EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE)

Phthalate migration from soft PVC toys and child-care articles

Opinion expressed at the CSTEE third plenary meeting Brussels, 24 April 1998

1. Summary

1.1 Terms of reference

The Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) has been invited by the EU Commission to give its opinion on the following points:

- The impact on children's health of the use of soft PVC containing phthalates in child-care articles and toys, which children of a young age could put into their mouths
- The limits which ought to be respected in relation to the migration of phthalates from these products
- The test method to be followed and the standards or parameters that should be taken into consideration to measure the phthalate migration level.

1.2 Procedure

The CSTEE established a Working Group in order to address these points. The Working Group agreed to the following process by which the health risks to children exposed to phthalates in toys being put in the mouth should be assessed. An exposure dose is calculated from the maximal amounts which are extracted when a surrogate for a phthalate-containing PVC-toy of 10 square cm is extracted for 6 hrs by a model saliva solution under dynamic conditions. Risk assessments are based on a body weight of an infant of 8 kg. This may be a worst-case approach since at present, a standardised and validated extraction method is not available. Critical effects for the phthalates were assessed from documentation made available to the Working Group, as well as by literature and data base searches. NOAEL (No-Observed-Adverse-Effect-Level) values were identified for 4 phthalates (DINP, DNOP, DEHP, DIDP), for BBP and DBP L(lowest)OAEL values were used. Where available, the same NOAEL values as identified by the EU Scientific Committee on Food (SCF), were employed, except for BBP because newer information has become available. A margin of safety was estimated by dividing the NOAEL values by the exposure dose. The Working Group noted that a margin of safety of at least 100 has been used in other exposure situations in order to identify a level of little concern.

1.3 Evaluation

The following maximum emission rates for DINP, DNOP, DEHP, DIDP, BBP and DBP were identified: 14 000, 1 500, 610, 280, 15 and 7 µg/10 cm^2/6hr, respectively. It is realised that for some of the lower emitting phthalates, these may not have been added to the products intentionally, but occur as by-products/impurities. The following intake doses were calculated for DINP, DNOP, DEHP, DIDP, BBP and DBP using a body weight of 8 kg for a teething infant: 1 700, 190, 75, 35, 1.9 and 0.81 µg/kg/day, respectively. The following NOAEL values were used for DINP, DNOP, DEHP and DIDP: 15, 37, 5 and 25 mg/kg/day, respectively. For BBP and DBP LOAELs (Lowest-Observed-Adverse-Effect-Level) of 171 mg/kg bw/day and 52 mg/kg bw/day, respectively, were used. Therefore an additional uncertainty factor of 5 was incorporated in the calculation of margin of safety.

For the 2 phthalates DINP and DEHP, the estimated margins of safety were below 100, namely 8.8 and 67, respectively. For DNOP, DIDP, DBP and BBP, the margins of safety were substantially higher (190, 710, 45 000 and 13 000, respectively). It is recognised that there are uncertainties with respect to assessing the actual exposures applying the current model values, both because these models differ considerably and have not been standardised and validated, and because the measured amounts show large variations throughout the various reported studies. On the other hand, the present assessment process has not taken into account that more than one phthalate may occur in children's toys or that there may be additional exposures through food, air and by dermal contact to these phthalates. Given these considerations and the possible enhanced sensitivity of young children to the effects of phthalates, the CSTEE concluded that the low margin of safety for DINP gives reason for concern. Although the margin of safety for DEHP was below 100, the CSTEE is less concerned with the estimated level of DEHP exposure, since humans appear to be less sensitive towards the critical effect of DEHP (hepatic peroxisome proliferation) identified in rats.

Given the contact of phthalate-containing PVC toys with lipophilic surfaces in the oral cavity, the CSTEE recommends that consideration should be given to applying more physiological extraction methods than have been used previously. The Committee is aware of an ongoing Dutch study in adult human volunteers which will in a more comprehensive fashion assess human exposure to phthalates from toys. Results of this study will be compared with *in vitro* extraction methods in order to arrive at a standardised method.

Besides the Dutch study, investigations on the kinetics of phthalate migration from soft PVC products are going on in several EU-countries, USA and Canada. The present evaluation of the CSTEE may be modified when the results of such studies become available. It should also be recognised that more extensive testing and evaluation of long-term effects of some of the phthalates may lead to a revision of the NOAEL values.

The CSTEE recommends that guideline values for extractable amounts of individual phthalates in toys be produced, incorporating a margin of safety of at least 100 from their respective NOAEL values. This would result in the following tolerable daily exposures for DINP, DNOP, DEHP, DIDP, BBP and DBP: 150, 370, 50, 250, 850

and 100 µg/kg, respectively (an additional uncertainty factor of 2 for BBP and 5 for DBP, respectively, because of starting from a LOAEL value). This would result in the following guideline values for maximum extracted amounts per 10 square cm and 6 hours for an infant weighing 8 kg: *DINP, 1.2 mg*; *DNOP, 3.0 mg; DEHP, 0.4 mg; DIDP, 2.0 mg; BBP, 6.8 mg;* and *DBP, 0.8 mg*. These values do not take into consideration that children may have additional phthalate exposures. The guideline values must be based on a standardised, validated extraction method. The Committee recommends that before introducing other plasticizers into toys which children can put into their mouths, the risk of their use should be assessed by the same process which has been applied to the phthalates discussed here.

In view of the importance in the human risk assessment of phthalates, the CSTEE recommends that an international expert meeting be convened in order to resolve the issue of using peroxisomal proliferation as a critical endpoint and how this should be extrapolated for assessment of human risk.

2. Introduction

2.1 Background

The Directive 92/59/EEC (OJ L No 228 of 11.08.92, p. 24) on General Product Safety, lays down in its Article 8 a procedure for the rapid exchange of information between Member States, on the emergency measures adopted or anticipated, in relation to products presenting a serious and immediate risk for consumers' health and safety.

In this context, the Commission services were approached by the Danish authorities, on 23 April 1997, regarding three emergency notifications (CSTEE/97/1-Add 1). These notifications concerned the measures taken against the commercialisation of various teething rings manufactured in China for the company "Chicco - Artsana".

According to these notifications the analyses carried out on behalf of the Danish authorities on these articles (CSTEE/97/1-Add 2), showed that they released certain phthalates (amongst others di-iso-nonyl phthalate, DINP), in quantities considered by them as unacceptable. Thus, after discussions with the competent authorities, the economic operators, in particular the Danish importer, withdrew these products from the market.

The Chicco company, while considering that its products were in conformity with Community legislation (Directive 88/378/EEC on toys safety) and did not present any danger, decided, on a preventive basis and awaiting the results of their own analyses, to voluntarily withdraw these products from the market of other Member States. It also carried out its own analyses taking into account the Document of CEN/TC-252/WG6 No.168, which is a working draft of CEN that proposes a test method to determine the migration of phthalates in child use and care articles. The results of the analyses conflicted with those of the Danish authorities (CSTEE/97/1-Add 3).

Moreover, the reactions of certain Member States - Belgium, England, Ireland and The Netherlands- to these notifications show that there are important differences

regarding the test methods used to measure phthalate migration (“dynamic” or “static” methods) and more specifically between the conditions or assumptions selected (period of exposure, surface contacts, type of stimulant...), to measure such migration (CSTEE/97/1-Add 4).

In their appraisals, certain Member States took account of the tolerable daily intakes (TDIs) fixed by the Scientific Committee for Food, in its Opinion on phthalates in infant formulae, expressed on 7 June 1996 (CSTEE/97/1-Add 5).

The differences mentioned above, had a considerable influence on the assessment of the risk related to such phthalate exposures which was made by the Member States, as well as on the determination of the measures they adopted. In addition, Belgium and United Kingdom required the Commission’s services to ask for the opinion of experts and/or of relevant Scientific Committees at the European level, on the risk presented to children’s health by toys and child-care articles made from soft PVC.

In the same context, the Commission’s services have been approached several times by organisations such as Greenpeace as well as by representatives of the toy industry (CSTEE/97/1-Add 7), concerning the problems presented by toys made from soft PVC and requesting information on the possible measures that could be adopted at EU level to cope with this problem.

2.2 Mandate

In view of this information, the divergence of opinions and the questions raised, the Scientific Committee on Toxicity, Ecotoxicity and the Environment has been invited to advise the Commission on the following points:

- *the impact on children's health of the use of soft PVC containing phthalates in child-care articles and toys, which children of a young age could put into their mouths;*

- *the limits which ought to be respected in relation to the migration of phthalates from these products;*

- *the test method to be followed and the standards or parameters that should be taken into consideration to measure the phthalate migration level.*

2.3 Working procedure

The CSTEE established a Working Group of five members plus an external expert to address these points. A preliminary position was formulated during the CSTEE plenary meeting in Brussels, 9 February, 1998. The Committee based its position on information given in the list of documents sent to it (CSTEE/97/1- Add 1 to Add 86), as well as in literature and data base searches. No-Observed-Adverse-Effect Levels (NOAEL) or L(lowest)OAEL were identified for the 6 phthalates identified in the highest amount in the analyses provided by Member States. The NOAEL values were identified for 5 phthalates (DINP, DNOP, DEHP, DIDP and BBP). For DBP a LO-AEL value was found.

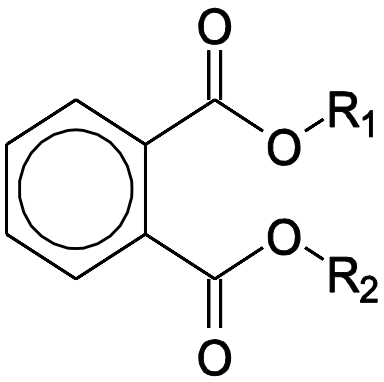
3. Exposure assessment

3.1 Types of phthalates in infants' toys

PVC is a rigid material which can be made soft by the addition of plasticisers. Plasticisers are in general high boiling compounds that, when incorporated into a polymer, cause a greater flexibility and workability of the material, brought about by an increased flexibility of the individual polymer chains. The most commonly used compounds for this purpose are esters of *o*-phthalic acid (phthalates).

The most used technique for the analysis of phthalic acid esters is gas chromatography with mass spectrometric detection. It is not difficult to extract total phthalates from PVC, but it is problematic to determine migration of phthalates from PVC.

Several esters of *o*-phthalic acid are used as plasticisers in PVC. The general structure of these are shown in Figure 1 where also the most important individual compounds, or groups of compounds, are listed.



Name	**Acronym**	**R_1**	**R_2**
Dibutyl phthalate	DBP	n-C_4H_9	n-C_4H_9
Dipentyl phthalate	DPP	n-C_5H_{11}	n-C_5H_{11}
Butylbenzyl phthalate	BBP	n-C_4H_9	-C_6H_5
Di(2-ethylhexyl) phthalate	DEHP	-$C_2H_4(C_2H_5)C_4H_9$	-$C_2H_4(C_2H_5)C_4H_9$
Di-iso-octyl phthalate	DIOP	-C_8H_{17}	-C_8H_{17}
Di-n-octyl phthalate	DNOP	n-C_8H_{17}	n-C_8H_{17}
Di-iso-nonyl phthalate	DINP	-C_9H_{19}	-C_9H_{19}
Di-iso-decyl phthalate	DIDP	-$C_{10}H_{21}$	-$C_{10}H_{21}$

Figure 1. General structure of esters of o-phthalic acid. The groups R_1 and R_2 are generally the same with the exception of BBP which contains one aliphatic and one aromatic side chain. The four first compounds in the table plus DNOP (according to CSTEE/97/1-Add 48, not used alone) are single

substances, while those with branched chains (designated iso-) are complex mixtures which can differ between products (see e.g. CSTEE/97/1-Add 75).

At the Laboratory of the Government Chemist, UK, 113 plastic teethers and toys have been investigated for their content of plasticisers (CSTEE/97/1-Add 19). The weight loss after diethyl ether extraction was used as a measure for the plasticiser content and losses around 50 % were quite common. In 82 samples containing phthalates, 45 had DINP as the major component, 20 DEHP and 11 DIDP.

In an investigation published by Greenpeace 1997 (CSTEE/97/1-Add 9) 71 toys were analysed. These samples were bought in 17 countries (9 from EU), but at least half of the toys were produced in China. In 12 of the 63 samples made of PVC, no or low levels of phthalates could be detected. In the remaining PVC samples one or several phthalates were detected, and the most common product was DINP (44 toys) followed by DEHP (32 toys), DIOP (2 toys) and DIDP (2 toys). DBP and BBP could be detected in 7 and 6 toys, respectively, but always at levels much lower than 1%.These latter two compounds probably occur as by-products/impurities and have not been added intentionally to the products.

Thirty teethers available on the Spanish market were analysed for DEHP and DINP (CSTEE/97/1-Add 20) and phthalates were detected in 12 of these.

The UK Government has followed the content of plasticisers in toys (CSTEE/97/1-Add 37) and the major phthalates found in PVC during the period 1990 and 1996 are shown in Table 1. The 29 teether samples tested in UK 1996 were from China, Thailand, Malaysia, Italy, Canada and UK. These results indicate that DINP is the most commonly used phthalate in teethers at present.

Table 1. Major plasticisers found in PVC articles in an investigation done by the UK Government (CSTEE/97/1-Add 37).

Phthalate	1990 (n=18)	1991 (n=27)	1992 (n=16)	1996 (n=29)
DBP	2			
DEHP	4	13	2	1
DINP	6	10	14	28
DIDP	6	4		

It should be noted that it can be difficult to detect the presence of e.g. DNOP in the presence of the multi-component product DINP, as has been pointed out by NERI in Denmark (CSTEE/97/1-Add 2, rev 1).

3.2 Leaching of phthalates from infants' toys

3.2.1 Articles of interest

Humans may be exposed to phthalates from toys in many different ways. The exposure via vapours in the air is probably rather small, especially for the higher

homologues as their vapour pressure is very low. The plasticiser can be transferred to the skin via direct physical contact and there is also an indirect exposure route via the food. These exposures will be discussed further below in Sections 3.2.5 and 3.2.6.

For small children, however, the oral exposure is probably the most effective route as they suck and "chew" the toys. The physical massaging of the products at the same time as there is a continuous flow of fresh saliva around the products will serve as an extraction procedure for the phthalates. The main focus of this report will therefore be concentrated on toys for small children, especially so-called teethers which are given to kids when their first teeth erupt.

3.2.2 Methods to study the leaching of phthalates

There is at present no standard method available to mimic the rather specific exposure during "chewing". CEN is working on a method and a "working draft" (CEN, 1997) proposes a static extraction of the plasticiser from the sample to an artificial saliva for 6 hours.

However, the Laboratory of the Government Chemist, UK (CSTEE/97/1-Adds 19 and 37) has compared leaching during static and dynamic conditions and report higher recoveries from the dynamic methods. The precision between parallel samples seems to be rather low in their study, which, according to the authors, may be explained by the release of particles from the sample. If this is the case in these experiments, it is probably a still larger problem in real life.

Several methods have been used to mimic chewing of the material. In a Dutch study the leaching with synthetic saliva was done in an ultrasonic bath (CSTEE/97/1-Add 5), while a study in UK tumbled the polymeric material in the saliva together with agate pellets (CSTEE/97/1-Add 19). Also, a report from the US shows an increase in phthalate leaching when the samples were impacted with a piston (CSTEE/97/1-Add 70). There has also been reference (e.g. in CSTEE/97/1-Add 37) to an Austrian study where actual sucking of the test material have been tested and the result indicated that amounts of phthalates migrated from PVC by the static method < the agitation method < sucking. In an ongoing Dutch study *in vitro* extraction tests will be compared with the results from human volunteers chewing and sucking the toys (CSTEE/97/1-Adds 63 and 64). The reporting of the results from this investigation is expected in August 1998.

It should also be remembered that the possibility exists that the phthalates may be hydrolysed by saliva and within the oral mucosa (Munksgaard and Freund, 1990). As a result, monoesters of released phthalates will also be taken up by the child. This is not taken into account when measuring the presence only of the parent compounds in the leachates.

The conclusion at this time must be that it is difficult to estimate the phthalate exposure of children chewing toys and teethers made of PVC containing these compounds as plasticisers. The available leachate data references in the following section are mainly produced in *in vitro* tests and may not properly reflect the real-life situation. Since it was not possible from a scientific viewpoint to evaluate which of the leachate data were more realistic than others, the CSTEE decided to use the

maximum emission rates as a starting point, representing the worst-case situation. Further knowledge about possible exposure to released particles and direct transfer to the oral cavity and ingestion is needed. Further there is a need of a harmonised test method for migration of phthalates (see point 7. *Conclusions*).

3.2.3 Calculation methods

In the CEN draft report (CEN, 1997) in their proposal for specific limits for products which are intended to be mouthed by children such as soothers (pacifiers, babies' dummies), the following criteria has been applied: exposure period, 12 h; product surface area, 10 cm^2; body weight, 5 kg. The CSTEE judged that these values were overly conservative for an infant using PVC-based teethers and decided to use the following more realistic criteria: exposure period, 6 hr; product surface area, 10 cm^2; body weight, 8 kg. The Committee selected in its calculations this scenario as a worst-case in the present evaluation.

The leached amount of phthalates thus have to be calculated per area and time. One of the studies (CSTEE/97/1-Add 12) report the extractions on a weight basis, but these have been recalculated using an average of the area per mass factors given in a report from Spain (CSTEE/97/1-Add 20). These values generally range between 6 and 21 cm^2/g, with an extreme value of 45. The arithmetic mean of all these is 15 cm^2/g which was used for the conversion of weight-based results.

3.2.4 Leached amounts

There are a number of determinations of phthalates leachate from toys reported. Most of them describe the techniques used very briefly and it is thus difficult to judge the quality of the results. Table 2 presents the available data from materials on the European market.

Table 2. Reported leaching of phthalates from toys under static and dynamic experimental conditions

Unit	DINP	DNOP	DEHP	DIDP	BBP	DBP	DPP	DNNP	Ref.
µg/dm^2/24h	nd-558233	nd-60677	nd-4193	na	nd-611	nd-259	nd-256	nd-1851	Add 2, rev 1
µg/cm^2/h	9-35	na	na	0.9-4.6	na	na	na	na	Add 5
mg/kg/6 h	0.094-0.29	na	nd	nd-0.084	nd	nd-0.06[B]	nd	na	Add 12[C]
mg/dm^2/6 h	3.89-4.51	na	1.79-2.13	na	na	na	na	na	Add 19
mg/cm^2/h	nd-0.022	na	0.00031-0.0072	na	na	na	na	na	Add 20
µg/dm^2/6 h	na	na	10.5-652.9	nd	na	na	na	na	Add 47
mg/dm^2/h	0.85-14	na	0.2-1[A]	na	na	na	na	na	Add 49
mg/dm^2/24 h	<0.02-<0.05	na	<0.004-0.01	na	na	na	na	na	Add 55[C] (LGA)
mg/dm^2/h	<0.1	na	<0.1	<0.1	<0.1	<0.1	na	na	Add 55[C]

									(TUV)
mg/dm^2/24 h	<0.05-0.25	na	<0.05-0.18	na	na	na	na	na	Add 55 (Budde)

nd: under unspecified detection limit.

na: not analysed.

[A] 4.0-22/24 h, ref. CSTEE/97/1-Add 49; [B] 0.12/12 h, ref. CSTEE/97/1-Add 12

[C] Static methods used for the leaching of phthalates

3.2.5 Intake doses from toys

To be able to compare the results given in Table 2, the maximum emission values are converted to a daily dose for a 8 kg infant mouthing 10 cm^2 of the investigated material for 6 hours every day. For the one study giving the results on a weight basis these are recalculated using the specific area 15 cm^2/g. The resulting maximum doses are presented in Table 3.

Table 3. Calculated maximum dose (µg/kg bw/day) of phthalates for a 8 kg baby mouthing 10 cm^2 of the toy during 6 h per day (Ref CSTEE/97/1- Adds indicated).

DINP	**DNOP**	**DEHP**	**DIDP**	**BBP**	**DBP**	**DPP**	**DNNP**	**Ref.**
1 700	190	13		1.9	0.81	0.81	5.8	Add 2, rev 1
260			35					Add 5
0.024			0.007		0.005			Add 12
56		27						Add 19
170		54						Add 20
		8.2						Add 47
1 100		75						Add 49
< 0.16		< 0.03						Add 55 (LGA)
< 8		< 8	< 8	< 8	< 8			Add 55 (TUV)
0.78		0.56						Add 55 (Budde)

The lowest results are reported by laboratories using static extraction methods [CSTEE/97/1-Add 12, Add 55 (LGA) and Add 55(TUV)]. Also the rest of the results cover a wide range, but it has to be kept in mind that these investigations represent many different toys and that different methods are used. In one report [CSTEE/97/1-Add 55 (Budde)] it is assumed that the same type of samples have been analysed as those investigated by Greenpeace (CSTEE/97/1-Add 49) and it is claimed that there are two to three orders of magnitude difference between the results. These observations further underline the need for standardised methods for the determination of leaching of chemicals from toys.

The Committee decided to use these maximum exposure doses in its following assessment reflecting a possible worst-case scenario. Such a starting point should also be warranted because more than one phthalate may occur in children's toys and because there may be additional exposures through food, air and by dermal contact to these phthalates.

3.2.6 Other exposures to phthalates

Humans are exposed to phthalates via air, water, food and dermal contact. Both in air and water these substances appear both in gaseous/dissolved form and adsorbed to particles. Few of the available data are actual measurements, the rest being results of different calculations or models. Unfortunately, it has not been possible to find any measured data on phthalate levels in breast milk which may be a source of exposure for many infants judged from the excretion of orally dosed phthalates in breast milk of rats (Dostal et al., 1987).

In the limited time available for the present risk assessment of phthalates in toys, it has not been possible to make a complete literature survey on other exposure routes for these compounds, but the information available in published risk assessments has been used. Also documentation from several ongoing assessments has been consulted, including those collected by *rapporteurs* for the European programme for existing chemicals where DBP, BBP, DEHP, DINP and DIDP are being assessed.

The CEN draft report (CEN, 1997) estimates the exposure from toys to be 10% of the total exposure for a given phthalate. The comparisons made here indicate that this is an ambiguous assumption and that perhaps 20-30% would be a more realistic figure (*v.i.*).

3.2.6.1 DINP

No specific information has been found regarding total exposure to DINP, other oral exposures or exposures from other routes to this compound.

3.2.6.2 DNOP

No specific information has been found regarding total exposure to DNOP, other oral exposures or exposures from other routes to this compound.

3.2.6.3 DEHP

The levels of intake of DEHP may have changed due to alterations in production and consumption of DEHP in PVC during the recent years. DEHP is the most widely used plasticiser [comprising 50% of all phthalate ester plasticisers that softens resins (WHO, 1992)]. The reports from UK and Greenpeace revealed that DEHP is being replaced by DINP in PVC-toys, to some extent.

DEHP has been quite extensively studied and thus it has been possible to estimate the daily intake for the general population. In a Canadian risk assessment (Canadian Environmental Protection Act, 1994a) the intakes given in Table 4 were calculated.

Table 4. Estimated daily intake of DEHP for small children in Canada (from Canadian Environmental Protection Act, 1994a)

Medium	**Estimated intake (µg/kg bw/day)**	
	Age group	
	0 - 0.5 year	***0.5 - 4 year***
Ambient air	0.00003 - 0.0003	0.00003 - 0.0003
Indoor air	0.86	0.99
Drinking water	0.13 - 0.38	0.06 - 0.18
Food	7.88	17.81
Soil	0.000064	0.000042
Total estimated intake	8.87 - 9.12	18.86 - 18.98
Children's products	<0.025 - 11.51	<0.0089 - 4.07

The estimated maximum intake of DEHP from children's products is smaller in the Canadian material than that has been reported in more recent studies. The Canadian estimate is, however, based on a leaching rate of 30 µg/h from the toys in the mouth. A 6 h exposure of a 8 kg child would give a dose of 23 µg/kg bw, day, which is an order of magnitude lower than the presently identified worst case. In The Netherlands the usual background load for infants has been estimated to 23 µg DEHP/kg body weight, and is a worst-case estimate (CSTEE/97/1-Add 40).

In the draft of an European risk assessment of DEHP there is a reference to a report from the industry (Chemical Manufacturers Association, 1984). In this it is claimed that, during contact with DEHP-containing material, the exposure to skin is 6.6 µg/cm^2/h. Absorption rates up to 1.06 µg/cm^2/h have been reported (ECETOC, 1994). Thus, if a child is playing with a DEHP containing toy of 1 dm^2 for 4 hours the transferred amount (100 x 4 x 6.6 = 2 640 µg) will be almost absorbed (2 544 µg) over 24 hours if the skin is not washed during that time. For a 8 kg child this corresponds to a dose of 199 µg/kg bw/day. The possible exposure from other DEHP containing surfaces (carpets, wallpapers, paint ....) is not possible to estimate at present, but the conclusion must be that the intake from toys may not be the major source for DEHP exposure.

The estimated oral exposure amount of young children to DEHP by contact with items used by children was estimated to be 62 mg to 665 mg (pacifiers, teethers, and vinyl toys) for the 0 to 36 month old child (CSTEE/97/1-Add 13).

In food the highest DEHP levels were found in milk (31 mg/l, fat basis) and cheese (35 mg/kg, fat basis) (WHO, 1992). In milk products with a fat content of 1%, 3%, or 35%, contents of 0.05, 0.12 or 1.4 mg/kg, respectively were found (Sharman et al., 1994). In a Danish study from 1996 the concentration of phthalates in infant formulae were rather low. The highest content of DEHP in various kinds of products was 60 µg/kg. This amount will give a daily intake of 30% of the daily allowance, assuming a 3 kg infant and consumption of 0.75 litre of milk (LST NYT, 1996).

Transfer of DEHP or other phthalates into milk of lactating rats has been shown by Dostal et al. (1987). No information about excretion and amount of phthalates in human milk has been found.

The estimated total daily consumption of commodities highly likely to be contaminated with DEHP, is estimated to vary between approx. 210 to a maximum of 2 000 µg/person/day (CSTEE/97/1-Add13; WHO, 1992). A number of recent estimates of the average daily lifetime exposure to DEHP gives values from 2.3 µg/kg bw to 6 µg/kg bw (ECETOC, 1994; MAFF 1996; Canadian Environmental Protection Act, 1997).

The levels of DEHP in community drinking water are thought to be low, although individual instances of contamination may be as high as 170 µg/l. In contaminated groundwater in the Netherlands levels from 20 to 45 µg DEHP/l have been reported (WHO, 1992).

In city air, concentrations of phthalates in atmospheric particulate matter range from 5 to 132 ng/m^3, but a concentration of 300 ng/m^3, has been reported. DEHP in indoor air might be substantially higher, levels of 50 µg/m^3, have been reported (WHO, 1992). Suspended particle exposure (phthalate concentration adsorbed to sedimented dust in dwellings) to the plasticiser DEHP has been shown to be one- to threefold higher than the estimated vapour phase exposure (Øie et al., 1997).

3.2.6.4 DIDP

No specific information has been found regarding total exposure to DINP, other oral exposures or exposures from other routes to this compound.

3.2.6.5 BBP

Based upon the maximum concentration reported in various environmental media (including ambient and indoor air, drinking water, food and soil), reasonable worst case estimates of exposure for the general population range from 2 µg/kg bw/day in 70-kg adults to 6 µg/kg bw/day in 7-kg infants (Canadian Environmental Protection Act, 1997). Food is by far the major source contributing over 90% of intake. Indoor air is second in importance, contributing 1 to 10 %. Also in this case, the above calculated maximum intake from toys (1.9 µg/kg bw/day) does not seem to be envisioned as a dominant source. The highest estimated intake from infant formulae in UK was 0.9 µg/kg bw/day (CSTEE/97/1-Add 8).

Food products on the US market have been shown to contain 0.5 to 53 mg BBP/kg, individually or in combination with other plasticisers (Castle et al., 1988). Retail samples of Canadian butter and margarine wrapped in aluminium foil-paper laminate were found to contain BBP at levels up to 47.8 mg/kg (Page and Lacroix, 1992). Danish studies of infant milk supplements have revealed levels of BBP up to 10 µg/kg (LST Nyt, 1996).

3.2.7.6 DBP

The daily intake of DBP has been estimated in a Canadian risk assessment (Canadian Environmental Protection Act, 1994b). The intakes were calculated for different age groups and the results for the youngest groups are shown in Table 5.

Table 5. Estimated daily intake of dibutyl phthalate (DBP) for young children in Canada (Canadian Environmental Protection Act, 1994b)

Medium	**Estimated intake of DBP (µg/kg bw/day)**	
	Age group	
	0 - 0.5 year	***0.5 - 4 year***
Ambient air	0.0002 - 0.0004	0.0003 - 0.0004
Indoor air	0.7	0.9
Drinking water	0.1	0.06
Food	1.6	4.1
Soil	<0.0005 - 0.007	<0.0004 - 0.005
Total	**~2.4**	**~5.0**

These Canadian calculations do not at all take leaching from toys into account and it can be seen that other routes are expected to result in doses as high (or even twice as high) as those described in Table 3.

In the draft of an ongoing risk assessment of DBP in Europe several sources of human exposure to this compound are identified. Nail polish, hair spray and adhesives are probably not important exposure sources for small children, but DBP is also used in food wrappings. Table 6 gives concentrations of DBP in some analysed food samples.

Table 6. Some reported concentrations of DBP in food

Food type	**DBP in mg/kg food**	**Reference**
Margarine	10.6	Page and Lacroix, 1992
Vodka	0.05-0.25	Hatanaka et al., 1994
Confectionery	0.02 - 14.1	Castle et al., 1989
Confectionery, meat pies, cake sandwiches	0.5 - 53	Castle et al., 1988
Cheese, salted meat, chips, milk, vegetable soup	0.07 - 2.80	Cocchieri, 1986
Butter	2 - 11	Morita et al., 1973

It is difficult from the data in Table 6 to judge the exposure of small children. The maximum likely human intake in UK in adults has been estimated to 31 µg/kg bw/day (WHO, 1997). This is about an order of magnitude more than our estimated worst case infant exposure from toys.

Exposure of the general population to DBP in food has also been estimated on the basis of data from a market-basket survey in Canada, it amounted to 7 µg/kg bw/day (WHO, 1997). The intake can vary considerably, depending upon the nature and

amount of packaged food that is consumed and the nature of use of food wrapping in food preparation.

The highest estimated intake of DBP from infant formulae was 0.014 mg/kg bw (CSTEE/97/1-Add 8). A recent study from Denmark reported maximum concentrations in the finished formulae of less than 0.1 mg/kg (LST NYT, 1996).

Based upon a survey of homes in California the medium daytime concentration of DBP in indoor air was 420 ng/m^3 (WHO, 1997).

4. Effect assessment

4.1 Mechanistic considerations

Phthalate esters, representing a variety of chain lengths and degrees of branching in the alcohol moiety, are able to induce peroxisome proliferation in rats and mice. In general, the longer chain esters are more potent than the shorter chain ones, and branched chain esters seem to be more potent than straight ones. The relative potency of DIDP, DEHP, DINP, DBP and BBP (calculated as nanomoles of palmitate oxidised per minute per milligram of homogenate protein divided by micromoles of chemical ingested per kilogram body weight per day) are 17, 15, 11, 3, 2 (after Barber et al., 1987). Carcinogenicity studies have been conducted in rodents on DINP, DEHP and BBP, only DEHP was found to be hepatocarcinogenic in rats and mice (Ashby et al., 1994). It is assumed that the carcinogenic effect is related to the peroxisome proliferation in rats. A carcinogenic effect solely related to peroxisome proliferation in rodents may have little relevance for humans (IARC 1995). MEHP, an active metabolite of DEHP, does not induce peroxisome proliferation in cultured human hepatocytes (Elcombe and Mitchell, 1986).

Inasmuch as induction of hepatic peroxisomal proliferation is the most sensitive change occurring in rats and mice after low doses of several of the phthalates, it can be argued that such changes also may have little relevance as critical endpoints for non-tumorigenic effects in humans. At least from a quantitative perspective, humans appear to be much less sensitive than rats and mice towards peroxisome proliferators (Ashby et al., 1994). The Scientific Committee for Food has chosen to use peroxisomal proliferation as the critical endpoint for DEHP and to apply an uncertainty factor (safety factor) of 100 as a matter of prudence (SCF, 1994). In concurrence with this decision, also the CSTEE will use peroxisomal proliferation as a critical endpoint for the phthalates under discussion when this is the most sensitive effect. The application of an uncertainty factor of 100 may be overly conservative given the apparent lower sensitivity in humans towards peroxisome proliferation. However, establishing a data-derived lower uncertainty factor is at present seen as difficult. Thus, the CSTEE recommends that an international expert meeting be convened in order to resolve the issue of using peroxisomal proliferation as a critical endpoint and how this should be extrapolated for assessment of human risk.

Severe testicular atrophy is observed after administration of phthalates with carbon chains of C_4-C_6. In general it is believed that the effect on testis is a specific effect on the Sertoli cells, but a recent publication suggests that there might be some

relationship between peroxisome proliferation and rat testis function (Mehrotra et al., 1997). In continuous breeding studies some phthalate esters reduce the fertility of both male and female mice, and in mice, teratogenic and embryotoxic effects are seen for some phthalates. The estrogenic effects of some phthalates has recently been investigated. A weak activity has been reported *in vitro* with BBP and DBP, but has not been shown to be of relevance in *in vivo* tests in laboratory animals (Coldham et al., 1997).

DEHP is a known reproductive toxicant and teratogen. In a recent publication it has been shown that the teratogenic effect is not related to activation of the peroxisome proliferator receptor since mice, homozygous wild type without the receptor, produced malformed offspring after gestational treatment with DEHP (Peters et al., 1997).

A recent publication hypothesises a relationship between testicular cancer and occupational exposure to polyvinyl chloride containing phthalate plasticisers (Hardell et al., 1997).

4.2.1 DINP

Two CAS Nos. exist for DINP: 68515-48 and 28553-12-0. Due to various production methods, different commercial products may vary in composition.

The overall NOAEL as used by the EU-SCF in its 1986 and 1988 evaluation is 0.03% in the diet from a chronic rat study (performed by Exxon, first published in 1998 by Lington et al.), corresponding to a dose of 15 mg/kg bw (this NOAEL was used in deriving the temporary TDI of 0.03 mg/kg bw using an uncertainty factor of 500). In 1996 the SCF re-evaluated DINP since new data had become available. In a 2 week study with male Wistar rats in which special attention was given to peroxisomal proliferation, a NOAEL of 200 mg DINP/kg diet corresponding to 18.2 mg/kg bw/day was established (SCF opinion expressed on 7-8 March 1996, SCF report not yet published). The SCF maintained the temporary TDI of 0.003 mg/kg bw/day awaiting on studies on teratogenicity and reproduction toxicity. Since then new data have become available.

The critical effects of DINP are on the liver. Two studies indicate a NOAEL for peroxisome proliferation or effects on liver enzymes in the range of 18.2 to 50 mg/kg bw (CSTEE/97/1-Adds 25 and 50). No effects have been observed on body and liver weight in male Wistar rats exposed for 2 weeks to 0, 20, 60, 200, 600, or 2000 mg/kg DINP/kg diet. Enzymes relevant for peroxisomal proliferation showed a NOAEL of 18.2 mg/kg bw. Effects on enoyl coenzyme A-hydratase were observed at a dose level of 5-7 mg/kg bw/day. In a study performed by the Hüls company a NOAEL could not be established, a LOAEL of 25 mg/kg bw/day was indicated (Hüls, 1993).

Male and female rats were fed DINP at dietary levels of 0, 0.03, 0.3 or 0.6% for a period up to 2 years. Using consumption assumptions, these dietary levels correspond to average DINP doses of approximately 0, 15, 150 or 300 mg/kg/ bw/day, respectively. Statistically significant and dose-related increases in liver and kidney weights were observed in the mid and high dose groups. Histopathologically, in the top dose centrilobular to midzonal hepatocellular enlargement was observed and in

the kidneys an increase in tubular cell pigment was observed. No peroxisome induction was observed in the livers of treated rats compared with controls. A slight, but statistically significant increase in mononuclear cell leukaemia in the mid and high dose groups was also observed. Based on this study the NOAEL for DINP is 15 mg/kg bw (Lington et al., 1997).

A recent published study of prenatal toxicity of 3 different types of DINP (0, 40, 200, 1000 mg/kg bw, gestational days 5 to 15 in rats) showed foetal effects (slight increased rates of skeletal retardation and soft tissue and skeletal variations, occurrence of some soft tissue and skeletal malformations). These effects were seen with one of the types of DINP at maternal toxic doses (1000 mg/kg bw/day), but not at the two lower doses (Hellwig et al., 1997).

Conclusion. The critical effect used for assignment of a NOAEL value for DINP is increased liver and kidney weight observed in an oral 2-year rat study (Lington et al., 1997). The NOAEL value is 15 mg/kg bw/day.

4.2.2 DNOP

CAS No 117-84-0. The most often used acronym is DNOP, sometimes also DOP has been used. One should be aware of the fact that DEHP also has been referred to as DOP several times in the literature.

No overall NOAEL could be established by the EU-SCF in its 1993 evaluation. In view of this DNOP was included in a temporary Group Restriction of 0.05 mg/kg bw/day, based on the data for DEHP together with those phthalates for which no specific TDI was established (SCF opinion expressed 16-17 June 1994, SCF report not yet published). In the SCF evaluation it is noted the results on DNOP concerning testicular effects were contradictory (subacute special studies showing no effect, whereas in 3 month and chronic feeding (2 years) studies such an effect was reported to occur).

In a very recent oral 13-week study in rats, DNOP was shown to cause mild microscopic changes in the liver (endothelial nuclear prominence, nuclear hyperchromicity, anisokaryosis) and thyroid (reduced follicle size, reduced colloid density) at 5000 ppm in diet . The NOAEL was found to be 500 ppm in the diet equivalent to 36.8 mg/kg bw/day (Poon et al., 1997).

The result of the in vivo screen for estrogenicity by Meek et al. (1997) was negative (no effect). *In vitro* estrogenicity assays were negative (including one assay carried out with the mono-ester).

In the recent 13-week study in rats by Poon et al. (1997), DNOP did not induce testicular damage at a dietary concentration of 5000 ppm equivalent to 350 mg/kg bw.

Conclusion. The critical effects used for assignment of a NOAEL value for DNOP are microscopic alterations in the liver and thyroid observed in an oral 13-week rat study (Poon et al., 1997). The NOAEL value is 37 mg/kg bw/day.

4.2.3 DEHP

CAS No.117-82-7. DEHP has also been referred to as DOP in the literature.

The Scientific Committee for Food has established a TDI for DEHP of 0.05 mg/kg bw/day (CSTEE/97/1-Adds 8 and 40).

Numerous studies have investigated the toxicity of DEHP following repeated oral administration in experimental animals, in particular the rat. In the liver of rats, the most striking effects observed are hepatomegaly, peroxisome proliferation, and hepatocellular tumours. Other effects include reduced body weight and body weight gain, mortality at relatively high dose levels, hypolipidemic effects (decreased plasma levels of cholesterol and triglyceride), alterations in clinico-chemical parameters, and effects on other tissues and organs than liver and testis. The lowest NOAEL for general toxicity (i.e. effects on liver and testis are excluded) observed in a well-performed 13-week-study in rats is 0.1% DEHP in the diet (corresponding to 63 mg/kg bw/day) based on increased relative kidney weight.

Hepatomegaly and peroxisome proliferation (characterised by increased number and size/volume of peroxisomes, changes in morphology, and alterations in peroxisomal associated enzyme activities (e.g. PCoA, catalase, CAT, LAH-11, LAH-12, and a-GD) have been observed in rats from about 14 days of exposure and throughout the exposure period at dose levels from 0.05% DEHP in the diet. Hepatocellular tumours were detected in rats and mice at higher dietary DEHP levels. There is a clear association between peroxisome proliferation and the occurrence of liver tumours in rats and mice after long-term exposure. The dose level necessary to induce hepatic tumours are from 300 to 430 mg/kg bw/day. Marked species differences are apparent in response to peroxisome proliferation. Rats and mice are very sensitive and hamsters are moderately sensitive, whereas guinea pigs and monkeys appear to be relatively insensitive or non-responsive at dose levels that produce a marked response in rats. In rat (the most sensitive species), the lowest NOAEL for peroxisome proliferation (based on light and electron microscopic morphometric analysis and induction of peroxisome associated enzymes) from a well-performed study is 5 mg/kg bw/day (RIVM, 1992).

One study has documented that DEHP is secreted into the milk of rats exposed to DEHP during the lactation period resulting in changes of the milk composition and also adverse effects in suckling pups (reduced body weight and induction of peroxisomal enzyme activities) (Dostal et al., 1987).

No data are available concerning peroxisome proliferation in humans exposed to DEHP. Data from studies of some other peroxisome proliferators (hypolipidemic drugs) do not indicate that humans are sensitive to peroxisome proliferation. Several oral studies in rats point to dose levels from 5 to 20 mg/kg bw being the dose level at which liver weight increases and peroxisomal proliferation is induced. The ability of DEHP to induce hepatocellular carcinomas in rodents has been linked to their capability for inducing peroxisomal activity in the liver.

The Scientific Committee for Food has established a TDI for DEHP of 0.05 mg/kg bw based on the absence of evidence for genotoxicity and the suggested relationship

between proliferation of liver peroxisomes and the occurrence of hepatomegaly and liver tumours in rats.

Testicular atrophy is the most obvious nonhepatic effect after oral, subcutaneous or intraperitoneal administration to male rodents, but also the ovary seems to be affected by DEHP exposure. The effects on gonads are influenced by the age of the experimental animals. Oral administration via diet seems to be the most sensitive exposure route. Swiss (CD-1) mice (20 animals of each sex) were dosed with 0.30% DEHP (150 mg/kg bw) in the diet (Morrissey et al., 1989). Continuous breeding studies were used to evaluate reproductive performance over a 98-day cohabitant period. Mice were separated by sex during the first 7 days of DEHP treatment. After detection of an adverse effect of DEHP treatment, a 1-week crossover mating trial was carried out between previously treated males and control females. Reproductive ability was assessed at 10 weeks of age in a single breeding trial over a 7-day period. At necropsy, the endpoint of target organ toxicity examined included organ, weights, per cent motile sperm, sperm concentration, and per cent abnormal sperm. In DEHP treated mice, there was a reduction in epididymal and testicular weights and sperm motility and sperm concentration were reduced. The number of abnormal sperm was increased.

In another fertility assessment by continuous breeding dietary levels of 0, 0.01, 0.1 and 0.3 % DEHP (0, 20, 200 and 600 mg/kg bw/day) were used. At the highest dose level a complete suppression of fertility was seen in the male mice. Sperm assessment showed that the per cent motile sperm and sperm concentration in cauda epididymis were significantly decreased. The group dosed with 0.1% (200 mg/kg bw) had significantly lower fertility and proportion of live pups than the control animals (Morrisey et al., 1989).

A two generation study in CD-1 mice (GLP) was performed with oral feeding of 0.01, 0.025 and 0.05 % of DEHP in the diet (NTIS 1988). The doses were equivalent to about 19, 48, and 95 mg/kg bw per day. The study was carried out to examine the effect of prenatally administered DEHP on the growth, development, and reproductive performance of the F1 generation. The F1 generation was mated within dose-groups of sexual maturity and F2-offsprings were evaluated for viability and growth through postnatal day 4. For F1-litters, the percentage of prenatal mortality was increased at the high dose (9% versus 26.4%). During the neonatal period, the per cent of viable pups was significantly decreased at 0.05% DEHP. No other effects of DEHP were observed upon growth, viability, age of acquisition for developmental landmarks (incisor eruption, wire grasping, eye opening, testes decent, vaginal opening, or spontaneous locomotor activity) on postnatal days 14, 21 or 50. A NOAEL for parental toxicity and for F2-offspring was both 95 mg/kg bw. A NOAEL of 48 mg/kg bw was concluded for F1-offspring.

Numerous studies have shown that DEHP is embryotoxic in rats in doses close to maternal toxicity. In mice, several studies have shown that DEHP is embryotoxic and teratogenic in doses not showing maternal toxicity.

Dietary levels of 0, 0.5, 1.0, 1.5 or 2% of DEHP were given to groups of F344/CrlBr rats (34-25) throughout gestation (day 0-20) (Tyl et al., 1988). The rats were sacrificed on day 20. Food intake was significantly decreased at all dose levels.

Maternal toxicity, reduced body weight gain, increased maternal absolute and relative liver weights and reduced foetal body weights per litter were observed at dietary level of 1.0%. There were no treatment-related differences in the number of corpora lutea or implantation sites per dam or in the percentage preimplantation loss. The number of resorptions, nonliving and affected implants per litter was significantly increased and the number of live foetuses per litter was significantly decreased at 2% DEHP. Mean foetal body weight was significantly reduced at all dose levels. The number and percentage of malformed foetuses per litter were not significantly different from control. The NOAEL for maternal and embryonal toxicity was 0.5% DEHP (357 mg/kg bw per day).

Dietary levels of 0, 0.025, 0.05, 0.10, 0.15 or 0.20 % of DEHP were administered to groups of 1-CR outbred mice, 30-31 per group throughout gestation days 0-17 (Tyl et al., 1988). The doses given were equivalent to 0, 44, 91, 190.6 or 292.5 mg/kg bw/day. Maternal toxicity, indicated by reduced maternal body weight gain, was noted in the two highest dose groups, mainly due to reduced gravid uterine weight. There were no treatment-related effects on the number of corpora lutea, implantation sites per dam, the percentage preimplantation loss, and sex ratio of live pups. The number and percentage of resorptions, late foetal death, dead and malformed foetuses were all significantly increased, and foetal weight and the number of live foetuses per litter was significantly reduced at 0.1 and 0.15%. The percentage of all foetuses with malformations and the percentage of malformed foetuses per litter were significantly increased from doses of 0.05%. External malformations observed were unilateral and bilateral open eyes, exophthalmia, exencephaly, and short, constricted, or no tail. Visceral malformations were localised predominantly in the major arteries. Skeletal defects included fused and branched ribs and misalignment and fused thoracic vertebral centra. The maternal NOAEL in this study appeared to be 0.05% (91 mg/kg bw/day), and the level for embryofetal NOAEL was 0.025% (equivalent to 44 mg/kg bw per day).

DEHP was given to ICR mice in the diet at 0, 0.01, 0.1 or 0.3% equivalent to 0, 20, 200 or 600 mg/kg bw per day in a continuous breeding experiment (Lamb et al., 1987). Both male and female mice were exposed during a 7-day premating period and were then randomly grouped as mating pairs. The dosing continued for 98 days. Exposure to DEHP produced a dose-dependent decrease in the number of litters and in the numbers and the proportion of pups born alive. The no effect level for both maternal and embryo/foetotoxic effects was equivalent to 20 mg/kg bw.

A contribution to DEHP´s developmental toxicity may be the developmental and teratogenic effect of the metabolites, MEHP or 2-ethylhexanol. MEHP (0, 35, 73, 134, or 269 mg/kg/bw on gestational days 0 to 17 to CD-1 mice, 25-27/group) was shown to cause developmental toxicity and malformations in doses from 35 mg/kg bw (NTP 1991)

Several reports are concerned with the effects of DEHP in the chick embryo (Woodward, 1988). In conclusion, the result suggest that DEHP is capable of causing damage to the central nervous system of the developing chick embryo. The studies demonstrated that phthalates have some potential for embryotoxic and teratogenic effects.

DEHP induces testicular atrophy in rats and mice. Young rats are more susceptible than older rats. Zinc-deficient and low protein diets enhanced the susceptibility to the gonadotoxic effect, but coadministration of zinc could not prevent the atrophy. To some extent co-administration of testosterone with DEHP to adult male rats appears to prevent testicular injury. MEHP is likely to be the active metabolite of DEHP affecting testes and reproductive functions, both *in vivo* and *in vitro*. However, the role of other metabolites has not been fully elucidated. There is a partial reversibility of DEHP-induced testicular atrophy in rats after cessation of exposure to DEHP, if the exposure was not too high and long lasting. From continuous breeding studies with mice it has been shown that both males and females are affected by DEHP.

The relative rapid onset of phthalate-induced testicular injury suggests a specific mechanism of action on Sertoli cells. The NOAEL for body, testes, epididymis and prostate weights and for endocrine and gonadal effects in male rats was considered to be 69 mg/kg bw/day of DEHP. The substance is embryotoxic and causes malformations in mice, but not in rats, when given orally in doses close to the maternal toxic dose. Very few teratogenicity studies has been performed in other species. Studies in rabbits are needed to conclude whether the teratogenic effect is seen in other species than in mice. In mice the maternal NOAEL level in dietary studies is 0.05% (91 mg/kg bw per day) when administered throughout pregnancy. In a continuous breeding experiment with male and female mice, the NOAEL for both maternal and embryo/foetotoxic effects was 0.01% DEHP in the diet (20 mg/kg bw/day). In the female rat the dietary NOAEL level for maternal toxicity is less than 0.5% (357 mg/kg bw/day). The dietary NOAEL for embryofetal toxicity in rats is 0.5% (357 mg/kg/bw/day), when administered throughout pregnancy.

In a recent 13-week oral study with DEHP in rats (Poon et al., 1997), hepatomegaly was observed at dietary concentrations of 500 and 5000 ppm, corresponding to doses of 36.8 and 345 mg/kg bw/day in males and 40.8 and 411 mg/kg bw/day in females, respectively. A NOAEL of 3.7 mg/kg bw/day was established from this study, this value confirms the NOAEL of 5 mg/kg bw/day from the RIVM-study (1992).

Conclusion. The critical effect used for assignment of a NOAEL value for DEHP is hepatic peroxisome proliferation in rats (RIVM, 1992). The NOAEL value is 5 mg/kg bw/day.

4.2.4 DIDP

CAS No 26761-40-0. Isomeric mixture containing primary aliphatic branched chains having predominantly 10 carbon atoms.

The overall NOAEL for DIDP as used by the EU-SCF in its 1995 evaluation is 500 mg/kg diet (25 mg/kg bw/day) based on increased liver weights seen in a 13-week study in rats (Hazleton, 1968). In 1996 the SCF established a temporary TDI of 0.05 mg/kg bw/day based on a NOAEL of 500 mg/kg diet (corresponding to 25 mg/kg bw/day) for increased liver weight in a 90-day study in rats and a uncertainty factor of 500. Similarly as for DINP, the SCF requested data on teratogenicity and reproduction toxicity for DIDP (SCF opinion expressed 7-8 March 1996, SCF report not yet published). New data have become available since then.

In another study of peroxisome proliferation male Wistar rats have been exposed to 0, 20, 60, 200, 600 or 2000 mg DIDP/kg diet for 2 weeks. No effect on liver and body weight was seen. Enzymes relevant for peroxisome proliferation were changed at 600 and 2000 mg/kg in diet. A NOAEL for enzyme changes relevant for peroxisome proliferation was 200 mg/kg diet equivalent to 18.4 mg/kg bw/day. Effects were also seen on carnitine acetyl transferase, the highest dose without effect was 5.6 mg/kg bw/day (CSTEE/97/1-Add 41).

No adequate study on fertility and teratogenicity had been published.

Conclusion. The critical effect used for assignment of a NOAEL value for DIDP is increased liver weight observed in an oral 13-week study in rats (Hazleton, 1968). The NOAEL value is 25 mg/kg bw/day.

4.2.5 BBP

CAS No 85-68-7.

The EU-SCF evaluated BBP in 1988 and established a temporary TDI of 0.1 mg/kg bw/day, based on a NOAEL of 140 mg/kg bw/day for changes in liver weight in a 26 week dietary study with rats. The SCF applied an uncertainty factor of 1000 since no adequate studies for teratogenicity and peroxisomal proliferation were available (CSTEE/97/1-Add 8). Since then several studies were performed with BBP.

A 26-week oral study in F344 rats resulted in decreased testicular weights, atrophy of seminiferous tubules, a near total absence of mature sperm production and marked hypospermia in the epididymides at a dose level of 2.5% in the feed (1417 mg/kg/day). Relative liver weight was increased at the 0.83% level (470 mg/kg/day), but not the 0.28% level (159 mg/kg/day) (Hazleton, 1985).

In an oral 90-day study in rats at dose levels of 151 to 1 069 mg/kg bw/day, increased liver weights were seen at all dose levels in females and at the mid and high dose in males. The increased liver weight in females was only slight at the two lower doses. Cecal weights were also increased at all dose levels in females, but not in males. Histopathological lesions of the pancreas were observed in males at the mid and high doses. The liver of high dose males had small areas of cellular necrosis. The LOAEL of increased liver and cecal weights in females was 171 mg/kg bw/day. The NOAEL for increased liver weight in males was 151 mg/kg bw/day, the NOAEL for pancreatic effects was 381 mg/kg bw/day (Hammond et al., 1987).

Biochemical evidence of liver peroxisomal proliferation in male F344 rats was determined at all dose levels in males and was apparent from a dietary level of 0.6% (approx. 300 mg/kg/day). Moderate electronmicroscopical evidence of liver peroxisomal proliferation was observed at the 2.5% dietary level (approx. 1250 mg/kg/day, this dose was the only one examined) (BIBRA, 1985). Also, a recent combined long-term toxicity and carcinogenicity assay identified a dose level of 300 mg/kg bw/day as the lowest effect level for peroxisome proliferation (NTP, 1997).

In a 2 year bioassay with F344 rats, females at the high dose (1.2% dietary level, 1200 mg/kg/day) had increased incidence of mononuclear cell leukaemia (18/50 vs. 7/49 in

control and also at 0.6% dietary level). There were no increased incidences of tumours in males, but they had to be killed at week 29-30 due to internal haemorrhaging (NTP, 1982). No increased tumour incidences were observed in B6C3F1 mice fed for 103 weeks with 0, 0.6 (840 mg/kg/day) or 1.2% dietary levels (1680 mg/kg/day) of BBP (NTP, 1982). The increased incidence of mononuclear cell leukaemia in rats (NTP, 1982) was not seen in a new two-year dietary study in F344/N rats (NTP, 1997). In this study, a statistically increased incidence of pancreatic acinar neoplasms was found in males.

BBP is not genotoxic in a number of *in vitro* tests or in a dominant lethal study in mice (Ashby et al., 1994).

BBP induces embryotoxicity and teratogenic effects in rats at a maternally toxic dose of 750 mg/kg at days 7-9 or 13-15 of gestation, this was not due to decreased maternal food intake (Ema et al., 1991; Ema et al., 1993). BBP is also embryotoxic and teratogenic in mice at maternally toxic dietary levels of 1.25-2.0% (NTP, 1990). An oral developmental reproduction study with BBP in Wistar rats is ongoing at the TNO, The Netherlands (Protocol P 470839).

BBP has shown oestrogen-like effects *in vitro* (Jobling et al., 1995). Sharpe et al. (1995) have reported that exposure of rats to BBP in drinking water at a level of 1000 µg/l (corresponding to a dose of 50 µg/kg bw/d) during pregnancy and lactation results in a reduction in testis size in the male offspring on postnatal day 90. However, administration of 1000 µg BBP/l to pregnant AP rats during gestation and lactation was not found to lead to changes in the sexual development of pups of either gender (Ashby et al., 1997). An as yet unpublished drinking water study performed by the TNO (sponsored by ECPI/CMA) with a similar design as the Sharpe et al.-study, has also not been able to duplicate the Sharpe et al.-findings.

BBP, given s.c. for three consecutive days up to an amount of 5 mg, did not induce increased uterine weight in prepubertal mice (Coldham et al., 1997).

The most sensitive clear critical effect reported in the repeated dose experiments was increased liver and cecal weights in female rats at the lowest dose of 171 mg/kg/day after 90-days feeding. No effects were found in males at 151 mg/kg/day (Hammond et al., 1987).

In a review document (BIBRA, 1992) it is stated that degeneration of testes were seen at 480 mg/kg bw/d, but the investigation itself was confidential. The study by Sharpe et al. (1995) have reported testicular toxic effects in the offspring at a very low dose (50 µg/kg bw/d), but Ashby et al. (1997) and ECPI/CMA (unpublished) failed to confirm these findings. It thus seems unwarranted to base a NOAEL on the findings of Sharpe et al. (1995).

Conclusion. The critical effects used for assignment of a NOAEL value for BBP are increased liver weights observed in an oral 90-day rat study (Hammond et al., 1987). This study did not identify a NOAEL value in female rats, the LOAEL value for the critical effect is 171 mg/kg bw/day. Because the LOAEL value is used and there was only a slight increase in liver weights, an additional uncertainty factor of 2 will be incorporated.

4.2.6 DBP

CAS No 84-74-2.

In 1988 the EU-SCF evaluated DBP and established a temporary TDI of 0.05 mg/kg bw based on a NOAEL of 50 mg/kg bw in a 3 month oral study with rats and an uncertainty factor of 1000. The SCF stated also that a definitive TDI could be established when an adequate 28-day oral study in rats become available with special attention to the effects on the liver including peroxisomal proliferation (SCF Publication No. 33, 1995).

Similar to other phthalate esters application of DBP to laboratory animals results in peroxisome proliferation, increased liver weight, liver tumours in mice, atrophic testes, impaired fertility and embryonal development. The monoester is seen as the active toxic metabolite. There is no indication that DBP or the metabolites are genotoxic (WHO, 1997).

In the gut and liver DBP is hydrolysed to phthalic acid, n-butanol and the monobutylphthalate (White et. al. 1983).The monoester is partially glucuronidised and excreted via the urinary tract. In rats the glucuronidation is 3 to 4 times lower than in hamsters, whereas glucuronidase activity in testes is higher, possibly explaining the higher sensitivity of rat testes to DBP toxicity.

Previous studies in rats receiving 120 and 1 200 mg//kg daily by gavage for three months or 0.6, 1.2 and 2.5 % in feed for 21 days showed increased liver weights, peroxisome proliferation, increased activity of palmitoyl-CoA-oxidase (PAL-CoA-oxidase) and testicular atrophy. Similar effects were seen in mice after 30 days' treatment by gavage (WHO, 1997). NOAELs have not been determined in these studies.

In more recent studies male and female rats were fed a diet containing 400, 2 000 or 10 000 DBP for 3 month, equivalent to daily doses of 30, 150 or 750 mg/kg bw (BASF 1992). At the highest dose reduced blood levels of triglycerides and triiodothyronine were found in both sexes, levels of glucose and albumin were increased and red cells, haemoglobin and haematocrit were reduced in males. Absolute and relative liver weights were increased in both sexes, the only histopathological finding was reduced or absent hepatocellular fat content. The activity of PAL-CoA-oxidase was also elevated. The NOAEL in this study was 150 mg/kg bw per day.

In a 28 days' study male rats were fed a diet containing 0.05, 0.1, 0.5, 1.0 and 2.5 % (25 000 ppm) DBP, corresponding to 51.5, 104, 515, 1 040 and 2 600 mg/kg bw/day. Body weights and food consumption were reduced at the highest dose. Increased liver weights and PAL-CoA-oxidase activities were seen at 515 mg/kg. Total hepatic protein-content was reduced at the lower doses only. The NOAEL was 104 mg/kg bw/day for induction of the PAL-CoA-oxidase and 515 mg/kg bw/day for atrophy of the testes (BIBRA 1990). The LOAEL for hepatic protein reduction was 51.5 mg/kg bw/day.

In an NTP study male and female F 344/N rats and B6C3F mice received DBP in feed for 13 weeks. Additional perinatal studies examined rats and mice exposed as pups in utero, for the 4 weeks of lactation, and for an additional 4 weeks post weaning. Additional studies examined the effects on rats of combining perinatal and adult subchronic exposure (Marsman 1995). In this 13 week toxicity study mice received 0, 1250, 5 000, 10 000 or 20 000 ppm DBP in the diet. The NOAEL of impaired body weight increase or increase of relative liver weights was 2 500 ppm for males and females. In the 13 weeks study in rats 0, 2 500, 5 000, 10 000, 20 000 and 40 000 ppm DBP have been given. In males NOAEL in anaemia, hepatomegaly and peroxisomal enzyme activities in male and female livers was 2 500 ppm. All other endpoints determined, e.g. testes and epididymal weights, hypercholesteremia, alkaline phosphatase activity, bile acid concentration, hepatic glycogen depletion, lipofuscin or histopathological changes showed higher NOAELs. The NOAEL of 2 500 ppm (approx. 130 mg/kg) was not altered when the adults first received the maximal perinatal exposure of 10 000 ppm, followed by the 4 different levels of DBP in diet during gestation and lactation and to the pups post weaning for four additional weeks.

Jobling et al. (1995) reported that DBP could reduce the binding of 17ß-estradiol to the oestrogen receptor and stimulate transcriptional activity. To evaluate the relevance of these findings for the intact animal, the reproductive toxicity of DBP was studied using the NTP's Reproductive Assessment by Continuous Breeding (RACB) protocol (Wine et al., 1997). Levels of 0.1, 0.5 and 1.0 % DBP in the diet were selected, which yielded average daily DBP doses of 52, 256 and 509 mg/kg for males and 80, 385 and 794 mg/kg for females. If the findings of Jobling et al. (1995) were correct one would see greater reproductive effects of DBP in second generation animals, because under the RACB protocol F0 rats are exposed only as adults, whereas F1 animals are born to mothers that are treated during maturation to sexual maturity and through mating. DBP consumption by F0 rats reduced the total number of live pups per litter in all treated groups by 8-17 % and live pup weights in the 0.5 % and 1.0 % dose groups by more than 13 %. In the pups reduced number of live pups, body weights, and in the F0 animals increased kidney and liver weights have been observed at the 1 % dose (509 mg/kg for males and 794 mg/kg for females). In the F1 mating trial, indices of mating, pregnancy, and fertility in the 1 % dose group were decreased, concomitant with a 13 % decrease in the dams' body weight. Necropsy resulted in decreased epididymal sperm contents and testicular spermatid head counts. No such effects have been seen at the 0.5 % dose (256 mg/kg in males, 385 mg/kg in females). The F2 pup weights were 6-8 % lower in all dose groups. This study shows that the reproductive/developmental effects of DBP on the second generation were greater than on the first generation. For reproduction the NOAEL was 256 mg/kg in males and 385 mg/kg in females. In the second generation the LOAEL for reduced F2 pup weights was 52 mg/kg for males and 80 mg/kg for females.

DBP given s.c. for three consecutive days up to an amount of 5 mg/kg bw did not induce increased uterine weights in prebubertal mice (Coldham et al., 1997).

Conclusion. The critical effect used for assignment of a NOAEL value for DBP is reduced F2 pup weights observed in a 2-generation reproductive study with rats (Wine et al., 1997). This study did not identify a NOAEL value, the LOAEL value for the critical effect was 52 mg/kg bw/day. Because the LOAEL value is used, an additional uncertainty factor of 5 will be incorporated.

5. Risk characterisation

5.1 Tolerable daily intakes established by The Scientific Committee for Food

The Scientific Committee Food (SCF/FCM has established tolerable daily intake (TDI's) for some of the phthalates. Only DEHP had a full TDI of 0.05 mg/kg bw. Temporary TDI of DINP (0.03), DIDP (0.25 in 1987, changed in 1988 to 0.05), BBP (0.1), and DBP (0.05) have been established. For other phthalates used in food contact material a TDI of 0.05 mg/kg with a temporary group restriction for migration had been established (CSTEE/97/1-Add 8 and Add 40)**.**

5.2 Margin of safety (MOS) approach

Principles for the conduct of risk assessment have been laid down in the Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances. In the Technical Guidance Document (TGD) to this regulation (1996) it is described that whenever possible, a quantitative risk characterisation is performed. This includes quantitative information on exposure for each population in comparison with the identified N/LOAEL. The ratio of the N/LOAEL to exposure is referred to as the "Margin of Safety" (MOS).

Here, the CSTEE has estimated MOS values by dividing the NOAEL values by the calculated dose for the 6 phthalates. It is realised that, due to the imperfection in the emission rate determinations and thus ascribing intake doses, the calculated MOS values may represent a worst-case scenario. However, exposure to more than one phthalate at a time and from other sources, may lessen the currently estimated MOS values.

Table 7. Critical effects, NOAEL values, maximum emission rates, intake doses and margins of safety for phthalates in toys

Phthalate	**Critical effects**	**NOAEL value mg/kg/day**	**Maximum Emission rate µg/10m^2/6 hr**	**Intake dose µg/kg/day**	**Margin of safety**
DINP	Increased liver and kidney weight	15	14 000	1 700	8.8
DNOP	Microscopic liver and thyroid changes	37	1 500	190	190
DEHP	Hepatic peroxisome proliferation	5	610	75	67
DIDP	Increased liver weight	25	280	35	710
BBP	Increased liver weight	171[A]	15	1.9	45 000[B]
DBP	Reduced F2 pup weights	52[A]	7	0.81	13 000[C]

[A] LOAEL value
[B] An additional uncertainty factor of 2 has been incorporated
[C] An additional uncertainty factor of 5 has been incorporated

5.3 NOAEL values

The NOAEL values presented in Table 7 are taken from the respective conclusion sections in Chapter 4. For DBP its designated LOAEL value is referred to.

5.4 Maximum emission of phthalates

The maximum emission values presented in Table 7 are taken from Table 2 and normalised per 10 cm^2 of mouthed material and 6 hr extraction period.

5.5 Intake doses

The intake doses presented in Table 7 are taken from Table 3, where the maximum emission values are converted to a dose by dividing by a body weight of 8 kg.

5.6 Margin of safety (MOS)

The MOS values presented in Table 7 are the results of dividing the respective NOAEL values by the intake doses. For BBP and DBP an additional uncertainty factor of 5 has incorporated due to the fact that the starting points are LOAEL values rather than NOAEL values (cf. 4.2.5 and 4.2.6).

5.7 Additional exposures

Toys are not the only source of phthalate exposure to children and other significant exposures may include via mother's milk, other types of foods, drinking water and direct contact with carpets and other soft PVC products. There are data available only for DEHP of the three most important plasticisers for toys. Air containing 50 µg DEHP/m^3 may result in an exposure dose of 13 µg/kg bw/day (inhalation volume per day 2.1 m^3, body weight 8 kg, 100% pulmonary absorption), however, the Canadian estimates are lower (approx. 1 µg/kg bw/day, Canadian Environmental Protection Act, 1994b). Unfortunately, no data on phthalates in human milk have been found. The intake of 0.2 L of other milk products can give a DEHP exposure of 23 µg/kg bw/day (concentration of DEHP on a fat basis 31 mg /kg, fat percentage 3%, intake volume 0.2 L, body weight 8 kg). Infant formulae can contain 0.06 mg DEHP/kg, which will result in an exposure of 6 µg/kg bw/day for an 8 kg infant consuming 0.75 litre.

These other exposures for DEHP will reduce the estimated MOS value of 67 calculated above (cf. Table 7). It is probable that there is a similar situation for the other major phthalates found in toys.

6. Guidance values for maximum extractable amounts of phthalates

6.1 Maximum tolerable extractable amounts

The CSTEE recommends that guideline values for extractable amounts of individual phthalates in toys be produced, incorporating a margin of safety of at least 100 from their respective NOAEL values. In Table 8 tolerable daily intakes have been calculated by dividing the NOAEL values by 100. For BBP, and additional factor of 10 has been incorporated (cf. 4.2.5) and for DBP, an additional factor of 5 has been incorporated (cf. 4.2.6). In order to arrive at a guidance value for maximum tolerable extractable amounts per 10 cm^2 and 6 h, the tolerable intakes have been multiplied by the bodyweight of 8 kg.

Table 8. NOAEL values, tolerable daily intakes and guidance values for phthalates in toys

Phthalate	**NOAEL value mg/kg/day**	**Tolerable daily intake µg/kg/day**	**Guidance value mg/10 cm^2 and 6 h/8 kg**
DINP	15	150	1.2
DNOP	37	370	3.0
DEHP	5	50	0.4
DIDP	25	250	2.0
BBP	171[A]	850[B]	6.8
DBP	52[A]	100[C]	0.8

[A] LOAEL value
[B] Additional uncertainty factor of 2 incorporated
[C] Additional uncertainty factor of 5 incorporated

7. Conclusions

For the 2 phthalates DINP and DEHP, the estimated margin of safety were below 100, namely 8.8 and 67, respectively. For DNOP, DIDP, BBP and DBP, the margin of safety were substantially higher (190, 710, 45 000 and 13 000, respectively). It is recognised that there are uncertainties with respect to assessing the actual exposures applying the current model values, both because these models differ considerably and have not been standardised and validated, and because the measured amounts show large variation throughout the various reported studies. On the other hand, the present assessment process has not taken into account that more than one phthalate may occur in children's toys or that there may be additional exposures through food, air and by dermal contacts to these phthalates. The CSTEE therefore concludes that the low margin of safety for DINP gives reason for concern. Although the margin of safety for DEHP was below 100, the CSTEE is less concerned with the estimated level of DEHP exposure, since humans appear to be less sensitive towards the critical effect of DEHP (hepatic peroxisome proliferation) identified in rats.

The Committee is aware of an ongoing Dutch study in adult human volunteers which in a more comprehensive fashion assess human exposure to phthalates from toys. Result of this study will be compared with *in vitro* extraction methods in order to arrive at a standardised method. The CSTEE recommends that consideration should

be given to applying more physiological extraction methods than have been used previously. The present evaluation of the CSTEE may be modified when the results of such studies become available. It should also be recognised that more extensive testing and evaluation of long-term effects of some of the phthalates may lead to a revision of the NOAEL values.

The CSTEE recommends that guideline values for extractable amounts of individual phthalates in toys be produced, incorporating a margin of safety of at least 100 between exposure and the NOAEL values for the respective phthalates. When applying the values for maximum extracted amounts per 10 square cm and 6 hours, and using an infant body weight of 8 kg the following guideline values would apply: DINP, 1.2 mg, DNOP, 3.0 mg, DEHP 0.4 mg, DIDP, 2.0 mg, BBP, 6.8 mg, and DBP, 0.8 mg. These values do not take into consideration that children may have additional phthalate exposures. The guideline values must be based on a standardised, validated extraction method. The Committee recommends that before introducing other plasticisers into toys which children can put into their mouths, the risk of their use should be assessed by the same process which has been applied to the phthalates discussed above.

In the view of the importance in the human risk assessment of phthalates, the CSTEE recommends that an international expert meeting be convened in order to resolve the issue of using peroxisomal proliferation as a critical endpoint and how this should be extrapolated for assessment of human risk.

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