadotropin releasing hormone
GOT	glutamine oxaloacetate transaminase
grn	granulin
hFSH	human (recombinant) follicle stimulating hormone
hNIS	human sodium/iodide symporter
HPG	hypothalamus-pituitary-gonad
HPRT	hypoxanthine guanine phosphoribosyl transferase
HSD, Hsd	Hydroxysteroid dehydrogenase; 3βHSD, 17βHSD
IARC	The International Agency for Research on Cancer (part of the WHO)
IFN	Interferons
Ig	Immunoglobulin
IL	Interleukin, a type of cytokine signaling molecule
Insl3	insulin-like 3 peptide hormone
IPCS	International Programme on Chemical Safety (WHO)
LABC	levator ani/bulbocavernosus muscle
LGLL	large granular lymphocytic leukemia (also called mononuclear cell leukemia or MNCL)
LH	luteinizing hormone
LOAEL	lowest observed adverse effect level
LOAEC	lowest observed adverse effect concentration
LOEL	lowest observed effect level

M	"molar" or "male", depending on the context
MBP	monobutyl phthalate (mono-n-butyl phthalate)
MBzP	monobenzyl phthalate
MCiNP	mono(2,7-dimethyl-7- carboxyheptyl)phthalate (also cx-MiDP; MCNP; mono(carboxyisononyl) phthalate)
MCiOP	mono(4-methyl-7-carboxyheptyl)phthalate (also cx-MiNP; 7cx-MmeHP; carboxy-MiNP; mono(carboxyisononyl) phthalate; MCOP)
MCF-7	human breast cancer cell line
MEHP	mono-2-ethylhexyl phthalate
MEP	monoethyl phthalate
MHiNP	mono(4-methyl-7-hydroxyoctyl)phthalate
MIBP	monoisobutyl phthalate
MiDP	Mono-isodecyl phthalate
MiNP	mono-isononyl phthalate (also MNP)
MiNP-G	monoisononyl phthalate glucuronide conjugate
MMP	monomethyl phthalate
MNCL	mononuclear cell leukemia (also called large granular lymphocytic leukemia or LGLL)
MNG	multinucleated gonocyte, multinucleated germ cell
MOiDP	monooxoisodecyl phthalate (also oxo-MiDP)
MOiNP	mono(4-methyl-7-oxooctyl)phthalate (also oxo-MiNP; 7oxo-MMeOP; mono(oxoisononyl) phthalate)
MOS	Margin of Safety
MPOA	hypothalamic medial preoptic area
mRNA	messenger RNA
N3	promoter of NIS
NIS	sodium/iodide symporter
NK cell	natural killer cell
NOAEL	no observed adverse effect level
NOAEC	no observed adverse effect concentration
NOEL	no observed effect level
NTP	US National Toxicology Program
NUE	enhancer for NIS
OECD	Organisation for Economic Co-operation and Development
p130	p130 is a tumor suppressor of the pocket protein family (other members of the "pocket protein family" are p107 and RB). They are involved in the coordinated regulation of cell cycle progression through modulation of the E2F family of transcription factors (Stengel 2009)
PBPK	physiologically based phramacokinetic
PC C13	rat thyroid cell line
PCR	polymerase chain reaction
phr	parts per hundred resin
PND	postnatal day
PNW	postnatal week
PPAR	peroxisome proliferator activated receptor
ppm	parts per million
PPS	balano-preputial separation, cleavage of the balano-preputial skinfold
PRL	prolactin
P450scc	cholesterol side-chain cleavage enzyme (encoded by *CYP11A1* gene)
PXR	pregnane X receptor
QSAR	quantitative structure-activity relationship
RAR	risk assessment report

RCR	risk characterisation ratio
RBA	relative binding affinity
rNIS	rat sodium/iodide symporter
Scarb1	Scavenger receptor class B member 1 (SR-B1) is encoded by the Scarb1 gene
SCCP	Scientific Committee on Consumer Products on phthalates in cosmetic products
SCENIHR	Scientific Committee on Emerging and Newly-Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SD	Sprague-Dawley
SDN-POA	sexually dimorphic nucleus of the preoptic area
SHBG	steroid (sex) hormone binding globulin
SR-B1	Scavenger receptor class B member 1 is encoded by the *SCARB1* gene
SRC-1	steroid receptor coactivator 1
StAR	Star
T	testosterone
T3	triiodothyronine (a thyroid hormone)
TCNES	Technical Committee for New and Existing Substances
TH	thyroid hormone
TNFα	tumor necrosis growth factor α
TSH	thyroid stimulating hormone
TSLP	thymic stromal lymphopoietin
US CPSC	United States Consumer Product Safety Commission
US EPA	US Environmental Protection Agency
VMH	ventromedial nucleus
VO	vaginal opening (landmark of puberty onset in female rats)
WHO	World Health Organization

Annexes

Annex 1 Opinion of the Committee for Risk Assessment (RAC) on ECHA's draft review report

8 March 2013

ECHA/RAC/A77-O-0000001412-86-10/F

Opinion of the Committee for Risk Assessment

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)"

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 an 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)".

	IUPAC NAME	EC NUMBER	CAS NUMBER
DINP	1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich	271-090-9	68515-48-0
	di-"isononyl" phthalate	249-079-5	28553-12-0
DIDP	1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich	271-091-4	68515-49-1
	di-"isodecyl" phthalate	247-977-1	26761-40-0

PROCESS FOR ADOPTION OF THE OPINION

Rapporteur, appointed by RAC: *Helmut GREIM*

The Executive Director of ECHA requested RAC on 25 April 2012 to provide an 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)".

ECHA's draft review report was made publicly available at http://www.echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report on 7 May 2012. Interested parties were invited to submit comments and contributions by 31 July 2012.

RAC was requested to assess ECHA's draft review report as well as the comments received on the report during public consultation and to adopt an opinion as soon as possible and not later than December 2012.

On 5 February 2013, the Executive Director of ECHA extended the deadline for adoption to 31 March 2013.

The RAC opinion was adopted by consensus on 8 March 2013. The opinion takes into account the comments of interested parties provided during public consultation.

TERMS OF REFERENCE

RAC is requested, pursuant to Article 77(3)(c) of REACH, to:

Adopt an opinion on ECHA's draft report "Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to Regulation (EC) No 1907/2006 (REACH)". Comments from the public consultation should be taken into account by RAC.

A) RAC should assess in its opinion the overall scientific quality of the report, its completeness, potential weaknesses, as well as the scientific validity of the conclusions drawn. If RAC disagrees with the conclusions, it is invited to elaborate on its reasons.

B) The opinion should in particular respond, based on the available evidence presented in the draft review report, to the following questions:

1) Is the selection of no observed adverse effect levels (NOAELs) and assessment factors (AF) to derive the derived no effect levels (DNELs) appropriate and sufficiently justified?

2) Does RAC support the assumptions and conclusions of the exposure assessment?

3) Does RAC agree to the conclusions of the draft review report that exposure to DINP and DIDP from mouthing of toys and childcare articles would present a risk, if the existing restriction was lifted?

4) Does RAC agree to the conclusions of the draft review report regarding consumer risk from the presence of DINP and DIDP in articles other than toys and childcare articles?

1) Does RAC agree to the conclusions of the draft review report regarding the risk from combined exposure[71] to DINP and DIDP?

[71] 'Combined exposure' includes all routes, pathways, and sources of exposure to multiple chemicals (as defined in the joint opinion of SCHER, SCENIHR and SCCS "Toxicity and Assessment of Chemical Mixtures" from 2011).

OPINION

Based on the evaluation of the information presented in the draft review report, and taking into account information from the public consultation, RAC responds as follows to the questions in the Terms of Reference. RAC refers to the supporting document to the opinion for more details and a better understanding to the opinion and its justifications.

A) RAC should assess in its opinion the overall scientific quality of the report, its completeness, potential weaknesses, as well as the scientific validity of the conclusions drawn. If RAC disagrees with the conclusions, it is invited to elaborate on its reasons.

RAC concludes that the overall scientific quality of the report is good, and the report is considered to be complete in that it addresses and discusses all necessary information to evaluate whether the existing restriction on DINP and DIDP in toys and childcare articles, which can be placed in the mouth by children is justified.

B) The opinion should in particular respond, based on the available evidence presented in the draft review report, to the following questions:

1) Is the selection of no observed adverse effect levels (NOAELs) and assessment factors (AF) to derive the derived no effect levels (DNELs) appropriate and sufficiently justified?

Modification of the dose descriptor for DINP and DIDP

RAC considers that adult rats orally absorb about 50-70% and humans 100%, and that therefore a modification of the dose descriptor with a factor of two can be justified.

RAC noted however, that the estimated absorption rate of 50% in adult rats might underestimate the actual absorption at low dose levels (see sections 1.1 and 1.5 of the supporting document). For that reason RCRs have been also calculated without the modification of the dose descriptor.

DINP

RAC agrees with the selected NOAELs and assessment factors applied to derive the DNELs for reproductive toxicity for DINP in the ECHA draft report.

With regard to repeated dose toxicity, RAC discussed two key studies for DNEL derivation, the Aristech (1994) and Exxon (1986) studies with NOAELs of 88 and 15 mg/kg/d respectively. Considering the dose spacing in those studies, in particular the Exxon study with 152 mg/kg as the next higher dose, the true NAEL (No Adverse Effect Level) could be argued to be somewhere between 88 and 152 mg/kg/day. However, there were differences in methodology between both studies: the Exxon (1986) study evaluated 4-5 liver sections, whereas the Aristech (1994) study examined 1-2 sections. It was argued that as a result of this methodological difference, the Exxon (1986) study was the most appropriate to use. RAC supported the NOAEL for DINP of 15 mg/kg as proposed by ECHA noting that the NAEL could be higher given the large dose spacing in this study.

Overall, RAC agrees with the selected NOAELs and assessment factors applied to derive the DNELs for repeat dose toxicity for DINP in the ECHA draft report.

DIDP

RAC agrees with the selected NOAELs and assessment factors applied to derive the DNELs for reproductive toxicity for DIDP in the ECHA draft report.

However, RAC questioned the LOAEL proposed in the ECHA draft report for DIDP repeated dose toxicity. As described in section 1.2.2 of the supporting document to the opinion, it can be questioned whether the LOAEL in the Cho et al. (2008/2010) study is dose related. Furthermore, RAC is aware that the relevance of spongiosis hepatis for humans has been questioned. Thus, RAC does not recommend to exclusively use the Cho et al. study to

identify the repeated dose NOAEL for DIDP. Instead, RAC proposes to use the NOAELs from the 90 days studies in dogs (Hazleton 1968b, NOAEL 15 mg/kg) and rats (BASF 1969, NOAEL 60 mg/kg) in addition, as described further in section 1.1 of the supporting document.

Based on these three studies and applying appropriate inter- and intra-species assessment factors, and extrapolation from subchronic to chronic exposure, RAC noted that the resulting DNEL for DIDP would be similar to the DNEL for DINP.

2) Does RAC support the assumptions and conclusions of the exposure assessment?

RAC generally supports the assumptions and conclusions of the exposure assessment for adults and for children.

It is noted that the exposure assessment for children is driven by exposure to DINP and DIDP from mouthing of articles, which heavily depends on the migration rates of phthalates from the mouthed article and the mouthing time per day. RAC notes that there is a high uncertainty of the migration rates and mouthing times. RAC took note of the error in the reported value from Greene (2002) in ECHA's draft review report, but concluded it did not have significant consequences on the mouthing time assumptions for a reasonable worst case exposure estimate. RAC supports to use a mouthing time of 2 hour per day for children until 18 months of age as a reasonable worst case.

RAC supports to lower the exposure estimates for sex toys containing DINP or DIDP (see section 2.2 of the supporting document).

3) Does RAC agree to the conclusions of the draft review report that exposure to DINP and DIDP from mouthing of toys and childcare articles would present a risk, if the existing restriction were lifted?

The reasonable worst case exposure estimates from toys and childcare articles alone, would result in RCRs exceeding 1 for all age groups for both DINP and DIDP (RCRs of 2.0 for 0-6 months, 1.6 for 6-12 months and 1.3 for 12-18 months respectively) based on DNELs of 0.075 mg/kg for both DINP and DIDP, which includes a modification of the dose descriptor of a factor 2. Combined exposure of the two phthalates based on reasonable worst case exposure estimates for toys, dermal contact, air/dust and food, results in a maximum RCR of 2.4 for 6-12 month old children (RCRs of 2.2 for 0-6 months, 2.4 for 6-12 months and 2.0 for 12-18 months respectively).

As an uncertainty assessment, taking into account that the RCRs based on combined exposure[71] of the two phthalates used the reasonable worst case exposure scenarios, RAC also calculated RCRs without use of the dose descriptor modification factor of 2 (thus using DNELs of 0.15 mg/kg). The resulting RCRs for combined exposure of the two phthalates are still slightly above 1 (RCRs of 1.15 for 0-6 months; 1.23 for 6-12 months and 1.04 for 12-18 months respectively). If in addition, in the case of DINP, a higher NOAEL were to be used, then the RCRs for children of all ages would be below 1 (see discussion on NOAEL selection in section 1.2.1 of the supporting document).

Overall, RAC concludes that a risk from mouthing of toys and childcare articles with DINP and DIDP cannot be excluded if the restriction were lifted.

4) Does RAC agree to the conclusions of the draft review report regarding consumer risk from the presence of DINP and DIDP in articles other than toys and childcare articles?

RAC agrees with the conclusion that the major exposure of adults to DINP and DIDP results from the use of sex toys (RCRs for the reasonable worst case of 0.4 for both phthalate esters). RAC noted that there are substantial uncertainties to exposure duration and migration rates of the phthalate esters from sex toys. A risk from use of sex toys can be considered unlikely. Dermal exposure for example from PVC garments is not anticipated to

result in a risk for the adult population and the developing foetus in pregnant women. Exposure from food and the indoor environment is not considered to constitute a risk for adults or children.

5) Does RAC agree to the conclusions of the draft review report regarding the risk from combined exposure[72] to DINP and DIDP?

RAC supports the concept to apply dose/concentration addition for assessing the risk from combined exposure to DINP and DIDP. However, as stated in the draft review report, for the purposes of the assessment of exposure to articles from mouthing or dermal contact (direct exposure to articles), it was assumed that the articles either contain DINP or DIDP. Therefore, dose addition does not apply to direct exposure to articles. It however applies to exposure via food and the indoor environment.

o0o

Basis for the opinion
The supportive document gives the detailed grounds for the opinion.

[72] 'Combined exposure' includes all routes, pathways, and sources of exposure to multiple chemicals (as defined in the joint opinion of SCHER, SCENIHR and SCCS "Toxicity and Assessment of Chemical Mixtures" from 2011).

ANNEX

SUPPORTING DOCUMENT TO THE OPINION

This supporting document shall be regarded as further reference material to the opinion of the Committee for Risk Assessment. It contains further details and assessment and may be used to better understand the opinion and its justifications.

Table of Contents

1. Human health hazard assessment (question 1)

RAC agrees with the conclusions drawn in the ECHA draft review report regarding all human health endpoints, with the exception of the repeated dose toxicity endpoint and the oral absorption part. These aspects are commented on below. In addition, RAC commented on the DNEL derivation by ECHA.

1.1 Toxicokinetics: oral absorption

1.1.1 DINP

Animal studies with DINP

Hazleton (1972) administered about 2500 mg/kg/day over 6 days to albino rats (4 treated, 2 controls). The amount excreted radioactivity in urine ranged from 8-18%. Considering the high dose, the absorption process was probably saturated (EC 2003a).

Midwest Research Institute (1983), also cited as McKee et al (2002), treated Fischer 344 rats with a single radioactive dose of 50 and of 500 mg/kg, with recoveries in urine of 49% and 43% respectively (after normalizing to 100% total recovery, which was 99 and 91% at 50 and 500 mg/kg, respectively). In a repeated dose study over 5 days with 50, 150 and 500 mg/kg, recoveries in urine were 52, 60 and 55 % respectively (after normalizing to 100% total recovery, which was 123, 117 and 115% at 50, 150 and 500 mg/kg, respectively).

Human volunteer studies with DINP

Koch and Angerer (2007) described elimination of major DINP metabolites via urine in a study where one human volunteer was dosed 1.27 mg/kg (n = 1). A recovery of 43.6% of the custom synthesised DINP-2 was calculated in urine measurements during 48h of four metabolites. Four metabolite 'groups' of structural isomers were measured. Other possible metabolites (with two or more functional groups or shortened side chains) were not measured. The recovered percentage is thus likely an underestimation of the actual elimination of DINP via urine (Koch and Angerer 2007).

Anderson et al. (2011) studied the kinetics of DINP and DEHP in 10 male and 10 female human volunteers (n = 20). Two dose levels were used of the deuterium labelled DINP and DEHP, which were for DINP 0.78 mg (0.010 mg/kg for males and 0.011 mg/kg for females) and 7.3 mg (0.090 mg/kg for males and 0.107 mg/kg for females). A recovery of 32.9 ± 6.4% of the labelled DINP was calculated in urine measurements during 48 h of four metabolites (the same metabolites as in Koch and Angerer).

1.1.2 DIDP

Animal studies with DIDP

From a gavage study with radiolabeled DIDP in Sprague Dawley rats (General Motors Research Laboratories 1983), the total absorbed dose was roughly estimated to be 55.6% after 0.1 mg/kg, 45.9% after 11.2 mg/kg and 17.3% after 1000 mg/kg. This seems to indicate absorption is saturable. The recovered radioactivity from urine and feces was >99%.

Human volunteer studies with DIDP

Not available.

1.1.3 DEHP

Animal studies with DEHP

Numerous studies have been performed to study the toxicokinetics of DEHP in different rat strains, and also in non-human primates, mice, hamster, guinea pigs, dogs, miniature pigs. Based on amongst others about 16 kinetic studies with DEHP in rats, RAC concluded in its opinion of 15 June 2012 on the Annex XV dossier proposing restrictions on four phthalates that the absorption of DEHP in rats can be estimated to be 70%.

In a first experiment studying kinetics, Sjöberg et al (1985) administered 1000 mg DEHP/kg to 25, 40 and 60 days old rats by gavage (9-10 animals per group). The mean AUC of MEHP of 25 day old rats (1213 µg h/ml) was significantly higher than that of the 40 and 60 day old rats (611 and 555 µg h/ml respectively). In a second experiment studying excretion, groups of 25 and 60 day old rats (6 animals per group) were administered 1000 mg ^{14}C-DEHP/kg by gavage. The cumulative excretion of radioactivity was 44% in 25 day old rats and 26% in 60 day old rats. The authors concluded that the observations suggest that the absorption, and therefore exposure, to MEHP and its metabolites was higher in young than in more mature rats.

In Study I of Kurata et al (2012), groups of 3 and 18 months old marmosets received 100 and 2500 mg/kg ^{14}C-DEHP by gavage (3 animals per group). At the low dose, the cumulative urinary excretion 7 days after dosing was higher in the younger (about 18%) than in the elder animals (13%). At the high dose the younger excreted about 10%, the elder 22% radioactivity in urine. Within one day after the low dose there was no difference between the two age groups (about 10% excretion), whereas at the high dose the excretion in the younger animals was less than in the elder ones (5 versus 15%). Two hours after dosing radioactivity in blood and bile was more than twofold higher in the younger animals at the low dose and about 40% lower at the high dose. Thus, within one day after the low dose, the younger animals absorb more than the elder ones. At the high dose younger animals show lower radioactivity in urine, bile and blood than the elder ones (about 40% less).

Based on the results from Study II of Kurata et al (2012), in which 4 week old rats and 3 months old marmosets received 100 mg/kg ^{14}C-DEHP by gavage, there might be large species differences in absorption between rat and marmosets. In rats at 1 day post-dose radioactivity excreted in urine accounted for 58% of the dose, whereas in marmosets this was only 8%.

Human volunteer studies with DEHP

Schmid and Schlatter (1985) studied excretion of DEHP taken orally by 2 volunteers (30 mg or about 0.4 mg/kg) and determined an excretion of 11 and 15% of the dose in urine by measuring 12 DEHP metabolites. DEHP taken by the same volunteers over a period of 4 days at a dose of 10 mg/day (about 0.13 mg/kg/day) resulted in 15 and 25% recovery in urine. The amount recovered for 5 of the 12 metabolites was less than 1%.

Koch et al (2005) measured 5 metabolites in one human volunteer after doses of 4.7, 28.7 and 650 µg/kg, with recoveries in urine of 66, 65 and 71% respectively (mean of 67%). This is indicative that at these low exposure levels there is no saturation of absorption.

Anderson et al (2011) studied 10 male and 10 female human volunteers (n = 20) given deuterium labeled DEHP (and DINP, see above) at dose levels of 0.31 mg (0.004 mg/kg for males and 0.005 mg/kg for females) and 2.8 mg (0.034 mg/kg for males and 0.041 mg/kg for females). The recovery in urine was 47% based on measurement of 4 metabolites. Using the same 4 metabolites from the Koch et al (2005) results, this would in comparison have given 65%. Anderson et al (2011) noted that the higher results seen in the Koch study can be explained because it is based on a single individual (with results still within the observed standard deviation). The authors also noted that the consequence of the difference is that

when calculating exposure from biomonitoring data the conversion factors and therefore the exposure will be slightly higher based on their results.

Kessler et al (2012) studied 4 male volunteers given 618-665 µg/kg labelled DEHP and found 31% of the dose excreted in urine based on measurement of 3 metabolites. The authors concluded that the results are in line with those from Anderson et al for the 3 metabolites (29.1 and 33.2%). The results from Koch et al gave 44.2% excretion in the urine of the 3 metabolites (Kessler et al 2012). The authors made the same remark as Anderson et al (2011) regarding the consequences to the estimation of exposure from biomonitoring results in urine.

1.1.4 Conclusion

Animal studies indicate that absorption of DINP and DIDP are saturable at high dose levels. Studies with DINP and DIDP indicate absorption rates of around 50%. A study with DINP indicates absorption of roughly 40-55% at dose levels as high as 500 mg/kg/day. As biliary excretion occurs, an unknown percentage of the radioactivity excreted in feces is to be added to the radioactivity excreted in urine to estimate the absorption. The absorption of DINP and DIDP can therefore be assumed to be in the range of 50-70% in the rat.

Human volunteer studies with DEHP clearly demonstrate that the amount 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. However, it is acknowledged that the studies in humans have not been designed to determine absorption.

RAC concludes that adult rats can be assumed to absorb 50-70%, whereas humans absorb 100% based on read-across from DEHP.

1.2 Repeated dose toxicity

1.2.1 DINP

In the RAR on DINP (EC 2003a) a number of repeated dose toxicity studies using rats, mice, rabbits, primates and dogs have been evaluated. The RAR concluded that "*...for effects on the liver and kidneys, a NOAEL of 88 mg/kg/day is determined in rats regarding results found in a chronic/carcinogenic study (Aristech, 1994)*". This NOAEL was taken because liver pathology unrelated to peroxisome proliferation was seen in this study.

In the Exxon study (Lington et al. 1997) using Fischer 344 rats, there was a dose-related increase in relative organ weights of liver and kidney in both males and females with a clear NOAEL of 15 (males) – 18 (females) mg/kg/day. In addition to the increased liver and kidney weights at the LOAEL of 152 (females) - 184 (males) mg/kg/day, males had increased incidences of spongiosis hepatis and serum levels of alkaline phosphatase and transaminases. Spongiosis hepatis was also seen in males in the Aristech study. In these studies the NOAEL/LOAEL for spongiosis hepatis are the same as for the increases in liver and kidney weights.

If both the Exxon and the Aristech studies would have been conducted under exactly the same conditions the dose response could have been expected to be the same in both studies. Considering the dose spacing in those studies, in particular the Exxon study with 152 mg/kg as the next higher dose, the true NAEL (No Adverse Effect Level) could be argued to be somewhere between 88 and 152 mg/kg/day. However, there were differences in methodology between both studies: the Exxon (1986) study evaluated 4-5 liver sections, whereas the Aristech (1994) study examined 1-2 sections. Comparison of Aristech (1994) data scaled to 4 slides, and another comparison with Exxon (1986) data scaled to one slide showed no statistical significant difference between the scaled data sets. This indicates that it is likely that

the dose response from both studies would have been the same if the studies would've examined the same amount of liver sections.

It was argued that as a result of this methodological difference, the Exxon (1986) study was the most appropriate to use. RAC supported the NOAEL for DINP of 15 mg/kg as proposed by ECHA noting that the NAEL could be higher given the large dose spacing in this study.

1.2.2 DIDP

The NOAEL for DIDP has been discussed in the RAR (EC 2003b) by EFSA (2005), SCCP (2007), SCHER (2008), and the US CPSC (2010). The studies used for NOAEL setting in the EU RAR were subchronic studies. Since the peroxisome proliferation effects in the liver of rodents are generally seen as species-specific, dog was considered to be a more relevant species for human risk assessment. The dog study by Hazleton (1968b) resulted in a NOAEL of 15 mg/kg/day. However, because of the limitations of the dog study, a NOAEL of 60 mg/kg/day from a 90-day rat dietary test was considered in addition (BASF 1969). The EU RAR carried out risk characterisation for both NOAELs.

According to the EU RAR (EC 2003b), the NOAEL in another 90 day rat study by Hazleton (1968a) was 0.3% (approx. 200 mg/kg/day) and the LOAEL 1% (approx. 650 mg/kg/day). As the NOAEL of 200 mg/kg/day in the Hazleton (1968a) study is higher than the LOAEL in the BASF (1969) study (120 mg/kg/day), it is the BASF study that determines the overall NOAEL for a study of that duration in the rat. Therefore, RAC considered it not appropriate to consider the Hazleton study for DNEL calculation. This is consistent with the approach in the EU RAR for DIDP. It could be noted that furthermore the Hazleton study used 10 animals per dose group versus 20 in the BASF study, and 3 dose levels versus 4 dose levels respectively. Industry argued that the 90 day rat study (Hazleton 1968a) should be used in addition to determine DNELs for DIDP as it was conducted with the substance which is produced commercially within the EU today (CAS number 68515-49-1). RAC did not consider this argument to be convincing, noting that read-across between the two forms of DINP and between the two forms of DIDP is general practise both by industry and by regulatory authorities, and furthermore, imported articles might contain either form of DIDP.

A new study by Cho et al (2008, 2010) reported a 2 years dietary study in male and female F344 rats at daily doses of about 22, 120 and about 500 mg/kg/day. Significant toxicity was observed at the highest dose level and similar to the previous studies, spongiosis hepatis occurred in male rats. Since the effect was seen at the lowest dose, no NOAEL could be derived by ECHA. Spongiosis hepatis occurred in 3/48 (6.3%), 3/49 (6.1%), and 5/39 (12.8%) male rats at the low, middle and highest doses, respectively but not in the controls. The ECHA report concluded that the negative findings in the controls do not contradict the experience from other studies using F-344 rats, where this lesion occurred between 0 and 34% of the male controls (Karbe and Kerlin 2002). Thus, ECHA proposed a LOAEL of 22 mg/kg/day to be derived from this chronic study. RAC questioned the reliability of the Cho et al. 2008 study to derive a LOAEL of 22 mg/kg/day DIDP as a starting point to set the DNEL (see also section 1.3).

The reliability of the Cho et al 2008 study has been extensively discussed. It can be argued that the incidences of the Cho et al study are all within the range of available controls from NTP studies, and that therefore the incidences should not be interpreted as a response to the administered dose. Alternatively, it can be argued that historical control data from NTP studies has limited relevance for evaluating the 0% incidence of spongiosis hepatis in the control of the Korean study by Cho et al: the uniformly low incidences seen in the study might be a consequence of the different breeder, possible differences in diagnosis, possible differences in amount of liver sections taken, etc. Thus, it could be argued that the statistical significance of the study is a relevant finding that is related to the dose. Moreover, a zero control incidence might not be a deviating finding, as the range of historical controls was reported to be 0-34% from 12 NTP studies, of which 1 showed a zero control incidence only. Moreover, in the 2 years' studies on DINP the incidences of spongiosis hepatis in the untreated Fischer rats were

24/81 animals (Exxon 1986) and 5/80 (Aristech 1994). Concerning the relevance of spongiosis hepatis for humans see section 1.3.

RAC recommended to use all three studies, i.e. the 90 day study in dogs (Hazleton 1968b), the 90 day and 2 year studies in rats (BASF 1969 and Cho et al 2008, 2010), in deriving the DNEL for DIDP.

1.3 Evaluation of human relevance of spongiosis hepatis

In the 2 year rodent carcinogenicity studies on DIDP (Cho et al 2008, 2010) and DINP (Exxon 1986 and Aristech 1994) histopathological changes in the liver included spongiosis hepatis at low but statistically significant incidences in all male treatment groups. The ECHA report discusses the relevance of these lesions in detail. The lesion occurs spontaneously in aging male rats and can be enhanced by genotoxic and non-genotoxic hepato-carcinogens but has not been described in dogs and non-human primates. According to the literature there is a controversial discussion whether spongiosis hepatis can be considered a proliferative change or may be regarded as a preneoplastic or even a benign neoplastic lesion.

ECHA concluded that the mechanisms of spongiosis hepatis are not known, but that they seem unrelated to peroxisome proliferation.

On behalf of the European Council for Plasticisers and Intermediates (ECPI) the significance of spongiosis hepatis for humans has been evaluated by Berry (2012), who concluded the following:

*"In my experience, there is no comparable human lesion, a view shared by expert human pathologists in this field. In Professor Sir Roderick MacSween's book the authors state "**to the best of our knowledge no human counterpart of spongiosis hepatis has ever been described**". The authors use the term **spongiocytic pericytoma** but are considering the lesion we discuss here and Bannasch is a contributor to the volume. Further, there was no evidence of a lesion resembling spongiosis hepatis in a review of 163 human livers conducted by members of the Bannasch laboratory (Su et al., 1997) nor in my autopsy study of 1500 livers at autopsy.*

The broad consensus of pathologists appears to support the view that spongiosis hepatis is a degenerative change. From NTP studies, spongiosis hepatis is a lesion that appears to be confined to rats, particularly male rats, and teleost fish."

The expert also questioned the reliability of the Cho et al. 2008 study:

"The authors make little note of the finding other than to note its presence and the overall conclusion made in the paper is that "The increases in the relative weights of the liver and kidney were not accompanied by any histopathologic lesions in those organs." There is little information presented in the paper to fully evaluate the lesion (i.e., correct diagnosis, information on severity, number of sections reviewed). As such, it would be difficult to utilize this endpoint as a point of departure in hazard identification and risk assessment. This is particularly the case, when as noted above, the changes observed have no relevance for human pathology."

He concluded that it would be difficult to utilize the endpoint spongiosis hepatis as a point of departure in hazard identification and risk assessment.

There are two publications reporting features resembling spongiosis hepatis in relation with hepatic adenomas that appeared in users of oral contraceptives (Nime et al. 1979 and Kaiserling and Müller 2005). Berry (2012) questioned the relation of these findings with spongiosis hepatis in rats however.

Considering the above, RAC noted that the relevance of spongiosis hepatis for humans has been questioned by some, while others have indicated that treatment-related lesions similar to spongiosis hepatic are described in human pathology (sinusoidal dilations or sinusoidal ectasia), but that the terminology differs.

1.4 Derivation of DNELs by ECHA

The DNELs for the different routes of exposures for adults, children (repeated dose effects and reproduction) and for foetal development in pregnant women as derived by ECHA are given in **Table 126** and **Table 127**.

Table 126 DNELs (mg/kg/day) for DINP proposed by ECHA

Route	Repeated dose toxicity		Reproductive toxicity children	Foetal development in Pregnant Women
	Adults	Children		
Oral (mg/kg)	0.15	0.075*	0.25*	0.5
Inhalation (mg/m3)	0.35	0.26	0.87	1.16
Dermal (mg/kg)	1.88	1.88	6.25	6.25

*includes a dose descriptor modification with a factor of 2 for absorption

Table 127 DNELs (mg/kg/day) for DIDP proposed by ECHA

Route	Repeated dose toxicity		Reproductive toxicity children	Foetal development in Pregnant Women
	Adults	Children		
Oral (mg/kg)	0.073	0.037*	0.26*	0.17
Inhalation (mg/m3)	0.17	0.13	0.904	0.38
Dermal (mg/kg)	0.92	0.92	6.50	2.06

*includes a dose descriptor modification with a factor of 2 for absorption

1.5 Comments from RAC on DNEL derivation by ECHA

1.5.1 Absorption

Based on a study from Sjoberg et al. (1985) which seemed to show a greater absorption of DEHP by the oral route in young rats compared to older ones (see section 1.1), the ECHA draft report differentiated between adults and children, assuming that the absorption rates in children are higher (100%) than in adults (50%). As a consequence, a lower DNEL for children was derived. This endpoint modification step is in line with the EU Risk Assessments for DINP, DIDP and DEHP. The RAC opinion on the Danish restriction proposal on four phthalates of 15 June 2012 also assumed 100% absorption in children.

RAC considers there is no indication that adults absorb less phthalate esters than children. The assumption of higher absorption by children in the draft ECHA review is based on DEHP data from adults (Koch et al 2005, Anderson et al 2011, Kessler et al 2012), from which a 50% absorption in adults has been estimated. However, these studies indicate a rather high absorption rate in adults taking into account that the amount recovered in the urine depends on the number of urinary metabolite measured, and the unknown amount of excretion via bile.

Since adults absorb almost 100% (see section 1.1) there is no need to assume an even higher absorption in children so that an additional factor to take into account differences in absorption between adults and children is not necessary. However, an endpoint modification is necessary considering the species differences in absorption: adult rats absorb about 50% whereas humans around 100%. As indicated in the ECHA guidance R.8, Appendix R.8.2-2, an endpoint modification is needed in that case. Indeed, the default situation, in the absence of information, is to assume the same bioavailability for experimental animals and humans for a particular exposure route. However, when available information indicates that at the relevant level of exposure humans absorb less (or more) than experimental animals, the dose descriptor needs to be corrected for this difference in bioavailability.[73]

RAC notes that Industry, by referring to the following text of the ECHA guidance R.8.4.3.1, page 24, questioned the justification for this endpoint modification: *"If no substance-specific data are available, the standard procedure for threshold effects would be, as a default, to correct for differences in metabolic rate (allometric scaling) and to apply an additional factor of 2.5 for other interspecies differences, i.e. toxicokinetic differences not related to metabolic rate (small part) and toxicodynamic differences (larger part). In case substance-specific information shows specific susceptibility differences between species, which are not related to differences in basal metabolic rate, the additional factor of 2.5 for 'remaining differences' should be modified accordingly".* Accordingly, Industry concluded that for DINP and DIDP substance specific data are available, which was said to support that there is no need for the default factor of 2.5, giving an interspecies assessment factor of 4 (which together with an intraspecies factor of 10 gives an overall assessment factor of 40).

RAC notes however that "toxicokinetic differences not related to metabolic rate (small part)" in the above citation does not refer to absorption but to the other aspects of toxicokinetics, i.e. distribution, metabolism and elimination. Indeed, the guidance clearly specifies that the dose descriptor needs to be corrected separately in the step prior to applying assessment factors in case there are differences in bioavailability between experimental animals and humans.

In summary, RAC considers that a modification of the dose descriptor with a factor of 2 is justified. RAC notes however, that the estimated absorption rate of 50% in adult rats might underestimate the actual absorption at low dose levels, in particular given the contribution of biliary excretion, and that therefore the modification of the dose descriptor with a factor of 2 might be considered to be conservative.

With regards to the assumption for inhalation, RAC agrees with the assumption in the ECHA draft report of 75% absorption in adults and 100% absorption in children. The assumption of 100% absorption in children could be considered conservative.

1.5.2 DINP

RAC supports most DNELs derived by ECHA for DINP for repeated dose toxicity and reproductive toxicity, as shown in **Table 128**. For the oral route, however, RAC considers that

[73] ECHA guidance R.8, R.8.4.2, point b) 'Modify, when necessary, the relevant dose descriptor(s) per endpoint to the correct starting point' clarifies as follows: *"In a few situations, the effects assessment is not directly comparable to the exposure assessment in terms of exposure route, units and/or dimensions. In these situations, it is necessary to convert the dose descriptor for the threshold effect (e.g. N(L)OAEL, benchmark dose, LD/LC50) into a correct starting point (i.e., correct the unit of exposure, e.g. corrected N(L)OAEL). This applies to the following situations:*
1. If for a given human exposure route there is a dose descriptor for the same route in experimental animals but for that particular exposure route there is a difference in bioavailability between experimental animals and humans at the relevant level of exposure."
This is exemplified also in Appendix R.8.2-2, point B, a modification of starting point is necessary amongst others "*If for a given human exposure route there is an effect parameter for the same route (in experimental animals or humans) but for that particular exposure route there is a difference in absorption between experimental animals and humans at the relevant level of exposure."*

the DNELs for adults and children should be the same. RAC further noted in section 1.2 that the NOAEL 15 mg/kg/day could be considered to be somewhat conservative.

Table 128 DNELs (mg/kg/day) for DINP supported by RAC

	Repeated dose toxicity		Reproductive toxicity	
Route	Adults	Children	Adults (pregnant women)	children
Oral (mg/kg)	0.075*	0.075*	0.25*	0.25*
Inhalation (mg/m3)	0.35	0.26	1.16	0.87
Dermal (mg/kg)	1.88	1.88	6.25	6.25

*includes a dose descriptor modification with a factor of 2 for absorption

1.5.3 DIDP

RAC supports the DNELs derived by ECHA for DIDP for reproductive toxicity, with the exception of the oral DNEL for pregnant women where RAC would apply a modification of the dose descriptor with a factor of 2 (see **Table 129**).

For the derivation of repeated dose DNELs for DIDP, RAC recommended (see section 1.2) the use of the NOAELs of the 90 days studies in rats (BASF 1969) and dogs (Hazleton 1968b) and the LOAEL of the 2 year study in rats (Cho et al 2008, 2010) by applying the appropriate interspecies scaling, the subchronic to chronic extrapolation and the intraspecies scaling. The oral DNEL without the modification of the dose descriptor can then be derived as follows:

- Dog 90 d study (Hazleton 1968b), NOAEL 15 mg/kg/day
  - Interspecies factor:
    a. AS (correction for differences in metabolic rate): 1,4
    b. remaining differences: 2,5
  Intraspecies factor general population: 10
  Exposure duration 90 day - chronic: 6[74]
  Issues related to dose-response: 1
  Quality of whole database: 1
  Total factor: 210
  DNEL: 0.07 mg/kg/day

- Rat 90 d study (BASF 1969), NOAEL 60 mg/kg/day
  Interspecies factor:
    c. AS (correction for differences in metabolic rate): 4
    d. remaining differences: 2,5
  Intraspecies factor general population: 10
  Exposure duration 90 day - chronic: 2
  Issues related to dose-response: 1
  Quality of whole database: 1
  Total factor: 200
  DNEL: 0.3 mg/kg/day

- Rat 2y study (Cho et al 2008, 2010), LOAEL 22 mg/kg/day

[74] The default assessment factor for sub-chronic to chronic extrapolation is 2 for a rat 90 day study. The lifespan of a Beagle dog is around 13 year; thus, a study duration of 90 days covers roughly 2% of its lifespan. As a comparison between dog and rat, a 28 day study (subacute) covers 4% of the lifespan and a 90 day study (sub-chronic) covers 12% of a rat's life. Thus, a 90 day dog study covers about half of the length of a subacute study in rats. This justifies a default assessment factor of 6 for subacute to chronic extrapolation for the 90 day dog study (see Table R. 8-5 in ECHA guidance R.8).

Interspecies factor:
e. AS (correction for differences in metabolic rate): 4
f. remaining differences: 2,5
Intraspecies factor general population: 10
Exposure duration 90 day - chronic: 1
Issues related to dose-response: 3
Quality of whole database: 1
Total factor: 300
DNEL: 0.07 mg/kg/day

The average of the 3 DNELs is 0.15 mg/kg/day (unmodified). Following the modification of the dose descriptor with a factor of 2, the DNEL is 0.075 mg/kg/day.

The DNELs for DIDP for repeated dose toxicity and reproductive toxicity supported by RAC are shown in **Table 129**.

Table 129 DNELs (mg/kg/day) for DIDP supported by RAC

	Repeated dose toxicity		Reproductive toxicity	
Route	Adults	Children	Adults (pregnant women)	children
Oral (mg/kg)	0.075*	0.075*	0.08*	0.26*
Inhalation (mg/m3)	0.35	0.26	0.38	0.90
Dermal (mg/kg)	1.88	1.88	2.06	6.50

*includes a dose descriptor modification with a factor of 2 for absorption

2. Exposure assessment (question 2)

In assessing exposure, the ECHA draft review report evaluated and documented a large number of studies and reports. RAC generally supported the exposure assessment in the ECHA draft review report, but provided comments on the exposure from toys and childcare articles, and on the exposure of adults from the use of sex toys (see below). In addition, RAC made a brief comment regarding the biomonitoring data for children.

2.1 Exposure from toys and childcare articles

Exposure is determined by the concentration of the leachable compound in the article, the migration rate and the duration of oral contact, which in the case of small children is mouthing time. The ECHA report presents a thorough evaluation of the available information. RAC noted the following regarding specifically the mouthing time assumption.

Mouthing time

The ECHA draft report used a study by Greene (2002) and Juberg et al. (2001) to derive a reasonable worst case mouthing time of 126 min/day.

During the opinion forming process, Industry commented that the ECHA report contained an error in the mouthing time it used from Greene (2002). In the room document RAC/23/2012/08, ECHA acknowledged that the draft report contained mistakes: ECHA had used a peer reviewed publication from Babich et al. (2004) which contained errors in the reported data from Greene (2002).

The correct 95th percentile mouthing time for the category "All soft plastic items except pacifiers" was not 127 minutes/day as reported by ECHA but 17.5 min/day.

In the room document ECHA assessed the corrected value in the context of all the available evidence, to derive a reasonable worst case estimate for mouthing of articles containing DINP or DIDP. Using a weight of evidence approach, ECHA referred to the following mouthing time results from the key studies:

- Greene (2002) reported 95th percentiles of 18 min/day for soft plastic items and 134 min/day for non-pacifiers for children of 3-11 months;
- Juberg et al. (2002) reported mean mouthing times for non-pacifiers of 70 min/day for 0-18 months old (without zeros, i.e. only taking into account children that mouthed, see Table 2 of Juberg et al.);
- Smith and Norris (2002) reported mean values for mouthing articles (excluding pacifiers and fingers) of 63 min/day for 6-9 month olds and 75 min/day for 5 year old children.

ECHA also pointed to a very large discrepancy between the maximum value of nearly 4 hours/day (227 min/day) for mouthing of toys by a child aged 6-9 months in Smith and Norris (2002) (with a mean of 39 min/day) and the highest 95th percentile of 18 min/day for 24-36 months old for mouthing of soft plastic items as calculated by a bootstrap procedure in Greene (2002).

Based on the above estimates, and considering the limitations and discrepancies in the data, as well as the skewness and difficulties to determine appropriate article categories, ECHA considered that a mouthing time of 2 hour is appropriate for a reasonable worst case scenario for mouthing of articles containing DINP or DIDP by children up to 18 months old. Further details are available from the room document.

RAC took note of the error in the reported value from Greene (2002) and of the assessment by ECHA in the room document RAC/23/2012/08. RAC is of the opinion that the assumption of 2 hour mouthing time per day is appropriate for a reasonable worst case scenario for mouthing of articles containing DINP or DIDP by children. RAC notes that a mouthing time of 3 hours/day was assumed in the EU RARs from 2003 and that 3 hours mouthing time is also the recommended value for risk assessment according to the ECHA guidance.

RAC notes that according to RIVM 1998 and CHAP (2001) pacifiers are rarely made of soft PVC. They are typically made of latex or silicone. According to Tonning et al 2009 PVC may be used in the mouth shield and the handle only (see p. 197 of the ECHA report). RAC agrees with ECHA's conclusion to exclude pacifiers for exposure assessment.

RAC notes that the (small) change in mouthing time from 126 min/day to 2 h/day results in slightly lower reasonable worst case exposure estimates (see **Table 130**) than presented in the ECHA draft review report.

Table 130 Estimated daily reasonable worst case exposures to DINP or DIDP of children at ages 0-6, 6-12, and 12-18 months from mouthing articles

	0-6 months	6-12 months	12-18 months
Body weight (kg)	6.21	7.62	9.47
Mouthable surface (cm^2)	10	10	10
Daytime mouthing (min/day)	120	120	120
Migration rate ($\mu g/cm^2/h$)	45	45	45
Exposure mouthing articles (excl. pacifiers) (µg/kg/day)	**145**	**118**	**95**

2.2 Sex toys

Sex toys are mainly made of soft PVC or rubber latex. Concentrations of up to 60% w/w DINP have been measured in soft PVC sex toys. Being aware of large uncertainties in the frequency and duration of use of sex toys, migration rates from the different products and their content of phthalate esters, the ECHA report estimated typical and reasonable worst case exposures of 10 and 113 µg/kg bw/day respectively. Since migration rates of DINP and DIDP are similar, these values are applicable to both phthalate esters. In the absence of data on the absorption rates of phthalates from the vagina or rectum, the absorption was assumed to be 50%.

The draft ECHA report assumed a migration rate of 140 µg/cm2/h for the typical case and 217 µg/cm2/h for the reasonable worst case from respectively the average migration rate and the 75th percentile migration rate of DIDP in VWA (2009). The draft report pointed out that the migration rates from VWA (2009) were high in comparison with results from migration experiments with toys and childcare articles. Several possible explanations for the differences were identified. It was considered plausible that the PVC matrix of the tested articles in the VWA study was of bad quality, and three out of 6 sex toys contained very high levels of DINP (50-55% w/w). ECHA considered also that experimental factors might have been a cause for the high results.

In the draft responses to comments from public consultation, ECHA stressed that phthalates are highly lipophilic, and therefore fatty simulants can produce significant migration in contrast with non-lipophilic media. ECHA considers this an important point since oil-based personal lubricants are frequently used with sex toys. ECHA however suggested calculating a median and 75th percentile from the combined DINP and DIDP data from VWA might be appropriate. A median of 65 µg/cm2/h, a 75th percentile of 121 µg/cm2/h and a 95th percentile of 250 µg/cm2/h can be calculated from 19 samples in VWA (2009) (13 DINP and 6 DIDP samples).

Considering the uncertainties discussed, RAC considered that a migration rate of 65 µg/cm2/h for the typical case and 121 µg/cm2/h for the reasonable worst case would be appropriate assumptions for risk assessment. The estimated exposure of DINP and DIDP associated with the use of sex toys would then become 4.8 and 63 µg/kg/day respectively for typical and reasonable worst case exposures (see **Table 131**).

Table 131 Estimated exposure of DINP and DIDP associated with the use of sex toys

	Typical case	**Reasonable worst case**
Body weight (kg)	60	60
Migration rate (µg/cm^2/h)	65	121
Surface (cm^2)	125	125
Duration (min)	2.14	15
Exposure (µg/kg/day)	4.8	63.0

2.3 Biomonitoring data

RAC agreed with the assessment for adults in the ECHA report but notes that it did not sufficiently highlight the biomonitoring data for DINP for children from Becker et al. (2009, as cited in Kransler et al. 2012). The study reported 95th percentiles of up to 39.62 µg/kg/day for children between 3 and 11 years, which is considerably higher than found in other EU studies for children of that age (up to 10.4 µg/kg/day). How to interpret these findings in terms of exposure of children below 18 months old is, however, difficult.

3. Risk characterisation (question 3, 4 and 5)

The previous sections demonstrate that DNELs derived for repeated dose toxicity (hepatotoxicity) are lower than those based on reproductive toxicity (**Table 128** and **Table 129**). For that reason RAC recommends to only use DNELs for repeated dose toxicity when evaluating the risks posed by DINP and DIDP.

3.1 Children

In view of the (small) change in mouthing time and RAC's recommendation for a different DNEL for DIDP for repeated dose toxicity as compared to the ECHA draft review report, RAC notes that the risk characterisation ratios (RCRs) in the ECHA report need adjustment.

For toys and childcare articles, the adjusted RCRs for the reasonable worst case exposure from DINP and DIDP would become as presented in **Table 132**.

Table 132 Risk characterisation for the reasonable worst case exposure from DINP or DIDP in toys and childcare articles (repeated dose toxicity)

	0-6 months	**6-12 months**	**12-18 months**
Oral exposure (µg/kg/day)	145	118	95
$DNEL_{oral}$ (µg/kg/day)	75	75	75
RCR_{oral}	**1.93**	**1.57**	**1.27**
Dermal exposure (µg/kg/day)	54	50	49
$DNEL_{dermal}$ (µg/kg/day)	1880	1880	1880
RCR_{dermal}	**0.03**	**0.03**	**0.03**
RCR_{total}	**1.96**	**1.60**	**1.30**

For aggregated exposure[75] (for which in the ECHA draft review report RCRs have been calculated by adding the RCRs for the typical exposures from the indoor environment and from food to the RCRs for the reasonable worst case exposures for toys and childcare articles), the adjusted RCRs (using the DNELs recommended by RAC) would become as presented in **Table 133** and **Table 134**.

[75] "Aggregated exposure" includes all routes, pathways, and sources of exposure to a given chemical (SCHER/SCENIHR/SCCS 2011).

Table 133 Risk characterisation for repeated dose toxicity from reasonable worst case exposure of children to DINP from toys and childcare articles combined with typical exposure estimates for exposure from the indoor environment and food

	0-6 months	6-12 months	12-18 months
RCR_{toys}	1.96	1.60	1.30
$RCR_{air/dust}$	0.039	0.112	0.091
RCR_{food}	<0.028	0.031	0.025
RCR_{total}	**2.03**	**1.74**	**1.42**

Table 134 Risk characterisation for repeated dose toxicity from reasonable worst case exposure of children to DIDP from toys and childcare articles combined with typical exposure estimates for exposure from the indoor environment and food

	0-6 months	6-12 months	12-18 months
RCR_{toys}	1.96	1.60	1.30
$RCR_{air/dust}$	0.019	0.056	0.045
RCR_{food}	0.013	0.016	0.013
RCR_{total}	**1.99**	**1.67**	**1.36**

When summing the RCRs for the reasonable worst-case exposures for all sources of exposure, the adjusted RCRs for aggregated exposure (using the DNELs recommended by RAC) would become as presented in **Table 135** and **Table 136**.

Table 135 Risk characterisation for repeated dose toxicity from reasonable worst case exposures of children to DINP from all sources

	0-6 months	6-12 months	12-18 months
RCR_{toys}	1.96	1.60	1.30
$RCR_{air/dust}$	0.135	0.365	0.300
RCR_{food}	0.028	0.144	0.169
RCR_{total}	**2.12**	**2.11**	**1.77**

Table 136 Risk characterisation for repeated dose toxicity from reasonable worst case exposures of children to DIDP from all sources

	0-6 months	6-12 months	12-18 months
RCR_{toys}	1.96	1.60	1.30
$RCR_{air/dust}$	0.067	0.183	0.149
RCR_{food}	0.013	0.072	0.084
RCR_{total}	**2.04**	**1.86**	**1.53**

As can be seen from **Table 133**, **Table 134**, **Table 135** and **Table 136**, the major contributor to the total RCR is the RCR from toys and childcare articles, with RCRs >1. The RCRs for the other exposure sources are well below 1.

Assuming the biomonitoring data by Becker et al (2009) for children is valid, an RCR of 0.53 for DINP for 3-11 years old children can be derived from the 95th percentiles. Most other studies report exposures of up to 10.4 µg/kg which corresponds to a RCR of 0.14 for children older than 2 years. The reasonable worst case exposure estimates from the indoor environment and food correspond very well with the 95th percentile estimates from Becker et al (2009) as can be seen in **Table 135** and **Table 136**.

The ECHA draft review report also addressed combined exposure[76] from DINP and DIDP. Risk characterisation for combined exposure is justified on the basis of the similar liver findings and since DINP and DIDP are both used in a wide variety of consumer articles and construction materials, with largely overlapping uses. However, as stated in the draft review report, for the purposes of the assessment of exposure to articles from mouthing or dermal contact (direct exposure to articles), it was assumed that the articles either contain DINP or DIDP. Therefore, dose addition does not apply to direct exposure to articles. It however applies to exposure via food and the indoor environment.

Simultaneous exposure is confirmed by biomonitoring data, showing that metabolites of both DINP and DIDP (MCINP and MCIOP) were detected in most of the tested persons. According to Frederiksen et al. (2010), it seems that participants with a high exposure to one phthalate were also highly exposed to other phthalates. This might justify to add reasonable worst case estimates, although it should be acknowledged that this might lead to overestimation of actual exposures.

The adjusted RCRs (using the DNELs recommended by RAC) for this combined exposure would become as presented in **Table 137**.

Table 137 Risk characterisation for repeated dose toxicity from reasonable worst case exposures of children to DINP and DIDP from all sources (combined exposure of DINP and DIDP)

	0-6 months	6-12 months	12-18 months
RCR_{toys}	1.96	1.60	1.30
$RCR_{air/dust}$	0.202	0.548	0.449
RCR_{food}	0.041	0.216	0.253
RCR_{total}	**2.20**	**2.36**	**2.00**

3.2 Adults

In view of the changed exposures from sex toys and RAC's recommendation for different DNELs for DINP and DIDP for repeated dose toxicity as compared to the ECHA draft review report, RAC notes that the RCRs in the ECHA report need adjustment.

For sex toys, the adjusted RCRs for the typical and reasonable worst case exposure from DINP and DIDP would become as presented in **Table 138**.

Table 138 Risk characterisation for vaginal/rectal exposure to DINP or DIDP in adult sex toys

	Typical case	Reasonable worst case
Vaginal/rectal exposure (µg/kg/day)	4.8	63.0
$DNEL_{oral}$ * 2 (µg/kg/day)	150	150
RCR_{sex}	**0.03**	**0.42**

[76] Combined exposure" includes all routes, pathways, and sources of exposure to multiple chemicals (SCHER/SCENIHR/SCCS 2011).

For aggregated exposure (combining the RCRs for the typical exposures from indoor environment, food and dermal exposure to articles to the RCR for the reasonable worst case exposure for sex toys), the adjusted RCRs (using the DNELs recommended by RAC) would become as presented in **Table 139**.

Table 139 Risk characterisation for reasonable worst case exposure of adults to DINP and for DIDP from sex toys combined with typical exposure estimates for exposure from the indoor environment, food and dermal exposure

	DINP	DIDP
$RCR_{sex\ toys}$	0.42	0.42
RCR_{dermal}	0.005	0.005
$RCR_{air/dust}$	0.008	0.005
RCR_{food}	0.002	0.001
RCR_{total}	**0.44**	**0.43**

When summing the RCRs for the reasonable worst case exposures for all sources of exposure, the adjusted RCRs for aggregated exposure (using the DNELs recommended by RAC) would become as presented in **Table 140**.

Table 140 Risk characterisation for reasonable worst case exposures of adults to DINP and for DIDP from all sources

	DINP	DIDP
$RCR_{sex\ toys}$	0.42	0.42
RCR_{dermal}	0.159	0.159
$RCR_{air/dust}$	0.030	0.018
RCR_{food}	0.053	0.027
RCR_{total}	**0.66**	**0.62**

The adjusted RCRs (using the DNELs recommended by RAC) for combined exposure to DINP and DIDP (with the exception of direct exposure to articles) would become as presented in **Table 141**.

Table 141 Risk characterisation for repeated dose toxicity from reasonable worst case exposures of adults to DINP and DIDP from all sources (combined exposure of DINP and DIDP)

$RCR_{sex\ toys}$	0.42
RCR_{dermal}	0.159
$RCR_{air/dust}$	0.048
RCR_{food}	0.08
RCR_{total}	**0.71**

References

Anderson WA, Castle L, Hird S, Jeffery J and Scotter MJ (2011). A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate. *Food Chem Toxicol.* **49(9)**: 2022-2029.

Aristech (1994). Aristech Chemical Corporation (1994). 2-Year Dietary Oral Toxicity Study in Rats with Diisononyl Phthalate. TSCA 8(e) Submission 8EHQ-0794-13083. CAS Number 68515-48-0. (as cited in EC 2003a).

Babich MA, Chen SB, Greene MA, Kiss CT, Porter WK, Smith TP, Wind ML and Zamula WW (2004). Risk assessment of oral exposure to diisononyl phthalate from children's products. *Regul Toxicol Pharmacol*. **40(2)**: 151-167.

BASF (1969). Bericht über den 90-Tage-Ratten-Fütterungsversuch mit PALATINOL Z (as cited in EC 2003b).

Becker K, Göen T, Seiwert M, Conrad A, Pick-Fuss H, Müller J, Wittassek M, Schulz C and Kolossa-Gehring M (2009). GerES IV: phthalate metabolites and bisphenol A in urine of German children. *Int J Hyg Environ Health.* **212(6)**: 685-692.

Berry C (2012). Expert Review: Evaluation of the significance of changes found in studies on DINP (Di-isononyl phthalate) and DIDP (Di-isodecyl phthalate) in the rat, 22 May 2012. Sponsor: European Council for Plasticisers and Intermediates. Submission of information via the public consultation on ECHA's draft review report by ECPI. Comment reference number 16, Attachment II. Public comments are available on http://echa.europa.eu/web/guest/addressing-chemicals-of-concern/restriction/consultations-draft-review-report

CHAP (2001). US Chronic Hazards Advisory Panel. *Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on Diisononyl Phthalate (DINP).* U.S. Consumer Product Safety Commission, Bethesda, MD. Available at: www.cpsc.gov/library/foia/foia01/os/dinp.pdf

Cho SC, Han BS, Ahn B, Nam KT, Choi M, Oh SY, Kim SH, Jeong J, Jang DD (2008). Peroxisome proliferator di-isodecyl phthalate has no carcinogenic potential in Fischer 344 rats. *Toxicol Lett.* **178 (2)** : 110-116.

Cho SC, Han BS, Ahn B, Nam KT, Choi M, Oh SY, Kim SH, Jeong J, Jang DD (2010). Corrigendum to "Peroxisome proliferator di-isodecyl phthalate has no carcinogenic potential in Fischer 344 rats" [Toxicol. Lett. 178 (2008) 110–116]. *Toxicol Lett.* **197**: 156.

EC (2003a). Risk Assessment Report for DINP . Final report, European Commission, EUR 20784EN, European Union Risk Assessment Report, Volume 35, Luxembourg: Office for Official Publications of the European Communities.

EC (2003b). Risk Assessment Report for DIDP . Final report, European Commission, EUR 20785EN, European Union Risk Assessment Report, Volume 36, Luxembourg: Office for Official Publications of the European Communities.

EFSA (2005a). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Di-isononylphthalate (DINP) for use in food contact materials. doi:10.2903/j.efsa.2005.244. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/244.htm

EFSA (2005b). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Di-isodecylphthalate (DIDP) for use in food contact materials. Available at: http://www.efsa.europa.eu/en/efsajournal/doc/245.pdf

Exxon (1986) Exxon Biomedical Sciences (1986). Chronic Toxicity/Oncogenicity Study in F-344 Rats. TestMaterial: MRD-83-260. Project No 326075 performed at Exxon Biomedical Sciences, Inc. Unpublished Laboratory Report (as cited in EC 2003a).

Frederiksen H, Jørgensen N and Andersson AM (2010). Correlations between phthalate metabolites in urine, serum, and seminal plasma from young Danish men determined by isotope dilution liquid chromatography tandem mass spectrometry. *J Anal Toxicol.* **34(7)**: 400-410.

General Motors Research Laboratories (1983). Effect of Dose on Di-isodecyl Phthalate Disposition in Rats. EPA Document N° 878213821, Fiche No OTS206315. (as cited in EC 2003b).

Greene M (2002). Mouthing times among young children from observational data. US CPSC, Bethesda, MD 20814, June 17, 2002.

Hazleton (1972). Metabolism Study of 14C Phthalate Ester in Rats. Unpublished Laboratory Report from Hazleton Laboratories submitted to Esso Research and Engineering Company,. Final Report, September 25, 1972.

Hazleton Laboratories (1968a). Three-Month Dietary Administration – Albino Rats DIDP – FDA Grade (Plasticiser) submitted to Dewey and Almy Chemical Division, WR Grace and Company.

Hazleton Laboratories (1968b). 13-Week Dietary Administration - Dogs Plasticiser (DIDP) submitted to WR Grace and Company (as cited in EC 2003b).

Juberg DR, Alfano K, Coughlin RJ and Thompson KM (2001). An observational study of object mouthing behavior by young children. *Pediatrics* **107(1)**:135–142.

Kaiserling E and Müller H (2005). Neoplasm of hepatic stellate cells (spongiotic pericytoma): a new tumor entity in human liver. (Case Reports). *Pathol Res Pract.* **201(11)**: 733-43.

Karbe E and Kerlin RL (2002). Cystic degeneration/spongiosis hepatis in rats. *Toxicol Pathol.* **30**: 216-227.

Kessler W, Numtip W, Völkel W, Seckin E, Csanády GA, Pütz C, Klein D, Fromme H, Filser JG (2012). Kinetics of di(2-ethylhexyl) phthalate (DEHP) and mono(2-ethylhexyl) phthalate in blood and of DEHP metabolites in urine of male volunteers after single ingestion of ring-deuterated DEHP. *Toxicology and Applied Pharmacology* **264**: 284-291.

Koch HM, Bolt HM, Preuss R and Angerer (2005). New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. *Arch Toxicol.* **79**: 367–376.

Koch HM and Angerer J (2007). Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. *Int J Hyg Environ Health.* **210(1)**: 9-19.

Kransler KM, Bachman AN, McKee RH (2012). A comprehensive review of intake estimates of di-isononyl phthalate (DINP) based on indirect exposure models and urinary biomonitoring data. *Regulatory Toxicology and Pharmacology* **62**: 248–256.

Kurata Y, Makinodan F, Shimamura N and Katoh M (2012). Metabolism of di (2-ethylhexyl) phthalate (DEHP): comparative study in juvenile and fetal marmosets and rats. *J. Toxicol. Sci.* **34(1)**: 33-49.

Lington AW, Bird MG, Plutnick RT, Stubblefield WA and Scala RA (1997). Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Fund Appl Toxicol.* **36**: 79-89.

McKee RH, El-Hawari M, Stolz M, Pallas F and Lington AW (2002). Absorption, Disposition and Metabolism of Di-isononyl Phthalate (DINP) in F-344 Rats. *J. Appl. Toxicol.* **22**: 293-302.

Midwest Research Institute (1983). Single and Repeated Oral Dose Pharmacokinetics of 14 C-Labeled Diisononyl Phthalate, with cover letter. Unpublished Laboratory Report from Midwest Research Institute prepared for Exxon Corporation, MRI Project No 7282-B, December 19, 1983 (as cited in EC 2003a).

Nime F, Pickren JW, Vana J, Aronoff BL , Baker HW, Murphy GP (1979). The Histology of liver tumors in oral contraceptive users observed during a national survey by the American college of surgeons commission on cancer. *Cancer* **44: 1481-1489.**

RIVM (1998). *Phthalate release from soft PVC baby toys.* National Institute of Public Health and Environmental Protection (RIVM). Report from the Dutch Consensus Group. RIVM Report 31 3320 002, Könemann WH (ed), Bilthoven, The Netherlands. Available at: http://www.rivm.nl/bibliotheek/rapporten/613320002.pdf

SCCP (2007). Opinion on Phthalates in cosmetic products. Scientific Committee on Consumer Products, SCCP/1016/06, 21 March 2007.

SCHER (2008). Opinion on phthalates in school supplies. Scientific Committee on Health and Environmental Risks. Opinion adapted by written procedure on 17 October 2008.

Schmid P and Schlatter C (1985). Excretion and metabolism of di(2-ethylhexyl)-phthalate in man. *Xenobiotica* **15(3)**: 251-256.

Smith S and Norris B (2002). *Research into the mouthing behaviour of children up to 5 years old*. Consumer and Competition Policy Directorate, Department of Trade and Industry (DTI), London.

Sjoberg P, Bondesson U, Kjellen L, Lindquist NG and Plöen L (1985). Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. *Acta Pharmacol. Toxicol.* **56**: 30-37.

Su Q, Benner A, Hofmann WJ, Otto G, Pichlmayr R and Bannasch P (1997). Human hepatic preneoplasia: phenotypes and proliferation kinetics of foci and nodules of altered hepatocytes and their relationship to liver cell dysplasia. *Virchows Arch.* **431**: 391-406.

Tønning K, Jacobsen E, Pedersen E, Strange M, Poulsen PM, Møller L and Boyd PB (2009). *Survey and health assessment of the exposure of 2 year-olds to chemical substances in consumer products.* Survey of Chemical Substances in Consumer Products, No. 102. Danish Environmental Protection Agency, Copenhagen.

US CPSC (2010a). Toxicity Review of Diisononyl Phthalate (DINP) Memorandum: United States Consumer Product Safety Commission (US CPSC) staff assessment of the potential toxicity associated with diisononyl phthalate (DINP). Available at: http://www.cpsc.gov/about/cpsia/toxicityDINP.pdf

US CPSC (2010b). Toxicity Review of Di(isodecyl) Phthalate. Memorandum: United States Consumer Product Safety Commission (US CPSC) staff assessment of the potential toxicity associated with di(isodecyl) phthalate. Available at: http://www.cpsc.gov/about/cpsia/toxicityDIDP.pdf

VWA (2009). Consumentenproducten in de eroticabranche. Fact sheet. Voedsel en Waren Autoriteit (VWA), Afdeling Signalering en Ontwikkeling, Regio Noord, Juni 2009.

Annex 2 Table with exposure of infants (6 months – 3 years) and newborn (0-6 months) to DINP and DIDP in toys derived in the EU Risk Assessments

	External measure of exposure	Internal dose	Reasoning underlying exposure estimate
DINP Oral	200 µg/kg/day	200 µg/kg/day Assumes 3 hours oral contact/day	Based on the highest observed release rate in a human volunteer study undertaken in the Netherlands to assess release of DINP to saliva from PVC samples which was combined with a child observation study to determine the oral contact time of young children with baby toys and an *in vitro* study with saliva stimulant.
DINP Dermal		1 µg/kg/day	Dermal absorption rate based on the dermal absorption of DEHP in experiments with rats and allowing a factor of 10 for the poorer penetration of skin by DINP than DEHP as determined in an in-vitro assay, assumes toy handled for 3 hours day and contact area is 100 cm^{-2}
DIDP Oral	200 µg/kg/day	200 µg/kg/day Assumes 3 hours oral contact/day	Highly variable rates of migration have been reported for DIDP in *in vitro* tests. The estimated intake is based on the worst case migration rate for DINP determined in the Dutch volunteer study that investigated DINP migration out of PVC toys. The predicted intake is about 10 times greater than the estimated intake based on the highest measured *in vitro* migration rates for DIDP.
DIDP Dermal		1 µg/kg/day	Dermal absorption rate based on the dermal absorption of DEHP in experiments with rats and allowing a factor of 10 for the poorer penetration of skin by DIDP than DEHP as determined in an in-vitro assay, assumes toy handled for 3 hours day and contact area is 100 cm^{-2}

Annex 3 Table with consumer exposures to DINP and DIDP in indoor air associated with building materials and furniture derived in the EU Risk Assessment

	External measure of exposure	Internal dose	Reasoning underlying exposure estimate	Comments
Adult (60 kg): DINP	40 µg/m^3	8.3 µg/kg/day (75% absorption) 20 hours exposure/day Inhalation volume 20 m^3/day	Worst case scenario based on DINP vapour pressure at 20°C (10 µg/m^3) and an assumption that DINP associated with particles is 3 times greater than the quantity present as vapour (based on findings of Norwegian study published in 1997). In comparison DINP concentrations in air in a laboratory where coatings contained DINP were reported to be 0.66 µg/m^3 suggesting that inhalation exposure was substantially over-estimated in the risk assessment.	The inhalation exposure is likely to have been over estimated as it seems highly unlikely that DINP concentrations in indoor levels reach the estimated levels. Total concentrations of particulate matter in indoor environments are generally <100 µg/m^3. Measurement data suggest that total daily intakes may be less than half the modelled values for indoor air. The exposure estimate neglects the contribution of inadvertent ingestion and dermal contact with settled dust in the indoor environment. Inadvertent ingestion of settled dust may be as or more important as a source of adult exposure to hazardous substances than inhalation of indoor air.
Adult (60 kg): DIDP	20 µg/m^3	4.2 µg/kg/day (75% absorption) 20 hours exposure/day Inhalation volume 20 m^3/day	Worst case scenario based on DIDP vapour pressure at 20°C (5 µg/m^3) and an assumption that airborne phthalate associated with particles is 3 times greater than the quantity present as vapour. It was noted that there were few measured data and maximum reported concentrations in indoor air were 0.02 µg/m^3.	The inhalation exposure is likely to have been over estimated as total concentrations of particulate matter in indoor environments are generally <100 µg/m^3. Measurement data suggest that total daily intakes may be less than half the modelled values for indoor air. The exposure estimate neglects the contribution of inadvertent ingestion and dermal contact with settled dust in the indoor environment

Children 3-15 years DINP and DIDP	Assumed same as adult	Assumed same as adult		Intakes in terms of µg/kg/day may be slightly higher than for adults because of relatively higher rates of metabolism, but difference would be small relative to the over-estimate of adult exposure.
Infants 6 months to 3 years: DINP	40 µg/m^3	42.6 µg/kg/day – based on 22 hours/day, 100% bioavailability Inhalation volume 9.3 m^3/day	Estimate as for adult exposure but assumes greater bioavailability	Exposure to DINP by ingestion of house dust is likely to be a more important route of exposure for small children than for adults. Toddlers may mouth all sorts of items, including hands, in addition to toys that may be coated with a thin layer of dust, particularly if they have been playing on the floor. Although it seems likely that the inhalation component of exposure is much smaller than estimated in the risk assessment (see comments on adults), it is likely that this is offset by significant exposure by the oral route.
Infants 6 months to 3 years: DIDP	20 µg/m^3	21.3 µg/kg/day – based on 22 hours/day, 100% bioavailability	Estimate as for adult exposure but assumes greater bioavailability	As for DINP, exposure to DIDP by ingestion of house dust is likely to be a much more important route of exposure for small children than for adults. Although it seems likely that the inhalation component of exposure is much smaller than estimated in the risk assessment (see comments on adults), it is likely that this is offset by significant exposure by the oral route.
Newborn (0-6 months) DINP and DIDP		Assumed to be same as for infants		

Annex 4 Table with estimated dietary exposures to DINP calculated in the EU Risk Assessment

	External measure of exposure	Internal dose	Reasoning underlying exposure estimate	Comments
Adult: consumer exposure	0.2 µg/kg/day	0.1 µg/kg/day based on 50% bioavailability in food and assumed 60 kg body weight	Based on the detection limit in a 1996 UK study (0.01 mg/kg food) intended to be representative of total diet which is reported to be equivalent to <1.7 µg/kg/day. In comparison the same survey reported a daily intake of DEHP of 5 µg/kg/day. It was noted that total phthalate levels reported in another study that included 3 EU countries but did measure DINP and was confined to dairy products were higher than those reported for the UK.	Levels of DINP in food are generally below the detection limits in published studies suggesting that the intake in food is likely to have been over-estimated. Most foods, including those with high fat contents appear to contain less than 0.005 mg/kg, although some oily foods may have been reported to have extremely high DINP levels as a result of contamination by food contact materials (≤740 mg/kg). Some individuals may have elevated DINP intakes if they are frequent consumers of oily foods supplied in small jars that happen to have PVC gaskets that contain DINP. The regulation of DINP content of food contact materials is likely to limit future exposure arising from food contact materials although the replacement of DEHP by DINP may lead to increased exposures in comparison to past levels. The increasing use of DIDP and aging of products containing DINP is however likely to lead to increased DINP contamination of the food chain.
Adult: environmental exposure – local sources		2-26 µg/kg/day*	Derived using the EUSES model. The highest predicted intakes – 25-26 µg/kg/day are associated with use of PVC and formulation of sealing compounds, printing inks and paints.	The EUSES model is designed to provide conservative estimates of exposure (i.e. over-estimates). There is no evidence that phthalate concentrations in raw food stuffs reach the levels predicted by the EUSES model.

Adult: environmental exposure – regional sources		1 µg/kg/day*	Derived using EUSES model – although the EU Risk Assessment notes that predicted pore water concentrations exceed solubility of DINP implying that exposures to DINP are likely to be over-estimated. Exposure almost entirely due to intake in fish and root crops.	Levels of DINP in food are generally below the detection limits in published studies suggesting that the intake in food is likely to be less than estimated by indirect exposure to environment. Given that phthalates are poorly soluble and lipophilic, it seems unlikely that root vegetables represent a major source of exposure
Children 3-15 years: consumer exposure	Assumed same as adult	Assumed same as adult		Intakes in terms of µg/kg/day may be slightly higher than for adults because of relatively higher rates of metabolism and food intake, but difference would be small relative to the over-estimate of adult exposure.
Infants 6 months to 3 years: consumer exposure	2.3 µg/kg/day	2.3 µg/kg/day based on 100% bioavailability, bodyweight of 8 kg and intake of formula milk of 141 g/day	Based on estimated intake in formula milk equivalent to 1.8 mg/kg/day and intake in food of 0.5 µg/kg/day based on detection limit in UK (1996) study and assumed intakes of 0.141 kg dried formula (detection level DINP content of 0.1 mg/kg) and a level of food consumption, one third of that in adults. The estimated intake is based on the bodyweight and milk intake at the bottom end of the age range as milk consumption reduces and bodyweight increases with age (giving a lower intake per unit bodyweight)	More recent studies of the DINP content of formula milk suggest that levels are <0.005 mg/kg implying the actual intake associated with formula milk is likely to be less 5% of the estimate used in the risk assessment – however the number of published data are few and the potential for DINP contamination to arise during the preparation of formula prior to feeding has not been investigated. Intakes in food are also likely to have been over-estimated (see comments on adult intakes)
Infants 6 months to 3 years: environmental exposure – local sources		6-141 µg/kg/day based on 100% availability	Derived using EUSES predictions of levels in different foods but assuming a different food basket (i.e. dietary mix) as described in the EU Risk Assessment	The evidence from the biomonitoring studies described later in this report indicates that the predicted intakes based on EUSES are vastly higher than the highest levels indicated by biomonitoring

Infants 6 months to 3 years: environmental exposure - regional sources		6.5 µg/kg/day	Derived using EUSES predictions of levels in different foods but assuming a different food basket (i.e. dietary mix) as described in the EU Risk Assessment	The evidence from the biomonitoring studies described later in this report indicates that the predicted intakes based on EUSES are higher than the levels indicated by biomonitoring
Newborn: 0-6 months: consumer exposure	2.4 µg/kg/day	2.4 µg/kg/day based on 100% bioavailability, bodyweight of 5.5 kg and intake of formula milk of 131g/day	Based on estimated intake in formula milk equivalent to 2.4 mg/kg/day based on detection limit in UK (1996) study. . The estimated intake is based on the average bodyweight and milk intake in this age range	See comments on DINP intake in formula milk above. DINP has not been detected in breast milk implying that concentrations are <1 mg/kg. Given the rapid metabolism of DINP, it is highly unlikely that maternal exposure to DINP would lead to significant quantities being excreted in breast milk. Exposure via breastfeeding is likely to be exceedingly small in comparison to the estimated intake in formula
Newborn: 0-6 months: environmental exposure	-	-	Not estimated as environmental exposure via milk considered negligible	It seems reasonable to assume that environmental exposure via formula milk is negligible

*It is assumed that this is an internal dose based on the same assumptions as for consumer exposure but this is not explicit in the EU Risk Assessment

Annex 5 Table with literature data on DINP in food

Food item	Number of samples, n	Number of samples above LOD	Limit of Detection (LOD), mg/kg	DINP concentration, mg/kg (of samples above LOD)			Country	Year	Data source
				Mean	Range	St.dev.			
Raw milk, pasteurized milk, yogurt with fruit, liquid infant formulae	27	0	0.005	-	-	`	Denmark	2005	Sørensen (2006)
Reconstituted infant formula from different parts of world	6	n.i.	0.005	n.i.	<0.005-0.012	n.i.	-"-	-"-	-"-
Vegetable oil	5	n.i.	n.i.	-	-	-	Denmark	2010	DVFA (2010)
Vegetable oil	165	n.i.	0.001*3	0.0017	<0.001-0.003 *[3]	n.i.	Italy	2009	Nanni et al. (2011)
Olive pomace	7	n.i.	0.001	0.0065	n.i.		-"-	-"-	-"-
Food in glass jars with plastic gaskets *[1]	19	1 (peanut butter)	1	99	-	-	Denmark	2004	Pedersen et al. (2008)
Food in glass jars with plastic gaskets *[1]	158	9 *[2]		175	120 – 270	n.i.	Switzerland	2005	Frankhauser-Noti et al. (2006)
Vegetable oil-containing products	365	5	1.5	13	4-22	n.i.	Austria	2007-2009	Grossgut, (2011)
Spicy sauces	24	1	n.i.	26	-	-	Austria	2009	BfG (2009)
Bread, pasta, rice, dairy products, meat, oil & fat, sauce, beer	40	n.i.	0.0006	n.i.	<0.0006 – 0.21	n.i.	The Netherlands	2011	VITO (2011) *[4]

LOD.: Limit of Detection
n.i.: not indicated
*[1] Ratio of lid surface area to weight of the food ranged from 0.14 to 1.17 dm^2/kg. DINP was detected in samples of soft cheese and sauce béarnaise.
*[2] DINP was detected in the food form all 9 packaging with gaskets with a substantial content of DINP
*[3] Limit of quantification for all samples indicated to be 3 mg/kg. Data presented with average and 0.05 significance level for each group of oil. Range represent the indicated range for averages for the different groups of oil.
*[4] Preliminary results – the data are expected to be published by the end of 2011.

Annex 6 Table with literature data on DIDP in food

Food item	Number of samples, n	Number of samples above LOD	Limit of Detection (LOD), mg/kg	DIDP concentration, mg/kg (of samples above LOD)			Country	Year	Data source
				Mean	Range	St.dev.			
Food in glass jars with plastic gaskets *[1]	19	6	1	62	8-173	-	Denmark	2004	Pedersen et al. (2008)
Food in glass jars with plastic gaskets *[1]	158	12 *[2]	5	approx. 175	5-740	n.i.	Switzerland	2005	Frankhauser-Noti et al. (2006)
Vegetable oil-containing products	365	3	1.5	159	4-469	n.i.	Austria	2007-2009	Grossgut (2011)
Vegetable oil	5	0	n.i.	-	-	-	Denmark	2010	DVFA (2010)
Raw milk, pasteurized milk, yogurt with fruit, liquid infant formulare	27	0	0.005	-	-	`	Denmark	2005	Sørensen (2006)
Reconstituted infant formula from different parts of world	6	0	0.005	-	-		-"-	-"-	-"-
Bread, pasta, rice, dairy products, meat, oil & fat, sauce, beer	40	n.i.	0.0008	n.i.	<0.0008 – 0.42	n.i.	The Netherlands	2011	VITO (2011) *[3]

LOD.: Limit of detection
n.i.: not indicated
*[1] ratio of lid surface area to weight of the food ranged from 0.14 to 1.17 dm^2/kg. DIDP was detected in samples of soft cheese and sauce béarnaise.
*[2] DIDP was detected in the food from 12 of the packaging. In 11 of the products, DIDP was the main plasticiser in the gasket.
*[3] Preliminary results – the data are expected to be published by the end of 2011.

Annex 7 Table with biomonitoring data for DINP metabolites

Population group	Specimen	Number of samples		Unadjusted metabolite concentration, µg/L, percentiles, Creatinine adjusted concentration in brackets, µg/g creatinine								Country	Sampling year	Data source
				MiNP		MCiOP		MHiNP,		MOiNP				
		n	% > LOD *[1]	50th	95th	50th	95th	50th	95th	50th	95th			
Urinary data														
Adult (21-29 y)	24 h Urine	60	*[2]					1.5 (1.2)	8.8 (7.6)	0.6 (0.5)	3.5 (3.9)	Germany	1988	Wittassek et al. (2007)
Adult (21-29 y)	24 h Urine	60	*[2]					1.8 (1.7)	14.9 (11.0)	0.8 (0.7)	7.3 (6.6)	-¨-	1989	-¨-
Adult (21-29 y)	24 h Urine	60	*[2]					2.2 (1.6)	31.5 (38.1)	0.8 (0.7)	3.2 (3.7)	-¨-	1991	-¨-
Adult (21-29 y)	24 h Urine	60	*[2]					1.8 (1.8)	10.0 (11.3)	0.8 (0.8)	5.3 (5.0)	-¨-	1993	-¨-
Adult (21-29 y)	24 h Urine	146	*[2]					2.0 (1.9)	12.0 (10.6)	1.0 (1.1)	5.6 (5.2)	-¨-	1996	-¨-
Adult (21-29 y)	24 h Urine	68	*[2]					2.1 (2.1)	47.9 (43.3)	1.1 (1.1)	26.6 (29.2)	-¨-	1998	-¨-

Population group	Specimen	Number of samples		Unadjusted metabolite concentration, µg/L, percentiles, Creatinine adjusted concentration in brackets, µg/g creatinine								Country	Sampling year	Data source
				MiNP		MCiOP		MHiNP,		MOiNP				
		n	% > LOD *[1]	50th	95th	50th	95th	50th	95th	50th	95th			
Adult (21-29 y)	24 h Urine	60	*[2]					1.9 (1.9)	11.6 (11.2)	1.0 (1.1)	9.6 (6.8)	-¨-	1999	-¨-
Adult (21-29 y)	24 h Urine	60	*[2]					2.1 (2.2)	13.9 (13.8)	1.1 (1.3)	5.7 (5.6)	-¨-	2001	-¨-
Adult (20-29 y)	24 h Urine	60	*[2]					2.3 (2.6)	13.3 (7.9)	1.6 (1.7)	10.4 (5.8)	-¨-	2003	-¨-
Children (3-5 y)	-¨-	137	n.i.			18.2	76.4	12.8	59.4	6.1	31.1	Germany	2003-2006	Becker et al. (2009)
Children (6-18 y)	-¨-	145	n.i.			16.6	58.8	12.5	61.6	6.1	28.2	-¨-	-¨-	-¨-
Children (9-11 y)	-¨-	149	n.i.			12.2	71.9	10.1	58.9	5.4	39.1	-¨-	-¨-	-¨-
Children (12-14 y)	-¨-	168	n.i.			9.6	43.4	9.2	38.4	4.6	21.1	-¨-	-¨-	-¨-

Children (3-14 y) total dataset	Morning urine	599	98,100,100			12.7	58.9	11.0	50.6	5.4	28.9	-¨-	-¨-	-¨-
Adults	Urine, morning	45	n.i			5.3	15.5	4.7	16.8	1.7	6.7	Germany	2007	Koch and Calafat (2009)
Women (14-60 y)	Urine	27						5.7	11.5	3.1	8.1	Germany	2005	Fromme et al. (2007a)
Men (14-60 y)	Urine *[3]	23 x 8						5.5	18.7	3.0	9.3	-¨-	-¨-	-¨-
Random population	Urine	25	96,96, 80			5.0	16.4	2.5	14.9	1.3	8.9	Germany		Koch et al. (2007)
Children (5-6 y)	Urine	111	99,96, 78			13.1 (19.7)	45.5 (91.6)	7.0 (10.8)	25.5 (41.9)	4.2 (5.8)	12.5 (27.6)	Germany	2007	Koch et al. (2011b)
Adults (19-29 y)	24 h urine	60				4.2	12.2	3.3	10.1	2.1	6.6	Germany	2002	Göen et al. (2011)
Adults (20-29 y)	24 h urine	60				3.2	15.1	2.8	16.5	2.1	10.0	-¨-	2004	-¨-
Adults (19-28 y)	24 h urine	60				4.1	29.0	3.5	20.4	2.2	15.9	-¨-	2006	-¨-
Adults (19-29 y)	24 h urine	60				3.6	27.4	3.6	20.6	2.3	16.2	-¨-	2008	-¨-
Children (3-14)	Urine	592	100			12.7	57.6	11.0	59.9	5.4	Ø'Ø	Germany	2002-2003	Seiwert (2010) as cited in UBA (2011)
Adult (20-29)	Urine	112	100			3.8	28.0	3.5	20.4	2.2	16.0	-¨-	2006-2008	-¨-

Plastisol workers, comparison group	Urine, pre shift	10	100			6.1 (5.4)	n.i.	5.7 (4.8)	n.i.	3.0 (2.0)	n.i.	Germany	n.i.	Koch et al. (2011a)
	Urine, post shift	10	100, 100,n.i.			6.5 (4.5)	n.i.	6.2 (3.8)	n.i.	2.8 (1.9)	n.i.	-¨-	-¨-	-¨-
Plastisol workers, exposed	Urine, pre shift	5	100			32.3 (15.5)	n.i.	26.0 (18.4)	n.i.	12.9 (8.0)	n.i.	-¨-	-¨-	-¨-
	Urine, post shift	5	100			57.8 (57.9)	n.i.	117 (117)	n.i.	44.3 (44.4)	n.i.	-¨-	-¨-	-¨-
Pregnant women (18-41 y)	Urine, day	99	98,96					2.5 (4.2)	38.3 (53)	2.2 (4.3)	30.0 (43.9)	The Netherlands	2004-2006	Ye et al. (2006)
Children (4-9 y), male	Urine	125/503 *[7]		0.6 (1.0)	n.i. *[7]	7.2 (10)	n.i. *[7]	6.6 (8.4)	n.i. *[7]	3.4 (4.1)	n.i. *[7]	Denmark	2006-2007	Boas et al. (2010)
Children (4-9 y), male	Urine	125/342 *[7]		0.5 (1.1)	n.i. *[7]	6.5 (12)	n.i. *[7]	4.9 (7.4)	n.i. *[7]	2.7 (3.9)	n.i. *[7]	-¨-	-¨-	-¨-
Men (18-26 y)	Urine	60	35,92, 95,82	<LOD	4.1	4.3	26.0	3.3	15.8	1.6	8.7	Denmark	2006	Frederiksen et al. (2010)
Pregnant women	Urine	287	92			2.7	17.2					France	2003-2006	Philippat et al. (2011)
Adults	Urine, day	129	97,100,87	<LOD	<LOD	8.4	46.2	13.2	43.7	1.2	6.6	USA	2003/2004	Silva et al. (2006a)
Overall population > 6 years *[6]	Urine	2,548		<LOD	2.3 (3.4)	5.1 (4.5)	54.4 (40.2)					USA	2005-2006	CDC (2011)

Overall population > 6 years *[6]	Urine	2,604		<LOD	2.0 (3.3)	6.4 (5.9)	63.0 (50.2)				-¨-	2007-2008	-¨-
Pregnant women	Urine	19	84			3.0					Israel	2006	Berman et al. (2009)
Pregnant women (25-35 y)	Urine	99	39, 31,40			<d.l. (0.4)	n.i. *4	<d.l. (0.4)	n.i.	<d.l. (0.3)	n.i.	Taiwan	2001-2002	Lin et al. (2011)
Children (5 y)	Urine	59	100, 98,100			9.4 (22.4)	n.i.	7.9 (17.7)	n.i.	4.3 (9.6)	n.i.	-¨-	2006-2007	-¨-
Children (2y)	Urine	26	99, 95,99			9.36 (21.2)	n.i.	6.2 (20.0)	n.i.	3.8 (9.2)	n.i.	-¨-	2003-2004	-¨-
Pregnant women (25-35 y)	Urine	50	7	<0.035	n.i.							Japan	2005-2006	Suzuki et al. (2009)
Children (6-16 y) and adolescents (17-21 y)	24 h urine	129	n.i.	ΣDINPm *5: 50th :31; 95th: 114								Denmark	2006-2008	Frederiksen et al. (2011)
	1st morning urine	129	n.i.	ΣDINPm *5: 50th :48; 95th: 236								-¨-	-¨-	-¨-
	2nd morning urine	129	n.i.	ΣDINPm *5: 50th :40; 95th: 209								-¨-	-¨-	-¨-
Other specimen														
Women (after birth)	Breast milk	30	0			<0.25	n.i.	<0.25	n.i.	<0.25	n.i.	Taiwan	2001-2002	Lin et al. (2011)

-¨-	Cord blood	30	0			<0.25	n.i.	<0.25	n.i.	<0.25	n.i.	-¨-	-¨-	-¨-
Men (18-26 y)	Serum	60	10,43, 2,2	<LOD	0.5	<LOD	1.7	<LOD	<LOD	<LOD	<LOD	Denmark	2006	Frederiksen et al. (2010)
-¨-	Seminal plasma	60	12,2, 0,0	<LOD	1.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-¨-	-¨-	-¨-

*[1] LOD – level of detection, in some studies indicated as level of quantification. The percentages are the respective percentages of the analysed metabolites in the same order as in the table
*[2] Indicated for the whole dataset that MHiNP and MOiNP were detectable in 99% and 92% of the samples and quantifiable in 95% and 77% of the samples, respectively.
*[3] Urine samples 8 consecutive days for each person
*[4] The 95th percentile is not indicated in the paper; the geometric mean with 95% confidence interval indicated
*[5] ΣDINPm: Sum of MINP, MCiOP, MHiNP and MOiNP adjusted for the molecular weights of the different metabolites.
*[6] The publication provides also the dataset by different age groups, sex and race/ethnicity,
*[7] MiNp and MCiOP measured in 503 males and 342 female samples; 125 male and 125 female samples were randomly selected for analyses of MHiNP and MOiNP. 75th percentiles are indicated in the paper.
n.i. = not indicated
empty cells indicate that the substance is not included in the studies

Annex 8 Table with biomonitoring data for DIDP metabolites

Population group	Specimen	Number of samples		Unadjusted concentration, µg/L, percentiles, Creatinine adjusted concentration in brackets, µg/g creatinine								Country	Sampling year	Data source
				MiDP		MCiNP		MHiDP		MOiDP				
		n	% > LOD *[1]	50th	95th	50th	95th	50th	95th	50th	95th			
Adults	Urine, morning	45	n.i			**0.7**	3.1	**1.0**	4.0	**0.2**	1.1	Germany	2007	Koch and Calafat (2009)
Children (5-6 y)	Urine	111	94,60, 30			**1.3 (2.2)**	4.5 (7.0)	**0.4 (0.6)**	4.9 (5.7)	**(<LOD)**	1.2 (1.6)	Germany	2007	Koch et al. (2011b)
Plastisol workers, comparison group	Urine, pre shift	10	100			**1.0 (0.7)**	n.i.	**0.6 (1.0)**	n.i.	**0.5 (0.6)**	n.i.	Germany	n.i.	Koch et al. (2011a)
	Urine, post shift	10	100, 100,n.i.			**1.1 (2.5)**	n.i.	**1.1 (0.8)**	n.i.	**0.7 (0.7)**	n.i.	-¨-	-¨-	-¨-
Plastisol workers, exposed	Urine, pre shift	5	100			**3.6 (2.5)**	n.i.	**2.2 (1.4)**	n.i.	**0.9 (0.7**	n.i.	-¨-	-¨-	-¨-
	Urine, post shift	5	100			**4.3 (5.3)**	n.i.	**16.8 (17.0)**	n.i.	**4.6 (5.2)**	n.i.	-¨-	-¨-	-¨-
Pregnant women	Urine	287	92			**1.7**	11.7					France	2003-2006	Philippat et al. (2011)
Adults	Urine, day	129	0,97, 100,87	<LOD	<LOD	**4.4**	104.0	**4.9**	70.6	**1.2**	15.0	USA	2003/2004	Silva et.al. (2006a)
> 6 years *[2]	Urine	2,548	90			**2.7 (2.5)**	17.5 (13.2)					USA	2005-2006	Calafat et al. (2011)
Pregnant women	Urine	19	68			**1.5**	n.i.					Israel	2006	Berman et al. (2009)

*[1] LOD – level of detection, in some studies it is the level of quantification (LOQ) which is indicated. The percentages are the respective percentages of the analysed metabolites in the same order as in the table
*[2] The paper provides also the dataset by different age groups, sex and race/ethnicity,
n.i. = not indicated
empty cells indicate that the substance is not included in the studies

EUROPEAN CHEMICALS AGENCY
ANNANKATU 18, P.O. BOX 400,
FI-00121 HELSINKI, FINLAND
ECHA.EUROPA.EU