European Chemicals Bureau

Institute for Health and Consumer Protection

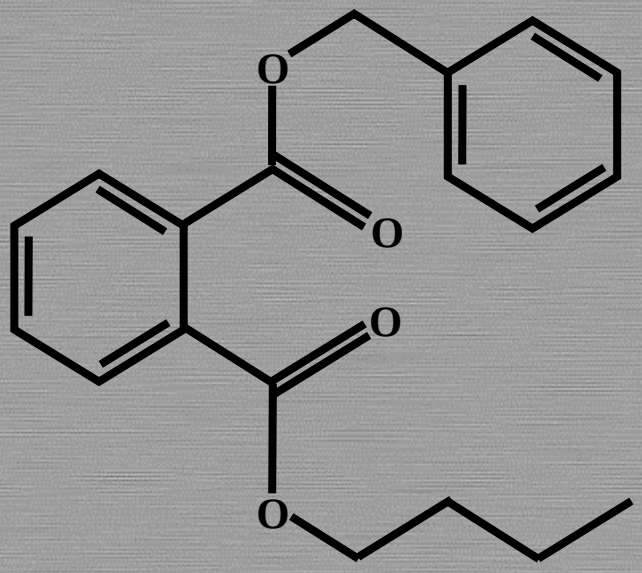
European Chemicals Bureau

Existing Substances

European Union Risk Assessment Report

CAS: 85-68-7 | **EINECS: 201-622-7**

benzyl butyl phthalate (BBP)



3rd Priority List

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RISK ASSESSMENT

Cataloguing data can be found at the end of this publication
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BENZYL BUTYL PHTHALATE (BBP)

CAS No: 85-68-7

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RISK ASSESSMENT

Final Report, 2007

Norway

The risk assessment of benzyl butyl phthalate (BBP) has been prepared by Norway on behalf of the European Union.

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Date of Last Literature Search:	**2003**
Review of report by MS Technical Experts finalised:	**2001**
Final report:	**2007**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

Roland Schenkel
Director General
DG Joint Research Centre

Mogens Peter Carl
Director General
DG Environment

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1 O.J. No L 084, 05/04/199 p.0001 – 0075

2 O.J. No L 161, 29/06/1994 p. 0003 – 0011

3 Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No:	85-68-7
EINECS No:	201-622-7
IUPAC Name:	benzyl butyl phthalate
Synonyms:	1,2-benzenedicarboxylic acid, butyl phenylmethyl ester; benzyl-n-butyl phthalate; phthalic acid, butyl benzyl ester; Santicizer 160; Sicol 160; Unimoll BB

Environment

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 is reached for the following life cycle steps/environmental compartments:

- For the use categories IIIa (flooring large and small site) and IIIh (non polymer use "confidential") at life cycle step III (processing and formulation) for surface water (including sediment)
- For the use categories IIIa (flooring large and small site), IIIc (PVC coated textiles) and IIIh (non polymer use "confidential) at life cycle step III (processing and formulation) for the terrestrial compartment

The exposure assessment for flooring (IIIa) and PVC coated textiles (IIIc) are based on the ESD "Plastics" (OECD, 2004). The recently updated ESD has passed the OECD process and is based on best available information. Further site specific data have not been obtained. The exposure scenario IIIh is based on information from Industry. The PEC/PNEC ratios for the aquatic (including sediment) and the terrestrial compartment are above 1, thus a risk to the aquatic and terrestrial environment can be expected.

Flooring sites were split into large sites with air treatment facilities in place and small sites without air treatment (in accordance with the ESD on Plastics Additives from 2004). Industry stressed that the estimation of plant size on the basis of BBP consumption may be misleading because BBP is usually not used alone but in a mixture with other plasticisers. Hence, small sites with respect to BBP are not necessarily small sites in terms of plasticiser use and industry has confirmed that the sites are actually not small sites in terms of plasticiser use. However, information from industry has also shown that there are actually sites without air treatment and hence the worst case ESD-scenario for small sites, which do not have air treatment in place, was not omitted even though the sites may not be small sites in terms of the definition of the ESD with respect to total plasticiser use.

According to industry emissions to waste water are an overestimation, both for the large sites and for the small site scenario, but as no site specific emission data have become available emission factors are takes from the ESD.

Conclusion (iii) is based on BBP consumption data from 2004. For 2005 there are only two producers left and industry provided estimations of the expected use volume of BBP for all use categories. These figures are confidential as there are only two producers left.

The total BBP volume used for flooring in 2005 has been further reduced, but the scenarios used in this risk assessment are still relevant. In 2005 it is still valid to use the ESD emission factors for a small site since sites without air treatment have been identified.

Applying the expected use volumes for 2005 to "PVC coated textiles" (IIIc) no risk to soil is to be expected.

Conclusion (i) There is a need for further information and/or testing.

A long-term fish study on reproductive and endocrine effects has to be performed.

Conclusion (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.

This conclusion is reached for the following life cycle steps/environmental compartments

- Production and distribution (Life cycle I and II) for all environmental compartments
- For the use categories IIIb, IIIc, IIId, IIIe IIIf and IIIg at life cycle step III (processing and formulation) for surface water (including sediment)
- For the use categories IIIb, IIId, IIIe IIIf and IIIg at life cycle step III (processing and formulation) for the terrestrial compartment
- For use and disposal (Life cycle IV and V) for all environmental compartments
- For the atmosphere (all life cycle steps)
- For STP at all production, formulation and processing sites
- For secondary poisoning (all life cycle steps)

Conclusions (ii) for surface water (including sediment) and the terrestrial compartment have to be seen as provisional until possible endocrine effects in fish have been resolved.

Human Health

Human Health (toxicity)

Workers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

It should be noted that the conclusion for "consumers" related to toys and childcare articles reflects the exposure situation at the time of data collection for this RAR. BBP is not intentionally used in toys and childcare articles in the EU but may be present as impurity in trace amounts. The possible situation that BBP might be used as a substitute for other phthalates in toys and childcare articles in the future has not been taken into account.

Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

It should be noted that recent epidemiological studies have indicated an association between maternal exposures to BBP as well as to other phthalates and the length of the anogenital distance (AGD) in newborn boys. These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to small sample size in the studies, this issue will have to be further investigated, and new studies in the future should be taken into account in the risk assessment of BBP.

CONTENTS

Euses Calculations can be viewed as part of the report at the website of the European Chemicals Bureau:

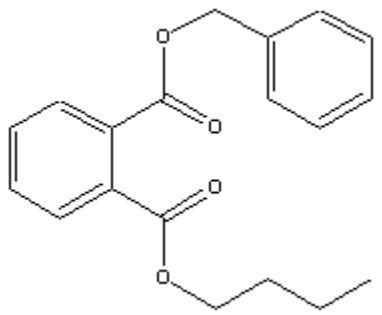
http://ecb.jrc.it

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No: 85-68-7
EINECS No: 201-622-7
IUPAC Name: benzyl butyl phthalate
Synonyms: 1,2-benzenedicarboxylic acid, butyl phenylmethyl ester, benzyl-n-butyl phthalate, phthalic acid, butyl benzyl ester; Santicizer 160, Sicol 160, Unimoll BB
Molecular formula: $C_{19}H_{20}O_4$
Molecular weight: 312.35
Structural formula:



1.2 PURITY/IMPURITIES, ADDITIVES

1.2.1 Purity/impurities

Degree of purity:

> 98.5% (w/w)

Identity and percentage (w/w) of impurities:

< 1.0% dibenzyl phthalate	(CAS No. 523-31-9)
< 0.5% benzyl benzoate	(CAS No. 120-51-4)
< 0.5% dibutyl phthalate	(CAS No. 84-74-2)
< 2 ppm α-clorotoluen	(CAS No. 100-44-7)
< 2 ppm α-α-diclorotoluen	(CAS No. 98-87-3)

1.2.2 Additives

< 0.5 ppm pentaerythritol tetrakis (3-(3,5-di-tert-butyl-4-hydoxyphenyl)propionate. (CAS No. 6683-19-8).

1.3 PHYSICO-CHEMICAL PROPERTIES

Data on the physico-chemical properties of BBP has been obtained from references in the HEDSET.

1.3.1 Physical state (at NTP)

Clear oily liquid with a slight characteristic odour and a bitter taste.

1.3.2 Melting point

<-35°C (Monsanto Internal data).

1.3.3 Boiling point

370°C at 10.10 hPa (Bayer AG MSDS).

1.3.4 Density

1.114 – 1.122 g/cm^3 at 25°C (Monsanto internal data 1988).

1.116 g/cm^3 at 25°C (Lewis, 1992).

1.3.5 Vapour pressure

The vapour pressure for BBP is low at environmental relevant conditions. As seen **Table 1.1**, estimates vary with a factor 10,000. However, the measurements of Hoyer and Peperle (1957) and Howard et al. (1985) using different techniques seem to substantiate each other. These values are also comparable to calculated estimates applying models (EPI suite and Mabey et al., 1982). The EPI suite vapour pressure model has been statistically compared with measurement data for 805 compounds and a correlation coefficient of 0.94 was estimated. Gledhill et al. (1980) do not quote a source for their value, but it seems likely that this is taken from Hoyer and Peperle (1957). The studies of Hoyer and Perperle and Howard et al are the only scientific published studies and the weight of evidence therefore indicates that the measured vapour pressure value of 0.00112 Pa is the most representative value. This value is used in the risk assessment.

Table 1.1 Vapour pressure for BBP

°C	Pa	Reference
20	0.00004 [1)]	Bayer AG (1987)
25	0.00011 [2)]	Howard et al. (1985)
20	0.00115	Gledhill et al, 1980
20	0.00112 [2)]	Hoyer and Peperle, (1957)
20	0.00329	QSAR (EPA:EPI suite)

Table 1.1 continued overleaf

Table 1.1 continued Vapour pressure for BBP

°C	Pa	Reference
25	0.00799	Mabey and Mill, 1978
20	0.5066 2)	Bayer –Denmark in Monsanto (1988)

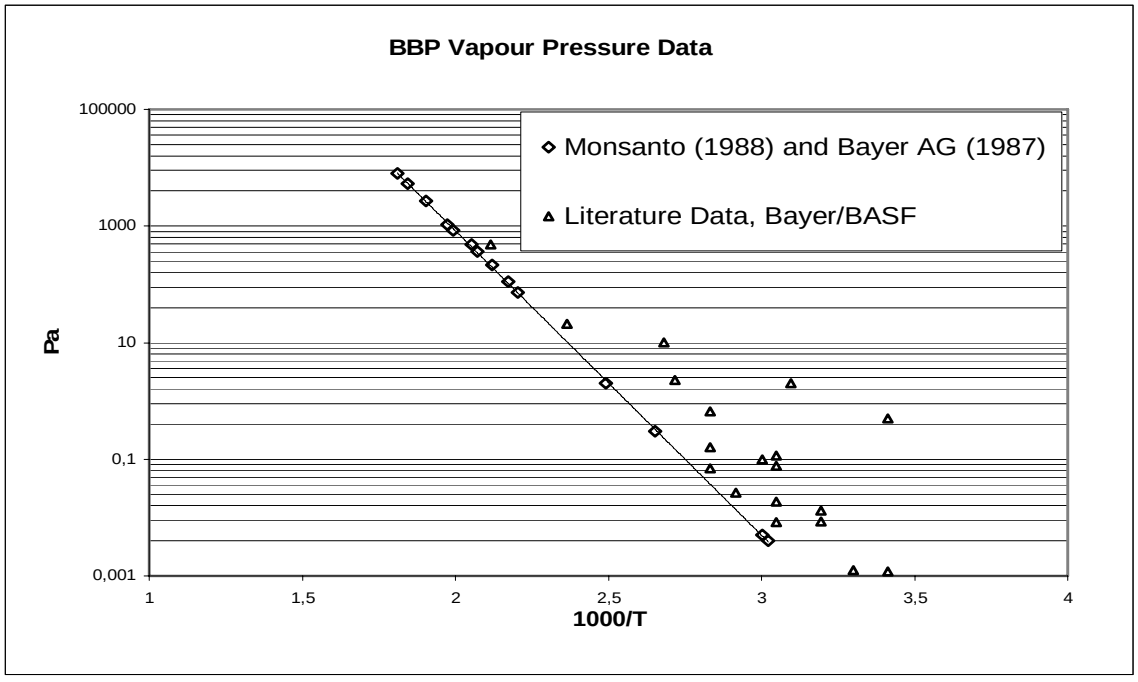
1) Value derived from extrapolation of measured data (see Figure 1.1)
2) Values for which measurement data are available

Table 1.2 Vapour pressure values for BBP at different temperatures

°C	Pa
20	0.00112
160	20*
180	72*
200	230*

* Values are interpolated from "best fit" line from Figure 1.1.

Figure 1.1 Vapour pressure data for BBP; combined studies of Monsanto (1988) and BAYER AG (1987) are used for the "best-fit" line. Data from various tests included in the HEDSET are included in the figure



1.3.6 Water solubility

Solubility values of 0.71 – 82.2 mg/L are found in literature (Mackay et al., 1994). Three high quality experimental data are identified in the HEDSET:

2.9 ± 1.2 - Source: Gledhill et al. (1980)
2.82 (20°C) - Source: Leyder and Boulanger (1983)
2.69 ± 0.15 - Source: Howard et al. (1985)

The mean value for these studies, equal to 2.8 mg/L, will be used.

1.3.7 Partition coefficient (log n-octanol/water)

Among the many log K_{ow} –values found in the literature (range 3.57-5.8) two experimental data (GLP-studies) using the shake-flask method with measured concentration of BBP in both phases are identified in the HEDSET. These two studies gave values of:

4.77 - Source: Gledhill et al. (1980)
4.91 - Source: Leyder and Boulanger (1983)

The mean value for these studies, equal to 4.84, will be used.

1.3.8 Other physico-chemical properties

1.3.8.1 Surface tension

Based on the chemical structure of BBP there is no indication that the substance has an influence on the surface tension of water.

1.3.8.2 Granulometry

Not applicable (liquid).

1.3.8.3 Flash point

The flashpoint is 198°C (390°F) (Lewis, 1992).

1.3.8.4 Autoflammability

The autoignition temperature is 425°C (Bayer AG, 1999).

1.3.8.5 Explosive properties

Based on the structure of the molecule BBP is not expected to have explosive properties.

1.3.8.6 Oxidising properties

With respect to chemical structure there is no indication for oxidising properties.

1.3.9 Summary

A summary of physico-chemical information of BBP to be used in further calculations is shown in **Table 1.3**.

Table 1.3 Summary of physico-chemical properties of BBP

Property	Value
Physical state	Liquid
Boiling point	370°C
Melting point	< -35°C
Vapour pressure	0.00112 Pa at 20°C
Water solubility (20-25°C)	2.8 mg/L
Octanol/water partitioning coefficient (log K_{ow})	4.84
Flash point	198°C at 390 F
Autoignition temperature	425°C
Density (25°C)	1.116 g/cm^3
Henry's Law constant (calculated)	0.176 Pa*m^3*mol^{-1}

1.4 CLASSIFICATION

Classification according to Annex I (29th ATP) of Directive 67/548/EEC[4]

Human Health

Repr. Cat. 2 ; R61	May cause harm to the unborn child
Repr. Cat 3 ; R62	Possible risk of impaired fertility
Symbol: T	Toxic

Environment

R50-53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Symbol: N	Dangerous for the environment

Labelling

T, N
R: 61-62-50/53
S: 53-45-60-61

[4] The classification of the substance is established by Commission Directive 2001/59/EC of 6 August 2001adapting to technical progress for the 29th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provosions relating to the classification, packaging and labelling of dangerous substance (OJ L 216/3, 16.06.2004, p. 123).

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION METHODS

Phthalate plasticisers are produced by esterification of phthalic anhydride in closed systems with a surplus of alcohol at temperatures of about 90°C. The vapour from the process is condensed and returned to the reactor. After virtually complete esterification the surplus alcohol is evaporated off under vacuum at 160°C. The second step involves the conversion of phthalic acid-monobutylester to BBP via reaction with benzylchloride. This step is slower than the first step. The product is then neutralised, washed and finally filtered. The reaction processes occur in closed systems. Process water is either treated in industrial wastewater treatment plants or discharged to the local municipal waste-water treatment plant. Liquid and /or solid waste fractions like distillation residues and used filter-papers are burned in an industrial combustion plant.

2.2 PRODUCTION VOLUMES

In the period 1994-1997 there were 3 producers of BBP in the EU. Reported production in this period was 45,000 tonnes/annum, with approximately 9,000 tonnes/annum being exported outside the EU. This resulted in a total use in the EU of 36,000 tonnes/annum. For the year 2004 industry has estimated that 19,500 tonnes of BBP are used within the EU. Only the total production volume is given and it was not further specified how much BBP is produced at the respective sites. According to predictions from industry, BBP consumption is further decreasing during the ongoing year 2005. However, these figures are confidential because one producer stopped production in 2005. There are therefore only two producers left and no information is available on which producer stopped production.

According to industry the considerable decline of BBP consumption over the recent years is due to its labelling as T; R61-62 and N; R50-53.

For the production sites there site specific information is only available for 1997 but not for 2004. Therefore the information from 1997 together with site specific production volumes of the respective sites in 1997 is used for PEC calculation for the production process. All other life cycle steps are calculated using 2004 consumption data. The production sites in EU (1997) are presented in **Table 2.1**.

Table 2.1 Production sites in Europe (1997-2004)

Company	Production site
(Solutia) Ferro	Belgium (Antwerp)
Bayer	Germany (Leverkusen)
Lonza SPA	Italy (Margherea plants)

2.3 USE PATTERNS

Figure 2.1 shows the industrial categories and use categories of BBP for the European market. This information originates from 1997 and as no other information is available it is assumed to be the same in 2004. Based on the available information the substance is mainly used (more than

95%) as a plasticizer of PVC or other polymers in the European Community. A softener (plasticizer) is incorporated into plastic in order to increase its processability, flexibility and extensibility.

In 2004 the main use of BBP (about 60%) is as a softener (plasticizer) in PVC products, with flooring as the largest single use category (41% of the total use volume). BBP is also used with other polymers in e.g. sealants, (polysulfide based, polyurethane based or acrylic-based), adhesives (based on polyacrylics and polyvinylacetate), paints (e.g based on polyurethane and polyacrylics), inks and lacquers (based on acrylics, nitrocellulose and vinyl resins).

For 2004 it is known that about 8,000 tonnes/annum of the total BBP production (19,500 tonnes/annum) is used in flooring and about 6,000 tonnes/annum in sealants while the remaining 5,500 tonnes/annum are not further specified. In 1997 industry submitted detailed figures for all the respective use patterns. The total sum in 1997 for all the use patterns together, except for flooring and sealants, was 5,400 tonnes/annum ("miscellaneous" in 1997). The tonnage for "miscellaneous" in 2004 (5,500 tonnes) is nearly the same as in 1997 and therefore it is assumed that the tonnages for all the separate uses in the "miscellaneous" categories in 2004 are the same as in 1997 (for tonnages see **Figure 2.1**).

Consumer products such as sealants, adhesives, car care products, and cosmetics may contain BBP. A relatively small but significant use is in the food wrap or food packaging area, which has diminished over recent years due to technological developments leading to no further requirement for BBP in one of the food wrap applications (i.e. regenerated cellulose film). The use of BBP in adhesives and cosmetics has also decreased during the recent years. Furthermore, BBP has been reported at low concentrations in baby equipment and children toys; however, in these products BBP probably occurs as byproducts/impurities and have not been added intentionally to the products. A recent Swedish survey on phthalates in cosmetic products in Europe (http://www.noharm.org/library/docs/Pretty_Nasty.pdf) confirms that BBP occurs in very few products and only in trace amounts. One of the other uses is of confidential nature and submitted information regarding this use is put in the confidential annex, Annex 2.

Information about BBP in products is presented in **Table 2.2**: Survey on chemical products based on a Norwegian survey on chemical products containing BBP in 1996 (SFT report 96:21, 1996). The total amount of BBP corresponded to about 50 tonnes/a in Norway, split into 12 tonnes in consumer products and 38 tonnes in products for occupational use in 1996 (SFT report 96:21, 1996). The Nordic database SPIN (substances in preparations in Nordic countries, http://www.spin2000.net/spin.html) provides data on the use of chemical substances in Denmark, Finland, Sweden and Norway from the year 2000. Most recent data on total use show a decline of BBP in the Nordic countries apart from Sweden. In Sweden 600 ± 100 tonnes/annum were used between 2000 and 2003, but an increase up to 820 tonnes/annum was registered for 2004. In Norway a clear decline from 63 tonnes/annum in 2000 to 13 tonnes/annum in 2004 was registered. The same trend is seen in Denmark, where the total number of products was 440 accounting for 881 tonnes/annum in 1998 compared to 93 tonnes/annum in 2004. **Table 2.2** is based on data from the Danish Product Register (1998).

Table 2.2 Survey on chemical products containing BBP on the Norwegian market based on (SFT report 96:21, 1996) and on additional information from industry.

Product type	Concentration in product [%]	Corresponding amount of BBP [tonnes]	Consumer use identified
Sealing compounds/ fillers(sealants for thermophane windows, acrylic-based sealants, polyurethane foam sealants)	1 – 30%	23	X
Plastics Plastic additives	5-30% 5-100%[2]	8.0	X
Anti fouling paints	1–20%	4.6	
Paints and laquers[1] Additives for paint and lacquers	1-30% 1-60% [3]	4.3	
Chemical products for textile industry	60– 100%[2]	4.1	
Adhesives/glues[1]	1–5% 10–30%	3.7	X[2]
Car care products, (other than paints, lacquers, under-coating)	0–30%	< 0.1	X
Cosmetics	-	< 0.05	X[3]
Other BBP containing products	60–100%[2]	4.1	

1) According to information from Norwegian importers BBP in these product types has been phased out/substituted to a considerable degree during recent years. It is not known if this is a general feature in Europe.
2) Industry has commented that they can not identify any product which contain BBP at this level and "that any data point with a higher percentage than 30% be caveated as not being confirmed". It should be noted that this survey would also identify chemicals intended for use in processing or formulation as "products".
3) According to information from the Norwegian Food Control Authority BBP is no longer used in cosmetics in Norway.

Table 2.3 Information on BBP containing products from the Danish Product Register (1998)

Product type	Concentration in product [%]	Corresponding number of products	Corresponding amount of BBP [tonnes]
Paints, lacquers and varnishes	0–1 1–5% 5–10% 10–20%	30 64 19 9	4 19 74 2
Fillers	0–1% 1–5% 5–10% 10–20% 20–50%	4 12 23 17 7	< 1 1 51 15 < 1
Process regulators (hardeners)	50-80%	3	< 1
Intermediates	confidential	confidential	confidential

2.4 LEGISLATIVE CONTROLS

The use of substances classified as carcinogenic, mutagenic or toxic to reproduction of category 1 or 2 according to Directive 67/548/EEC is regulated and the placing on the market is subject to restriction for sale to the general public according to Directive 76/769/EEC. The marketing and use of BBP and preparations containing BBP intended for consumer use is prohibited through the 29th amendment[5] to the Directive on Restrictions on the Marketing and Use of Certain Substances and Preparations (76/769/EEC).

The marketing and use of BBP and preparations containing BBP in toys and childcare articles is prohibited through the 22th amendment [6] to the Directive (76/769/EEC).

It bans the use of DEHP (Di-2-ethylhexyl) phthalate, DBP (dibutyl phthalate) and BBP in toys and childcare articles and DINP (Di-"isononyl" phthalate), DIDP (Di-"isodecyl"-phthalate) and DNOP (di-n-octyl phthalate) in toys and childcare articles which can be placed in the mouth by children.

The use of substances classified as carcinogenic, mutagenic or toxic to reproduction of category 1 and 2 according to Directive 67/548/EEC is prohibited in cosmetic products according to Directive 76/768/EEC concerning cosmetic products. Substances classified as CMR of category 3 should be prohibited unless they are evaluated by the SCCP and found acceptable for use in cosmetic products. Through the amendment [7]of Directive 76/768/EEC, BBP is listed now in Annex II to the directive and must therefore not form part of the composition of cosmetic products.

[5] DIRECTIVE 2005/90/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 January 2006 amending, for the 29th time, Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (substances classified as carcinogens, mutagens or substances toxic to reproduction — c/m/r)

[6] DIRECTIVE 2005/84/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 14 December 2005 amending, for the 22th time, Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (phthalates in toys and childcare articles)

[7] DIRECTIVE 2005/80/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 November 2005 amending Council Directive 76/768/EEC concerning cosmetic products, for the purposes of adapting Annexes II and III thereto to technical progress.

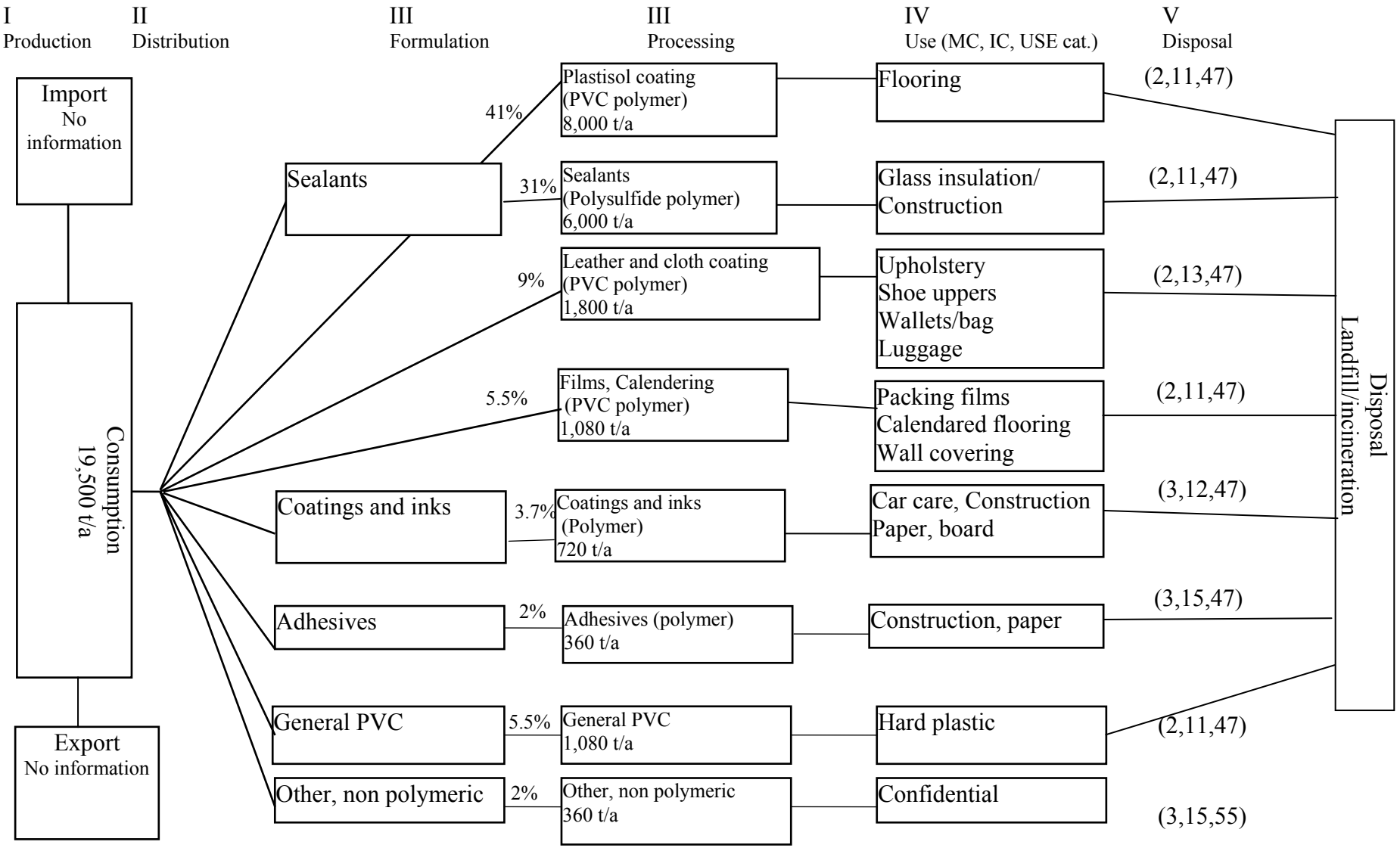
Only authorised substances should be used in the manufacture of all types of regenerated cellulose film intended to come into contact with foodstuffs. BBP is deleted from the list of authorised substances due to the Directive 2004/14/EC [8]amending the directive on materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs (93/10/EEC).

The Directive 2002/72/EEC relates to plastic materials and articles intended to come in contact with foodstuffs. Substances or groups of substances listed in Annexes II to VI, can be used for the manufacture of plastic materials and articles, subject to the restrictions therein. However the list of additives established through Directive 2002/72/EEC shall be considered to be an incomplete list until the Commission decides (by 31 December 2007 at the latest) that it shall become a positive Community list of authorised additives, to the exclusion of all others. BBP is not included in the current, but yet incomplete list of additives set out by the 2nd amendment [9] to the Directive 2002/72/EEC.

8 DIRECTIVE 2004/14/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 January 2004 amending Directive 93/10/EEC relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs

9 DIRECTIVE 2004/19/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 1 March 2004 amending for the 2 nd time, Council Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs

Figure 2.1 Flow scheme of life cycle stages of BBP and % distribution between use areas for 2004



3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General

The following life cycle description is assumed representative of the life cycle stages of BBP. This information originates from 1997 and is assumed to be the same in 2004 as no other information is available. Emissions of BBP to the environment in this risk assessment are based on the appropriate tables in the TGD when site specific information has not been available. The OECD Emission Scenario Document on Plastics Additives from 2004 is used for the life cycle steps flooring (IIIa), PVC coated textiles (IIIc), polymer films (IIId) and general PVC (IIIe).

I	Production
II	Distribution (road tankers and ships)
IIIa	Processing of PVC flooring by plastisol coating
IIIb-1	Formulation of PVC sealants
IIIb-2	Processing of PVC sealants
IIIc	Processing of PVC coated textiles
IIId	Processing of polymer films
IIIe-1	Formulation of general PVC
IIIe-2	Processing of general PVC
IIIf-1	Formulation of paints and inks
IIIf-2	Processing of paints and inks
IIIg-1	Formulation of adhesives
IIIg-2	Processing of adhesives
IIIh	Formulation of non polymer
IVa	Interior use of PVC products and polymer films
IVb	Interior use of sealing
IVc	Use of, paints and inks and adhesives
IVd	Use of non polymer
V	Incineration and landfill disposal of BBP containing polymer products

For life cycle stages I and III, site specific and/or generic emission scenarios are used for calculating the predicted local environmental concentrations (PECs) in the various compartments. Emission in connection with distribution (Stage II) is assumed to occur locally at the production site in one region based on the information that the road tankers are cleaned at the production site. Stages IV and V can be regarded as diffuse sources of BBP. The emission of BBP from these latter stages will be considered in the establishment of regional PECs. Although the TGD does not have specific guidelines for the risk assessments of emissions from landfills (stage V), available data give some indications of the amount that may be released from landfills and this has therefore been included in the EUSES model as a contribution to regional PECs.

Generic scenarios are mainly based on the EU-Technical Guidance Documents (TGD, European Commission 2003) defaults except when expressively stated in the text. Regional concentrations are evaluated with the model SIMPLE BOX included in the program EUSES. However, with regard to emission factors for PVC products, the OECD published “Emission Scenario Document on Plastics Additives” (ESD “Plastic”, OECD 2004) has been applied.

3.1.2 Local Exposure Scenarios

3.1.2.1 Production (life cycle stage I)

There are 3 plants in Europe producing a total of approximately 45,000 tonnes/annum of BBP (based on 1994 – 1997 data). About 9,000 tonnes/annum are exported outside Europe. Some information has been submitted concerning releases at all three production sites. In **Table 3.1** below emissions and environmental concentrations expected are presented using site specific information, and, where information was lacking, the TGD default factors for emission of BBP to air and wastewater have been used. Emission factors for air and water are taken from TGD, Table A1.1, however, the air emission factor of zero to air, does not take into account a higher than ambient temperature during production. According to information from industry a maximum working temperature of 160°C is expected. According to Section 1.3.5, BBP has a vapour pressure of 20 Pa at this temperature. The emission factor at this vapour pressure gives a more realistic emission scenario as one production site actually does report measurable emissions to air. Sludge from production site WWTP (waste water treatment plant) is either deposited in landfill or incinerated. Therefore the risk assessment of local concentrations through sludge application to soil is not considered relevant. Since sludge from production sites is eliminated and the indirect release of BBP to air is negligible in WWTP, the WWTP step is omitted for production sites in the EUSES calculations. Instead all the release of BBP is directed to surface water. As nothing is known about the present locations of the production sites in 2004 the summarised release for all three sites is included in the regional compartment.

The reported releases to surface water from all 3 production sites (see **Table 3.1**) are summarised and result in a release of 1.6 kg BBP/day to surface water. For air the sum of the releases to air from the three sites (kg/annum) is divided by 300 days in order to give an estimated release per day to air of 0.41 kg/day. All release to air and water is expected to occur in one region. This seems to be the most conservative approach. These release values are estimated based on non-detect values in effluent WWTP monitoring data.

For 2004 industry has estimated that 19,500 tonnes BBP are used within the EU. It is not known how much of it is produced at the sites and there is no site specific information available for 2004. Therefore the figures and information from 1997 are used for the calculation of Predicted Environmental Concentrations (PEC) for the production process. **Table 3.2** summarises the local PEC values for production sites which are used in the risk characterisation.

Table 3.1 Estimation of local concentration in air and water for production sites (1997 data)

Sites	Site A	Site B	Site C
Production volume	Confidential	Confidential	Confidential
Main category	1b	1b	1b
Number of days	300	300-320	300
Fraction of main source	1	1	1
Emission to air [kg/annum]	5	115	1.75

Table 3.1 continued overleaf

Table 3.1 continued Estimation of local concentration in air and water for production sites (1997 data)

Sites	Site A	Site B	Site C
Production volume	Confidential	Confidential	Confidential
Local conc. air 100m from source [µg/m^3]	0.0014	0.0320	0.00049
Emission to STP [kg/annum]	99,000	34,500	81,000
Conc. in STP [mg/l]	165	5.3	19.0
Conc. in effluent [mg/l]	$3.3 \cdot 10^{-3}$	< 0.02	< 0.1
Release to surf. water [kg/day]	0.007	< 0.4	< 1.2
Local conc. surface water [mg/l]	$3.3 \cdot 10^{-4}$	$4.7 \cdot 10^{-6}$	$1.35 \cdot 10^{-4}$
Local conc. sediment [mg/kg]	0.076	$1.1 \cdot 10^{-3}$	0.031

Table 3.2 Local PECs for different compartments at production sites (1997 data)

Compartment	Site A	Site B	Site C
	PEC	PEC	PEC
Surface water [µg/l]	0.50	0.18	0.31
Sediment [mg/kg wwt]	0.14	0.07	0.10
Oral fish [mg/kg]	0.22	0.08	0.14

3.1.2.2 Release from different industrial uses

Processing of polymers for flooring (life cycle stage IIIa)

BBP consumption in flooring and number of sites

It is assumed by ECPI (European Council for Plasticisers and Intermediates) that 41% (8,000 tonnes) of the 19,500 tonnes of BBP used in EU in 2004 are used in flooring using mostly the plastisol spread coating process and to a lesser extent calendering. For the ongoing year 2005 industry expects an even lower amount of BBP used in this use category due to its labelling as T; R61-62 and N; R50-53. It was estimated by industry that there were approximately 20 flooring plants in Europe in 1997.

Size of the sites

BBP consumption for flooring has decreased from 21,600 tonnes in 1997 to 8,000 tonnes in 2004. No information on the exact number of flooring sites using BBP in 2004 is available. However, it is assumed that the number of sites has decreased considerably during the last years due to the serious decrease in BBP consumption. Information from 2005 show that the number of sites is now considerably reduced compared to 1997. It is therefore assumed that 1,600 tonnes/annum were used for flooring at a single large site in 2004 (20% of total) and 250 tonnes/annum at a small site. The definition of a small site is taken from the ESD "Plastics". The ESD "Plastics" distinguishes between large sites which have fume elimination equipment in operation and smaller sites without air treatment facilities. A small site is defined as a site at which less than 250 tonnes plasticiser are processed per year and which has a 10-fold higher emission factor to air and water than a large site.

This information is supported by information given at TC NES IV'04 where industry confirmed that a distinction could be made based on smaller sites using BBP in the range of one hundred to a few hundred tonnes/annum and large sites using BBP in the range of one thousand to a few thousand tonnes/annum. However, industry has stated that the estimation of plant size on the basis of BBP consumption may be misleading because BBP is usually not used alone but in a mixture with other plasticisers. Hence, small sites with respect to BBP are not necessarily small sites in terms of plasticiser use and industry has confirmed that the sites are actually not small sites when the total plasticiser use is taken into account. However, information from industry has also shown that there are actually sites without air treatment (see results of the EuPC survey below) and hence the worst case ESD-scenario for small sites, which do not have air treatment in place, could not be omitted even though the sites may not be small sites in terms of the definition of the ESD with respect to total plasticiser use.

Emission control equipment

According to industry the layout of a modern European PVC flooring production plant, using plastisol spread coating process, is primarily of the "discontinuous" type. That means separate process lines and reeling up of the flooring at several points in the coating and printing process and transfer to reel at the beginning of the next process line. Key process steps involve open coating with plastisol on the flooring substrate or heating of the coated "web" to gel to fully polymerise the PVC. BBP is normally delivered and stored in bulk and pumped directly from bulk storage and metered into high shear mixers to produce plastisol. This formulation is done in closed systems with vapour containment and air purification equipment and is performed at the processing site. During processing, the plastisol is spread on "web" and is heated in ovens; the off gas is treated with air purification systems. According to ECPI/CEFIC report "Phthalate esters used in Plasticised PVC" (1996) about 53% of the plants in Western Europe using plastisol spread in coating processes have air purification equipment with filter treatment, 22% use incineration treatment and 25% have no treatment.

The ESD "Plastics" from 2004 states that already by 1989, 75% of all plasticiser in spread coating applications was used in production lines with air treatment and that this proportion has certainly increased since then and is probably approaching 100% under the influence of national and European regulations. At TC NES III '05 industry stated that the EU Plastics Converters reported that this level was approaching 92%.

EuPC survey

EuPC (European Plastics Converters association) conducted a survey on emissions of BBP plasticizers during processing/formulation of vinyl flooring in Europe (EuPC Report of September 2005). The survey aimed at giving an overview of the emissions of BBP and treatment systems installed in converting plants for air, water and soil.

The vinyl flooring market is very concentrated with about 16 companies representing almost 100% of the production in the EU. The consumption of BBP in flooring in the EU 25 is estimated as 8,000 - 10,000 tpa in 2004. The lower figure is used for PEC calculations in the draft RAR because we got it confirmed by industry. Companies representing together 80% of the BBP consumption in vinyl flooring have answered the EuPC questionnaire.

Information on the consumption and emission treatment of the remainder of the companies (20% of the BBP consumption in flooring) has been provided to the rapporteur by ECPI. These companies have air treatment facilities. In total less than 20 vinyl flooring manufacturing plants (locations) are using BBP in the EU 25.

95% of the plants using BBP in vinyl flooring manufacturing in the EU 25 have exhaust air treatment, 5% have no air treatment (combined EuPC and ECPI data). Incineration is the most common treatment system and also filtration is widely used. Plants not having installed an exhaust air treatment system are processing by calendaring, not by spread coating.

75% of the plants interrogated by EuPC do not produce any aqueous effluent. Emissions to water are occasional and may occur as the machinery is being cleaned. Only minimal contact with water happens as cooling water is usually trapped in closed loop systems. When discontinuous emissions to water were reported, two third of the plants sent waste water away for treatment and one third treated it in the plant itself. The sludge from waste water would then mainly be incinerated. No emissions to soil were reported.

The estimation of plant size on the basis of BBP consumption may be misleading because BBP is usually not used alone but in a mixture with other plasticisers. Hence, small sites with respect to BBP are not necessarily small sites in terms of plasticizer use. In fact all flooring plants have production capacities at least an order of magnitude greater than that which the Emission Scenario Document on Plastics Additives classifies as a "small site".

Emission factors used for PEC calculation

It is estimated that there were approximately 20 flooring plants in Europe in 1997. A survey of 3 plants (Tucker and De Bie, 1998) with respect to air concentrations gave concentrations in the range of 0.006 to 2.8 mg/m^3. In one plant, emission concentrations were measured at 6 points and were found to be in the low range 0.006-0.08 mg/m^3, with a mean of 0.035 mg/m^3. Using the mean emission concentration of the 6 emission points to air in this plant, it was estimated that the plant had an emission of 1.1 g/h. Using the maximum concentration of 0.08 mg/m^3, gives an output of 3g/hour, and assuming a 7,200 hour operation/year the total emission would be 21.6 kg. Using the same volume for the emission value of 2.8 mg/m^3 would give a total emission of 756 kg/annum. Assuming that each of these plants uses 1/20 of the BBP used for flooring leads to estimated emission factors of 0.00002 and 0.0007. However, without information on the actual amount of BBP processed at these plants it is not possible to derive an emission factor from these data. Moreover information is only available for 3 out of approximately 20 sites. These data refer to the situation in 1997 and nothing is known about the 2004 situation.

Based on the information aggregated in the above mentioned EuPC survey it is not possible to overwrite the ESD scenarios. No site specific emission data are available and hence no site specific emission factors could be derived. Industry stated that it was too a rough split to assume that 50% of the emissions go to air and 50% to waste water and that the emissions to waste water are therefore considerably overestimated. However, as we do not have site specific emission data the assumptions from the ESD from 2004 are applied.

Moreover, 5% of the plants (combined EuCP and ECPI data) questionnaire does not have air treatment. Therefore the small flooring site scenario with higher emission factors could not be omitted even though the sites may not be small sites in terms of the definition of the ESD with respect to total plasticiser use.

The ESD "Plastics" defines three volatility groups for the various types of plastics additives; high, medium and low volatility group. BBP is explicitly stated and classified into the high volatility group. For high volatility plasticizers an emission factor of 0.125% (to air and waste water) is proposed for spread coating for large sites with fume elimination equipment in operation. It is recommended to increase this factor by a factor of 10 for small sites (< 250 tonnes) assuming no air treatment equipment. The emission factors for calendering are

the same as for spread coating. A default emission factor to industrial soil is taken from Table A 3.11 in TGD.

According to the plastisol-spread coating process described in the ESD "Plastic" it can be assumed that 75% of the consumption is performed at sites with adequate air treatment. Therefore for calculation of PECregional and PECcontinental the use category flooring has been split into two use scenarios, with 75% of the flooring tonnage consumed at large sites with air treatment and 25% of the flooring tonnage consumed at small sites without air treatment.

Local PECs for flooring at small sites (IIIa-1) and at large sites (IIIa-2) are presented in **Table 3.2**.

Formulation and processing of sealant (III b)

Industry estimates that about 31% (= 6,000 tonnes) of the total BBP amount within EU was used for sealants in 2004. Industry informed further that the amount used for sealants in 2005 had considerably decreased compared to 2004 and that this reduction was due to the labelling of BBP as T; R61-62 and N; R50-53.

BBP is used as plasticisers in polysulfide sealants, which are used for providing airtight fittings for thermopane double glass windows. BBP is also used in sealants that are available to consumers such as polyurethane foam sealants (e.g. window frame insulation) and acrylic based sealants (water based). These products may be characterised as building or construction products.

Industry indicates that there are about 6 formulation sites within EU, with the 5 larger sites formulating 95% of the total polysulfide sealant made. There is at present information from two large sealant formulation plants, accounting for approximately half of the BBP used for sealants. The following is a description for the larger of the two which according to industry information from 2001 is supposed to formulate "at least 1/3 of all BBP used for sealants" and which has the highest releases and therefore represents a local worst case scenario. This information refers to the 1997 situation and it is not clear whether this is still up-to-date.

The formulation occurs routinely in closed systems, where in static vessels BBP is mixed with prepolymer and inorganic materials under vacuum. No cooling water is used and maximum temperature attained during the formulation is 55^{o}C. Process water is exclusively used as a Bernouilly agent for vacuum pumps. Between the reactor and the vacuum pumps a trap is installed to capture possible mist or vapour. The total amount of water used is 25 m^{3}/day, which is rejected into the municipal sewer system. Assuming the water is saturated with BBP this gives a total release of 0.07 kg/day for this site or a release factor of 0.00001 to waste water. The formulated sealant is filled into plastic lined drums for shipment to processing sites. Recycling of these drums is relevant for some sites, in which case it is assumed that the drums have to be cleaned before re-use. Cleaning of formulation equipment occurs on a biannual basis, generating 200 kg of solid waste. Most of this is recycled with about 5 kg of waste being incinerated. Information from the other site indicates even lower emissions, the larger site is therefore assumed to be representative for all sealant formulation sites.

As industry has informed that the largest site formulates 1/3 of all BBP used for sealants, this site is used both as regional tonnage (= 1,800 tonnes/annum in 2004) and as local site and the fraction of main source is therefore 1. For emissions to air and soil the default emission factors from the TGD (Table A 2.1) are used. The default number of working days (300) is assumed. Local PECs are calculated with EUSES and presented in **Table 3.2**.

During processing the polysulfide sealant is extruded at low temperatures at numerous sites. Water is not used during this process, cleaning of equipment is performed with solvents. The contaminated solvent is cleaned by distillation and solid waste is incinerated. Processing therefore gives no release to water or soil. With respect to air the default value from the TGD (Table A3.11) could be used, however, due to low work temperature a reduced air emission factor of 0.001 is considered realistic.

The authorities are aware of two products intended for use as "grouting agents" or "sealing agents". The total use of BBP for these two products is about 450 kg/year in Norway, which is not corresponding to the total use in EU (50 kg/year). The sealant typically contains about 25% BBP. According to the safety data sheet the application area for these products are for watertight sealing of cracks in rocks and filling of cracks in concrete constructions. Release of BBP during formulation and processing is expected to be similar to that described above with the exception of the use as grouting agent. According to industry, only one manufacturer of grouting agents has been identified. The grout contains typically 25% BBP and the total use of BBP for this product is approximately 50 kg/year in the EU.

There are no available studies on the release of BBP from grouting/sealing agents. One monitoring study in Norway monitored the release of Dibutylphthalate (DBP) from a grouting agent during use in a railroad tunnel (Sverdrup et al., 1999). A total release factor of 0.16% was found during the monitoring study (release to surface water). This release factor is probably too conservative for BBP due to the lower water solubility of BBP compared to DBP, but it may be used for assessing the release of BBP from grouting agents.

Assuming that 60% of the total production of the BBP-containing grout is used in a single scenario, 30 kg will be injected with the grout. A release factor of 0.16%/year gives a release of 48 g/annum of BBP to the surface water. This seems to be negligible even at a local scale.

Processing of PVC coated textiles (life cycle III c)

It is assumed that 9% of total EU use of BBP was used for this purpose in 2004. This leads to a total amount of BBP of 1,800 tonnes in this use pattern.

According to industry the production of synthetic PVC based leather involves on site formulation in a closed system with vapour containment. Industry has suggested that due to extensive use of vapour phase control, the emission factor to air should be reduced to 0.001, however, this would need to be confirmed by site specific information from the processing plants. A laboratory study referred to in the ECPI/CEFIC report (1996) gave an emission of 0.5-1% for plasticisers using this process without vapour containment. An emission factor of 0.0025 is estimated in the ECPI/CEFIC report (1996) for processing of spread coated products made by the plastisol technique. This was established by taking the mean emission for a number of sites in Europe.

According to the ESD "Plastics" (OECD, 2004) coating of textiles is performed through the extrusion process and it is recommended to use an emission factor of 0.025% to air and water for large sites with fume elimination equipment. For small sites (< 250 tonnes) it is recommended to increase the factor by a factor of 10. Therefore an emission factor to water and air equal to 0.25% is used in the EUSES model. The emission factor for soil is taken from Table A 3.11 in the TGD. Industry has estimated that the number of sites using BBP is less than 10. Furthermore, formulation is performed at the processing site and the fraction of main source is therefore set to 1 (default for formulation; TGD Table B 2.9).

Local PECs as calculated with EUSES are presented in **Table 3.2**.

Processing of polymer films (life cycle III d)

It is assumed that 5.5% of the total BBP amount within EU was used for polymer films in 2004. This leads to a total amount of BBP of 1,080 tonnes in this use pattern.

BBP is mixed into the polymer to provide flexibility of the polymeric material. These processes would be characterised as hot melt calendering systems, the process temperature is 180°C according to the ECPI/CEFIC report (1996). According to industry the production of polymeric films involves on site formulation in well ventilated systems with vapour control. Film processing is performed in closed extrusion machines with vapour control. The off gas is either incinerated or otherwise controlled, however, no site specific information has been provided and the number of sites is not known.

The ESD "Plastics" (OECD, 2004) recommends an emission factor to air and water of 0.025% for film extrusion (BBP is grouped into the high volatility group) during conversion. For small sites (< 250 tonnes) they recommend to increase this factor by a factor of 10. Therefore an emission factor to water and air equal to 0.25% is used in the EUSES model. The emission factor for soil is taken from Table A 3.11 in the TGD. The number of sites for other use categories is limited and the same is assumed for this use category. As formulation takes place at the processing sites the fraction of main source is assumed to be 1 (default for formulation, TGD, Table B2.9). Local PECs as calculated with EUSES are presented in **Table 3.2**.

Formulation and processing of general PVC (life cycle stage III e)

About 5.5% of the total use of BBP within EU was assumed for this purpose in 2004. This leads to a total amount of BBP of 1,080 tonnes in this use pattern.

It is assumed that PVC formulation is performed separately and at elevated temperatures. It is thought that processing involves moulding of hot PVC in casts. According to industry air-cooling is the standard in moulding and water is normally not used in the production of PVC products, apart from cooling of cables after extrusion. However, industry could not exclude that water cooling of moulded PVC may be used at some sites. No detailed site specific descriptions of formulation and processing of general PVC are available. The ESD "Plastics" (OECD, 2004) suggests an emission of 0.025% to air and water for formulation (compounding, high volatility group). With respect to processing the suggested emission factor for injection moulding (high volatility group) is 0.025%. For small sites it is suggested to increase this factor by a factor of 10. Therefore the emission factor used in the EUSES model is 0.25% for both water and air. The emission factor for soil is taken from Table A 2.1 with regard to formulation and from Table A 3.11 in the TGD with regard to processing. Default values are used with respect to fraction of main use (TGD, Table B2.9). Local PECs assuming the default emission factors in these tables are presented in **Table 3.2**.

Formulation and processing of coatings and inks (life cycle stage III f)

It is assumed that 3.7% of the total use of BBP within EU was used for this purpose, which includes paints lacquers and inks in 2004. This leads to a total amount of BBP of 720 tonnes in this use pattern.

It is presumed that these inks are used for printing e.g. colour printing inks for board, paper and textiles. This use is evaluated as industry category 12 (pulp, paper and board). The main function of BBP is to impart flexibility to the coatings and inks. This prevents chipping and flaking of the coatings and inks from the surface of an object. Information from Norwegian importers indicates that BBP use in paints is considerably declining. It is not known if this is a general feature in

Europe. No site-specific information is available. BBP's assumed use is as a softener (use category 47), however, this is not a specified use category in IC 12, and therefore the use category 48 (solvent) is used to find appropriate emission factors. It can be assumed that the users are relatively small in size and number. Processing occurs at ambient temperatures. Emission factors during formulation of coating and inks are assumed to be represented by Table A 2.1 in the TGD, however, as water is not directly involved in the formulation process, the emission factor of 0.003 to waste water seems sufficiently conservative when compared to the emission factor suggested by ESD on Plastics (OECD, 2004) for PVC which is 0.00025 (see use category "general PVC" above). The generic scenario for processing makes use of the default emission factors from the TGD Table A3.12. The local PECs are presented in **Table 3.2**.

Formulation and processing of adhesives (life cycle III g)

It is assumed that adhesives accounted for about 2% of the total use of BBP within the EU in 2004. This leads to a total amount of BBP of 360 tonnes in this use pattern.

The purpose of adding BBP to adhesives is to impart flexibility of a polymer component i.e. polyacrylics or polyvinylacetate in the adhesives. It is presumed that BBP is used for dispersion adhesives, which are used for paper, packaging, wood, building industry and the automobile industry. The most important use area of these adhesives is for paper and packaging. Information from Norwegian importers indicates that use of BBP in adhesives has decreased during the last years, it is not known if this is a general feature in Europe.

There is no site-specific information available for formulation and processing of adhesives. Formulation and processing is assumed to occur separately. BBP is assumed to be used as a softener in polymers. Table A 2.1 in the TGD is deemed representative for the formulation life stage, however, as water is not directly involved in the formulation process, the emission factor of 0.003 to waste water seem sufficiently conservative compared to the emission factor suggested by ESD "Plastics" (OECD, 2004) for PVC which is 0.00025 (see use category "General PVC" above). Due to the lack of a particular scenario processing of adhesives, in this case the use as glue, has been grouped in IC 16. Main category II (matrix) seems relevant for this process. Emission factors to air and soil are taken from Table A 3.16, however, the emission factor of 0.01 to water seems excessive in a process not utilising water. Therefore the emission factor of 0.001 from Table A 3.11 (II softener) in the TGD is used. The local PECs are presented in **Table 3.2**.

Formulation and processing of non-polymer products (life cycle stage III h)

2% of the total BBP amount in the EU was assumed to be used within this group in 2004. This leads to a total amount of BBP of 360 tonnes in this use pattern.

The main application in this category is confidential and industry has provided information about it. The use is described in Annex 2, confidential.

Industry has recommended the release factors to be used as well as the release scenarios. A local site using 0.66 of total tonnage is used to assess the regional emissions.

Release factors for air, water and soil are taken from TGD Table A 2.1, however, as water is not directly involved in the formulation process, the emission factor of 0.003 to waste water seems sufficiently conservative compared to the emission factor suggested by ESD on Plastics (OECD 2004) for PVC which is 0.00025 (see use category "General PVC" above). Processing is not relevant. A release factor of 0.05 for air, water and soil is used during service life. The local PECs are presented in **Table 3.2**.

3.1.2.3 Release during distribution and use of end-products

In this section the release during distribution/transport (life cycle stage II), service life (life cycle stage IV) and incineration/disposal (life cycle stage V) are considered. The results will be used in combination with the release estimates from life cycle stage I and III to derive regional and continental PECs for the various environmental compartments. Because BBP is almost exclusively used for indoor applications no release from waste or fixtures remaining in the environment is assumed. BBP differs from the other phthalates in this respect.

Distribution (life cycle stage II)

Almost all of the phthalates consumed within EU, including BBP, are transported by road tankers (Cadogan, 1994; ECPI, 1996). During distribution losses occur during cleaning of the tanks. Two estimates are available for the losses of BBP to the aquatic environment from tank cleaning activities: 0.05% of total production (ECETOC, 1985) or 0.01% of total consumption (Cadogan, 1994). These estimates give a release of 18 tonnes/year or 3.6 tonnes/year and relate to the 1997 situation.

ECPI (1996) has estimated the loss on washing to be 1 kg per tanker. According to information from the manufacturers 55% of the road tanker fleet is dedicated, which minimises washing needs (Tukker et al., 1996). Assuming that 90% of the BBP used within EU is shipped by road tankers, results in 32,400 tonnes which are transported by tankers per year. Each tanker carries 22 tonnes which gives a total of 1,472 tanker loads per year. Estimates based on the assumption that 45% of the tankers are non-dedicated and that loss during washing is 1 kg for each load (and that the 55% dedicated fleet use no washing) would give a total loss of 0.66 tonnes/year. In addition to losses due to washing, ECPI (1996) estimated that 1 kg was lost to the environment during loading/unloading–which is assumed to be a maximum estimate of losses due to spillage. Total estimated losses due to spillage would then be 1.472 tonnes/year. Adding release due to spillage and washing gives a total estimated loss of approximately 2.1 tonnes/year. This release is all assumed to occur at the regional production site to a WWTP.

Release due to distribution is 2.1 tonnes/year to wastewater.

Interior use of PVC products and polymers (life cycle stage IV a)

Most of the identified PVC products and polymer utilising BBP as plasticisers have mainly indoor use, therefore no assessment has been performed with respect to direct release to the environment during service life. It is assumed that emission from flooring is also representative for general PVC, polymer films and PVC coated textiles. PVC coating has many applications connected to products with water-resistant surfaces, i.e. wallpaper, textiles, leather and other similar products. Flooring is the largest product group (8,000 tonnes/annum in 2004) and all other PVC products (3,960 tonnes/annum from the use of BBP in leather and cloth coating, films/calendaring and general PVC in 2004) are assumed to give similar emissions. This makes together a sum of BBP of 11,960 tonnes/annum used in PVC products. During use, emissions from flooring are expected due to abrasion, washing and evaporation. ECPI (1996) has estimated phthalate emissions to air and water due to water extraction during washing of flooring. From estimates of general release of plasticisers from flooring due to water extraction an emission rate of $4 \cdot 10^{-4}$ per year was calculated. Similarly they also estimated the loss due to evaporation, arriving at a factor of $1.6 \cdot 10^{-5}$ per year. Abrasion of PVC flooring has been evaluated in the RAR of DEHP (draft September 2001) on the basis of industrial information. There a lifetime loss (lifetime = 20 years) of 6.2% due to abrasion is assumed and a similar procedure is used in this RA. This loss is assumed to occur on surfaces with frequent walk (50% of surface). The

particles produced by abrasion are very small particles. The distribution of these particles is unknown, but it is assumed that 50% is removed by wet cleaning and ultimately released to WWTP. The rest is either removed by vacuum cleaner or ventilated out. Airborne abrasion particles may be expected to contribute to humane indoor air BBP concentration.

The emission rates of $1.6 \cdot 10^{-5}$ to air, $4 \cdot 10^{-4}$ to waste water via water extraction and $7.75 \cdot 10^{-4}$ (=0.0031 · 0.5 · 0.5) to waste water due to abrasion are used in EUSES. It is assumed that all BBP emitted to water enters the wastewater treatment system. A total use of BBP in PVC products equals to 11,960 tonnes in 2004 and assuming a lifetime of 20 years, the following emissions can be estimated for indoor use of PVC:

To air:	$11{,}960 \cdot 20 \cdot 1.6 \cdot 10^{-5}$	= 3.8 tonnes/year in EU
To wastewater:		
Due to water extraction:	$11{,}960 \cdot 20 \cdot 4 \cdot 10^{-4}$	= 96 tonnes/ year
Due to abrasion:	$11{,}960 \cdot 0.0031 \cdot 20 \cdot 0.5 \cdot 0.5$	= 185 tonnes/ year
Total release to waste water in 2004		= 281 tonnes/year.

Use of window sealants with BBP (life cycle stage IV b)

For building construction products an emission similar to that of life cycle stage IV c would be realistic. However for these products no information about the tonnage is available. Because the window sealant is closely confined within the window frame, any evaporation of BBP from window sealants to air is thought to be negligible.

Use of paints, inks and adhesives (life cycle stage IV c)

It is assumed that air is the main release compartment for these use categories. These products together accounts for a total of 6% or 1,080 tonnes of the BBP use within EU in 2004. Because BBP is used as a plasticiser in polymers within these products, an emission factor to air is based on the evaporation rate found for flooring. It is assumed that the ink, adhesive or paint thickness is 0.01 mm and that evaporation therefore is a factor 100 higher than for flooring. This gives a release fraction to air of 0.0016 per year. Further a lifetime of 5 years is assumed which will give the following estimate for emission to air.

To air: $1{,}080 \cdot 5 \cdot 0.0016 = 8.6$ tonnes/ year in EU

Use of non-polymer products (life cycle stage IV d)

This product group makes 2% of BBP use equal to 360 tonnes/year in 2004. According to industry a loss of 5% is assumed for air, water and soil on a yearly basis.

To air: $360 \cdot 0.05 = 18$ tonnes/year in EU
To wastewater: $360 \cdot 0.05 = 18$ tonnes/year in EU
To soil: $360 \cdot 0.05 = 18$ tonnes/year in EU

3.1.2.4 Release during disposal of end-products

Incineration

About 35% of all waste including BBP containing waste is assumed to be incinerated, the rest is assumed to be accumulated in landfills (Ejlertsson, 1997). During incineration most of the BBP will be combusted, however, fly ash from municipal incinerators has shown BBP concentrations

in the range of the detection limit of 1.2 mg/kg. Incinerator ash is most frequently disposed off in landfills, but it is not expected to contribute significantly to the BBP leaching from landfills. Without knowing the amount of ash deposited in landfill a more exact evaluation is not possible.

Landfills

In addition to disposal of PVC waste, landfills are also used for deposition of sludge from WWTP (Mersiowsky et al., 1999). Although the concentration in sludge as found in the study by Furtmann (1996) is low (n.d-3.5 mg/kg dwt) compared to PVC products which contain 10-30% BBP, the BBP present in sludge may leach out faster. In a model landfill system concentrations of up to 48 µg/l BBP were found in leachates from landfill during the first year (Ejlertsson, 1997). This was reduced to 2-7 µg/l during the second and third year. 8 µg/l were also found in an actual landfill in Sweden (Gryt landfill as quoted by Ejlertsson (1997)). The mean value of the measurements of BBP in the landfill lysimeter of Ejlertsson also gives a value of 8 µg/l. In a study of 9 landfills in Europe (Mersiowsky et al., 1999), BBP was measured in the seepage and a maximum value of 7 µg/l was found. One of these landfills had no treatment of leachate. However, this particulate site was not active; therefore it is assumed that all leachate is treated in WWTP. As 8 µg/l is the highest concentration measured in an active landfill, it will be assumed representative for all landfills. Using the landfill situation of UK as a model for the entire EU represents a worst case approximation as UK has a larger proportion of garbage going to landfills. Scaling up to include the whole of Europe is performed as follows:

Volume of leachate: (2000 landfills in UK) (10ha/landfill) (2,000 m^3 leachate/ha)
Population correction: 372 million in EU/57 million in UK
BBP conc. in leachate: 8 µg/l (Ejlertsson, 1997)

Release from landfills to WWTP:

$$\frac{(2{,}000\ \text{landfill})(10\text{ha/landfill})(2{,}000\text{m}^3\ \text{leachate/ha})(372/57)(8\ \text{mg/m}^3)}{1 \cdot 10^9\ \text{mg/tonne}} = 2.09\ \text{tonnes BBP/year}$$

In the landfill simulation study by Ejlertsson (1997) also the monoesters were monitored. An assessment of these monoesters is given in Annex 1.

3.1.3 Calculated local PECs in water, sediment, air and soil

Table 3.2 gives an overview over the input parameter used for calculation of the local PECs for life cycle stage III (formulation and processing) in 2004, as well as the corresponding PECs for water, sediment, air and agricultural soil.

Table 3.3 Calculated local PECs for water, air, soil and secondary poisoning and input parameters for life cycle stage III (formulation and processing) applying estimated 2004 tonnages

Scenario	IIIa-1	IIIa-2	IIIb-1	IIIb-2	IIIc	IIId	IIIe-1	IIIe-2	IIIf-1	IIIf-2	IIIg-1	IIIg-2	IIIh
Type of use	Plastisol Flooring Large site	Plastisol Flooring Small site	Sealants		PVC coated textiles	PVC Films and sheet	General PVC		Paints and inks		Adhesives		Confidential
Industry and Use category	11 Polymer 47 Softener	11 Polymer 47 Softener	11 Polymer 47 Softener		11 Textiles 47 Softener	11 Polymer 47 Softener	11 Polymer 47 Softener		12 Pulp,Paper Board 47 Softener (48)		0 Others 47 Softener		0 Others 0 others
Life cycle step	Processing	Processing	Formulation	Processing	Processing	Processing	Formulation	Processing	Formulation	Processing	Formulation	Processing	Formulation
Application (%use)	31	10	31	25	5	3	3	3	2	2	1	1	1
Tonnage [t/a]	6,000	2,000	6,000	6,000	1,800	1,080	1,080	1,080	720	720	360	360	360
Regional tonnage [t/a]	1,600	250	1,800	1,800	180	108	108	108	72	72	36	36	238
Main category	II	II	Ib	II	II	II	Ib	II	III	III	III	II	III
No. days (B-table)	300	300	300	300	300	300	300	65	300	36	300	115	300
Fraction of main source	1	1	1	0.1	1	1	1	0.15	1	0.05	1	0.8	1
Release to air	0.00125 ESD	0.0125 ESD	0.0005 A2.1	0.001[1]	0.0025 ESD	0.0025 ESD	0.00025 ESD	0.0025 ESD	0.00025 A2.1	0.05 A3.12	0.00025 A2.1	0.0001 A3.16	0.0025 A2.1
Release to water	0.00125 ESD	0.0125 ESD	0.00001[1]	0.0[1]	0.0025 ESD	0.0025 ESD	0.00025 ESD	0.0025 ESD	0.003[1]	0.0005 A3.12	0.003[1]	0.001 A3.11	0.003[1]
Release to ind.soil[2]	0.0005 A3.11	0.0005 A3.11	0.0001 A2.1	0.0[1]	0.0005 A3.11	0.0005 A3.11	0.0001 A2.1	0.0005 A3.11	0.0001 A2.1	0.0015 A3.12	0.0001 A2.1	0.005 A3.16	0.0001 A2.1

Table 3.3 continued overleaf

Table 3.3 continued Calculated local PECs for water, air, soil and secondary poisoning and input parameters for life cycle stage III (formulation and processing) applying estimated 2004 tonnages

Scenario	IIIa-1	IIIa-2	IIIb-1	IIIb-2	IIIc	IIId	IIIe-1	IIIe-2	IIIf-1	IIIf-2	IIIg-1	IIIg-2	IIIh
To air [kg/d]	6.67	10.4	3.0	0.60	1.5	0.90	0.09	0.62	0.60	4.97	0.30	0.03	1.98
To water [kg/d]	6.67	10.4	0.06	0.0	1.5	0.90	0.09	0.62	0.72	0.05	0.36	0.25	2.37
Standard STP yes/no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Dilution	10	10	10	10	10	10	10	10	10	10	10	10	10
PECair [µg/m^3]	1.52	2.38	0.69	0.14	0.34	0.21	0.02	0.03	0.14	0.14	0.07	0.002	0.45
PECstp [mg/l]	0.27	0.43	0.002	0	0.06	0.04	0.004	0.03	0.03	0.002	0.01	0.01	0.10
PECwater [µg/l]	27.1	42.3	0.41	0.17	6.23	3.81	0.54	2.69	3.08	0.38	1.63	1.18	9.75
PECsediment [mg/kg wwt]	6.20	9.67	0.09	0.04	1.43	0.87	0.12	0.61	0.71	0.09	0.37	0.27	2.23
PECagr. Soil [mg/kg wwt]	8.79	13.7	0.08	0.001	1.98	1.19	0.12	0.82	0.95	0.07	0.48	0.33	3.13
Oral worm [mg/kg]	15.4	24.0	0.15	0.009	3.47	2.08	0.22	1.44	1.67	0.12	0.84	0.58	5.47
Oral fish [mg/kg]	5.05	7.84	0.12	0.08	1.20	0.75	0.14	0.18	0.61	0.08	0.35	0.15	1.84

ESD) Release factors taken from the ESD “Plastics” (OECD, 2004)
1) For explanation see text in Section 3.1.1
2) No further assessment is made for industrial soil.

3.1.4 Calculation of regional and continental PECs

The calculations of regional and continental PECs were performed using the EUSES model. The contribution of the different life cycle stages to the regional and continental PECs is presented in **Table 3.4**. Regional releases are estimated based on that 10% of the EU consumption occurs within a region. The exceptions are with respect to flooring, where 20% is assumed to be used in one region, formulation of sealants (IIIb), where 30% is assumed to be used within one region and to production, where there are only 3 sites and the entire tonnage is assumed to be produced in one region and site specific release is used. Industry has specified that with respect to use category "confidential" (IIIh) there is one site that uses 0.66 of the total tonnage. This site is also used for assessing regional releases.

The EUSES WWTP defaults for production sites have been substituted with site-specific information with regard to release to surface water according to **Table 3.1**. Instead of applying a WWTP at production sites it is assumed that monitored amount is released directly to surface water (see Section 3.1.2).

Table 3.4 shows the daily release to air and wastewater for all life cycle stages used for calculating regional and continental PECs.

Table 3.4 Regional and continental releases for all life cycle stages

	Regional [kg/d]		Continental [kg/d]	
	air	water	air	water
I Production	0.41	1.60	0	0
II Distribution	0	5.75*	0	0
IIIa-1 PVC flooring, large site	5.48	5.48	15.1	15.1
IIIa-2 PVC flooring, small site	8.56	8.56	59.9	59.9
IIIb-1 Form. sealants	2.47	0.05	5.79	0.12
IIIb-2 Proc. sealants	4.93	0	11.5	0
IIIc PVC textiles	1.23	1.23	11.1	11.1
IIId Polymer films	0.74	0.74	6.66	6.66
IIIe-1Form. gen. PVC	0.07	0.07	0.67	0.67
IIIe-2 Proc. gen. PVC	0.74	0.74	6.65	6.65
IIIf-1 Form. inks	0.49	0.59	4.44	5.33
IIIf –2 Proc. inks	9.80	0.10	88.3	0.88
IIIg-1 Form. adhesive	0.25	0.30	2.20	2.70
IIIg-2 Proc. adhesive	0.01	0.10	0.09	0.88
IIIh Form. non-polymer	1.62	1.95	0.84	1.0
IVa use PVC products	1.95	141	8.46	607
IVb use sealing	negligible	negligible	negligible	negligible
IVc use inks adhesive	2.27	negligible	20.4	negligible

Table 3.4 continued overleaf

Table 3.4 continued Regional and continental releases for all life cycle stages

	Regional [kg/d]		Continental [kg/d]	
	air	water	air	water
IVd use non polymer	4.90	4.9	44.1	44.1
V Disposal	0	1.02	0	4.39
Total	45.9	168	286	766

* Not included for calculation of regional and continental concentrations

Table 3.5 Regional and continental PECs estimated by EUSES

Compartment	Regional	Continental
Year	2004	
PEC in water [µg/l]	0.17	0.01
PEC in sediment [mg/kg wwt]	0.07	$4.5 \cdot 10^{-3}$
PEC in air [µg/m^3]	$6.3 \cdot 10^{-4}$	$5.8 \cdot 10^{-5}$
PEC in agri. soil [mg/kg wwt]	$3.0 \cdot 10^{-3}$	$1.6 \cdot 10^{-4}$

3.1.5 Monitoring data

3.1.5.1 Measured concentrations of BBP in the air

There are few data available on air concentrations of BBP. One study from Spain sampled coarse and fine aerosol particles both during the summer and winter season (Aceves and Grimault, 1993). The samples were taken in Barcelona City centre. The data ranged from 0.25 ng/m^3 up to a maximum of 8.0 ng/m^3.

Recent data from several sites in EU show values equal to or below 2 ng/m^3 in 12 samples and a value of 3 ng/m^3 in 4 samples (RIVM/CEFIC).

In Denmark deposition of BBP from air was measured. Measurements were made both in the countryside and at an urban site (Århus). They found deposition rates of 11.2 µg/m^2/year in the countryside and 45.9 µg/m^2/year in Århus. Scaling up to include the entire area of the EU ($3.6 \cdot 10^6$ km^2) would account for between 40.3 and 165 tonnes of deposition of BBP within EU on a continental basis. A more recent study by Vikelsøe et al. (2001) gives a mean deposition of 17 µg/m^2/year at Roskilde. This value results in a deposition of 61 tonnes when averaged for the whole of EU. These values are between 33 and 136% of the estimated total direct release (121 tonnes/year) to air (see **Table 3.4**). That such a high fraction of release to air is deposited may indicate that BBP mainly is deposited rather than degraded in air.

Outdoor air monitoring of BBP close to two flooring plants

CEFIC took an initiative to monitor the air release from the two types of use categories representing the main contributions of BBP to air, use categories flooring and sealants. The following presentation of the results of this monitoring study is an edited and compressed version of the report (CEFIC, 2003) submitted to the Rapporteur. When evaluating the results of an air monitoring study, whose main aim is to sample a representative air sample 100 m apart

from a point source, many uncertainties must be taken into account. Variables such as wind speed and direction, turbulence, height above ground of outlet point relative to sampling point and topography of the area are all examples of variables that influence the degree of dilution observed at the sample point, relative to the concentration in ventilated air.

Samples were collected on polydimethysiloxane by passing 6-15L of air through sorption tubes. After desorption samples were analysed by GC-MS. Sample values are subtracted blank values and the limit of detection was 2 ng/m^3. **Table 3.6** and **Table 3.7** gives the results for two flooring plants.

Table 3.6 Air concentration at a flooring plant 1 taken approximately 100 m from point source at Plant 1 at 14-15.30 hours for all sample dates

Samples	Wind conditions	Weather	Temp [°C]	BBP [ng/m³]
Sampling time: 10 December 2002				
1 and 2	In wind direction.	Sunny, no clouds, nearly no wind	-2	14 9
3 and 4	Opposite to wind direction	Sunny, no clouds, more windy than for samples 1 and 2	-2	403 399
Sampling time: 13 January 2003				
1 and 2	30° out of wind direction,	Clouded, windy	5	8 10
3 and 4	Opposite to wind direction	Clouded, very windy	5	319 297
5 and 6	Opposite to wind direction	Clouded, very windy	6	82 129
Sampling time: 1 September 2003 after installation of a new "droplet" trap				
1 and 2	Opposite to wind direction	Clouded, windy	22	14 17
3 and 4	In wind direction	Clouded, windy	22	38 17

Sampling at plant 1 was done at three different occasions, twice in the winter and once in the summer. It should be noted that prior to the last sampling plant 1 had taken some actions to reduce emissions and a new "droplet trap" was installed on the ventilation system. According to information from industry most ventilation from the production hall is only "filtered" by the "drop-trap" and the air is then emitted outside. The chimney is only 1 m high and the emitted air is projected against the roof at a throughput of 35.000 m^3/hour.

If the wind direction descriptions given in the table are correct then all observed values > 38 ng/m^3 seem to originate from other sources than the flooring plant. This does not seem logical and therefore the high values are assumed to be a result of emissions from the flooring plant. Turbulence may give different wind directions at ground level than above the plant. Industry has suggested that the observed anomaly may be due to irregular local wind flows between the buildings. Parallel samples show fairly good consistency and are therefore likely to be representative for the conditions at the sampling time. The report describes that the sampling in January and September was done after a period with intense showers.

The results from the sampling in January fit quite well with the results from the sampling in December. Between the second (January) and third (September) sampling time plant 1 had taken

some actions to reduce emissions and a new droplet trap was installed on the ventilation system. All samples at the September sampling time show consistently low concentrations and this is probably mainly due to the emission reduction actions. However, it may be questioned whether the sampling is truly representative considering the difficult wind systems at the plant and the relatively few number of samples taken.

The results from plant 2 are given in **Table 3.7**. The emissions from Plant 2 seem to behave in a more "normal" manner in relation to wind direction. The monitoring values at plant 2 are comparable to the values for plant 1 with respect to high concentrations in the December sampling. Measurements taken 27th August in the wind direction from the chimneys after a shower seem to have "missed" any emissions from the plant. Whether the low levels are mainly due to the reported 70% reduction in BBP consumption cannot be ascertained. The low levels may partly be due to wet deposition or due to non representative sampling (few samples). More sampling would be needed in order to document the effect of the reduced BBP consumption.

Apparently the summer levels around the plants are lower than the winter levels. Whether this is altogether due to reduced emissions in both plants or may also partly be due to the different weather conditions is uncertain.

Table 3.7 Air concentration at a flooring plant 2 taken approximately 100m from point source at Plant 2 at 12.30-14.30 hours for all sample dates

Samples	Wind conditions	Weather	Temp [°C]	BBP [ng/m³]
Sampling: 11 December 2002				
1 and 2	In wind direction, 75-150 m from chimneys (*)	Sunny, no clouds, very windy, very cold	-5°C	285 237
3 and 4	90° out of wind direction	Sunny, no clouds, very windy, very cold	-5°C	12 10
Sampling: 27 August 2003, a 70% reduction in BBP use was reported compared with first sampling time				
1 and 2	Opposite to wind direction, 75-150 m from chimneys (*)	Moderate wind, very cloudy, after shower	14°C	10 4
3 and 4	In wind direction, 200 m from chimneys	No wind, cloudy, after shower	18°C	7 5

* At Plant 2, the production halls have in total 5 exhaust chimneys

Outdoor air monitoring of BBP close to one sealant plant

CEFIC took an initiative to monitor air release from the two types of use representing the main contributions of BBP to air, use categories flooring and sealants. The following presentation of the results of this monitoring study is an edited and compressed version of the report (CEFIC, 2003) submitted to the Rapporteur.

The intention was to measure emissions from two sealant sites, but industry reported that "due to changes in the use pattern of BBP it will not be possible to measure emissions from two sealant sites." Results from the monitoring at one sealant producer, plant 3, are presented in **Table 3.8**. According to plant 3 phthalates are used in a "closed circuit", "no special ventilation". At the time of sampling only BBP was used. Samples were taken from 14.00-15.00 on 21st August 2003. Air samples from the sealant plant were taken only 25-50 m from production hall, and depending on height of chimney/ventilation pipe contaminated air might not have been fully mixed down to ground level at this point. It is therefore not possible to assess whether these sample can be assumed to be representative of emissions from this type of use. Samples

downwind from the release site are higher than at the opposite side, indicating that sampled air contains BBP released from the plant.

Table 3.8 Air monitoring at a sealant plant in Germany.

Session 1	Location: Plant 3	Weather	Temp [°C]	BBP [ng/m³]
1 and 2	In wind direction, 25-50 m from production hall	Sunny, very warm, dry	28°C	38 6
3 and 4	Opposite to wind direction	Sunny, very warm, dry	28°C	1 1

With respect to BBP used in sealants, the monitoring values are much lower than the estimated values, however, the 2 samples taken downwind vary greatly in measured amount and also the sample point is too close to the emission point. It is therefore thought that these samples may not be representative for the actual emission situation.

3.1.5.2 Measured concentrations of BBP in the aquatic environment

Because phthalates occur in plastics used in laboratories and sample collectors, measurements may sometimes be overestimated. There is rarely information available in older reports on how such contamination had tried to be avoided. One may assume that more recent measurements are less affected by such problems. One should have this in mind when reading the summary tables below giving BBP concentrations in various compartments.

Measurements of BBP in sewage treatment plants

Influent and effluent BBP concentrations in sewage treatment plants (STP) are available for several countries (see **Table 3.9**). It is assumed that none of the measurements presented in this table represent BBP specific local activities in the EU.

In the USA there are measurements of influent concentrations in WWTPs of up to 0.5 mg/l which probably reflects industrial use of BBP (US EPA, 1982).

In a survey of 3 municipal STPs in Norway, concentrations in the range of 0.26-0.58 µg/l were found in the influent (Braaten et al., 1996).

Based on differences in the USA between influent and effluent BBP-concentrations in wastewater more than 80% of STPs had a 90% removal of BBP in secondary STP treatment, while 10% had less than 40% removal (US EPA, 1982). Similar results for the Norwegian STP are 77% removal in the best STP and only 14% removal in the worst STP (no secondary treatment). Much of the reduction may be caused by adsorption to the sludge. Sludge concentrations in the Norwegian STP were in the range of 0.14 to 1.4 mg/kg dwt (see **Table 3.15**).

The fate of several chemicals was monitored in a Danish STP, Søholt at Silkeborg (Boutrup et al., 1998). BBP was analysed in the influent and effluent and in sludge on a weekly basis during week 46, 47 and 48 in 1997. BBP was found in the range of 0.6-1.7 µg/l in the influent and < 0.5 µg/l in effluent. Sludge concentrations were between 100-410 µg/kg dwt. It was calculated that 52-95% were degraded in the STP. Agricultural soil amended with sludge did not have detectable concentrations (< 50 µg/kg dwt) of BBP.

Applying the TGD defaults for BBP in a sewage treatment plant with biological treatment give 92% removal with 42% directed to sludge. In EUSES calculations the TGD defaults for BBP have been used.

Table 3.9 Measured BBP concentrations in various STP and WWTP influent and effluent

Location	Concentration	Source
Netherlands, 12 STP Municipal	Influent:5-1 µg/l Effluent: < 0.1-1 µg/l	Vethaak (2002)
Netherlands, 10 STP Industrial	Influent:1-2 µg/l Effluent: < 0.1-1 µg/l	Vethaak (2002)
Norway, 3 STP	Influent:0.260-0.583 µg/l Effluent: < 0.060-0.500 µg/l	Braaten et al. (1996)
Sweden	1-10 µg/l range influent 1989-91 1.2 µg/l average influent 1989 2.1 µg/l average influent 1990 1.1 µg/l average influent 1991 ND in effluent 1989-91	Paxéus, et al. (1992)
Effluent from Sweden's 3 largest STPs	6 µg/l Henriksdal STP 17 µg/l Goteborg Regional STP ND1 Sjolunda STP	Paxéus (1996)
Netherlands 5 WWTP	0.3-2.0 µg/l in influent 5 samples	Van der Velde et al. (1999)
Germany 5 WWTP	0.062-0.284 µg/l in effluent 5 samples	Spengler et al. (1999)
Germany WWTP	0.15 µg/l in 1 of 42 sampled effluents the other were ND	Braun et al. (2000)
Germany 2 WWTP industrial	< ND-0.72 µg/l influent < ND in effluent.	Braun et al. (2000)
Germany 2 WWTP	0.8 µg/l influent mainly household 2.4 µg/l influent mainly industrial < 0.04 µg/l in effluent	Furtmann (1996)
Denmark, municipal STP	0.6-1.7 µg/l influent, < 0.5 effluent	Boutrup et al. (1998)
USA	8 µg/l influent, 1.3 µg/l effluent	Gledhill (1980)
USA	Influent: max 560 (µg/l) 57% with conc. > 2 µg/l Effluent: max 34 µg/l 11% with conc. > 1 µg/l (89% non-detect at 1 ug/L)	US EPA (1982)

1) ND Not Detected

A Norwegian survey of organic pollutants in wastewater includes measurements of BBP in effluents from small industry (Nesgård et al., 1998). It should be noted that all small type industry effluents were collected and diverted to the local sewage treatment plant. This plant had an inflow concentration of BBP of 1.3 µg/l in the same sample period. A summary of the results is presented in **Table 3.10**.

Table 3.10 Effluent concentrations of BBP from different types of small industry measured in Norway (Nesgård et al., 1998)

Industry type	BBP Emissions [µg/l] in effluents from industry					
	Average	Median	Min	Max	SD	n
Gas station with car wash	1.4	1.2	0.1	5.3	1.3	11
Wash hall for heavy transport	2.6	---	2.2	3	---	2
Wash hall for technical equipment, before treatment	106	---	52	160	---	2
Wash hall for technical equipment, after treatment	7.6	0.7	< 0.3	22	12.5	3
Motor vehicle workshops	8.3	6.9	< 0.3	20	8.2	6
Metal and machine workshops	3.6	2.1	< 0.3	11	4.4	6
Laundries	9.6	9.5	8.4	11	1.2	4
Graphical Companies	2	---	0.4	3.6	---	2
Paint and paintspray industries	4.1	---	1.8	6.3	---	2
Paintspray companies	0.3	---	< 0.3	0.6	---	2
Chemical Industry	3.7	---	---	---	---	1
Laboratories	0.5	---	---	---	---	1
Hospitals	< 0.3	---	---	---	---	1
Technical College	< 0.3	---	---	---	---	1
Food control company	1.4	---	---	---	---	1

A comparative study of BBP waste water from different diffuse sources in Denmark was reported by Vikelsøe et al. (1998), see **Table 3.11**. The range in BBP concentrations found in effluents from car washing of 0.5-150 µg/l was much higher than the range detected in Norway (0.1-5.3 µg/l). However, BBP concentrations found in laundry effluents in Norway were in the range of 8.4-11 µg/l and higher as in laundry effluents in Denmark (< 2 µg/l). The absolute maximum concentration measured in Europe is 320 µg/l found in one sample from the effluent of a kindergarden.

Table 3.11 Concentration ranges of BBP in waste water from different diffuse sources in Denmark

Diffuse source	Car Wash	Hospital	Laundry	Kindergarden
No. of samples	26	12	2	1
BBP [µg/l]	0.5-150	< 2	< 2	320

Another study in Norway covered exclusively the contribution from household (Nesgård and Lima-Charles, 1998). The effluent of 240 houses, with an estimated population of 800 persons, was collected directly at certain collection basins. A summary of these results with respect to BBP emission is shown in **Table 3.12**. The influent concentration in the receiving sewage treatment plant had an average concentration of 1.6 µg/l, equal to a pro capita release of 1.168 g/year. Scaling up the release measured here to include the whole of Norway gave an annual release from households of 4.67 tonnes/year of BBP. Scaling up this value to cover EU (Norway: a population of 4 million, EU: a population of 372 million) would indicate a total release of 434 tonnes of BBP within the EU directed to STP, which is less than the 600 tonnes/year calculated for life cycle stage IVa (see Section 3.1.2.3).

Table 3.12 Concentrations of BBP in sewage from households in Norway (Nesgård and Lima-Charles, 1998)

BBP emissions from household effluent [µg/l]					
Average	Median	Min.	Max.	SD	n
1.6	1.4	< 0.3	3.9	1.1	23

Measurements of BBP in surface water

There are no monitoring data available that may presently be considered as local water concentrations for production or formulation sites. BBP measurements in surface water are available for a variety of locations from heavily industrialised locations in Germany to more pristine locations in Norway and Sweden. A summary of available studies is shown in **Table 3.13**. Most of the samples show levels of BBP of less than 1 µg/l. The exceptions are the samples taken from industrial areas in Germany and the marine measurements in the North Sea. Both the Rhine and Emscher locations gave samples with BBP concentrations > 1 µg/l. The maximum measured concentration in the Rhine was 3.4 µg/l and in a tributary river, Wupper, concentrations up to 13.9 µg/l have been measured (Furtmann, 1996).

The highest concentrations were measured in the Wupper. The actual measurements for Wupper are presented in **Figure 3.1**. The mean concentration during the sampling period 1991/92 was 3.45 µg/l with 6 samples > 10µg/l.

Recently a new monitoring survey (Braun et al., 2000) of phthalates in industrial parts of Germany was reported, covering much of the same area as the Furtmann (1996) study. The report concluded that background contamination in surface water with phthalates continued at the same level as in early 1990. Because of the prior problem of high values in the Wupper, an intensified program was launched to identify a possible local source. First a monitoring screening was performed. Water samples from the river Rhine and its tributaries were taken at 8 different places at three times (May, June, November 1999). BBP concentrations were below detection limit (0.05 µg/l) at 6 sites, but for two places, Bimmen and Wupper/Opladen, BBP concentrations of 0.35-13.0 µg/l were detected. The data from the Wupper/Opladen, taken in 1999, showed some samples with similar high BBP concentrations (1.7-13.0 µg/l) as in the Furtmann (1996) study. In a second monitoring survey in 1999 water samples were taken at 10 stations along the Wupper with stainless steel buckets in June and October. Measured concentrations were in the range of 0.09-1.1 µg/l in June and 0.18-1.6 µg/l in October, with the highest concentration found at the end of the stream course during the June sampling and the opposite concentration profile in the October sampling. This could indicate an intermittent release of point sources. The lower BBP values in the range of 0.09-1.6 µg/l are more in accordance with monitoring data from other German rivers. The report concludes that the increased BBP concentrations in Wupper may probably be attributed to industrial activities and a high population density in this area. The small runoff for the Wupper might also contribute to increased BBP concentrations in this river. It was suggested that the difference in measured BBP concentrations at Wupper/Opladen might perhaps be due to the different sampling methods used. Water samples taken with automated sampling equipment gave higher BBP concentrations (1.7-13.0 µg/l) than samples taken with a steel bucket (0.40-0.46 µg/l). Therefore a parallel analysis of water samples, taken with the two different sampling equipments was performed, which indicated that the automatic sampler contaminates water samples, this gave a 2.9 µg/l higher BBP concentration. However, the detected value of 2.9 µg/l could not explain measured BBP concentrations up to 13 µg/l by cross-contamination only.

Based on the former findings a new monitoring study for BBP in the Wupper was performed in 2003 by the Nord Rhine Westfalian authorities (for results see also **Table 3.13**). Water samples were taken twice a month from February to December either by an automatic gaging station or per hand with a steel bucket. The gaging station situated at Wupper/Opladen (5.5 km right border) and sampling point for "hand samples" was Wupper/ Lev-Rheindorf (0.6 km). Detection of BBP was performed by gas chromatography with a detection limit of 0.063-0.080 µg/l. Comparison of the two different sampling methods did not show any problem due to contamination via equipment. Out of 43 values were 41 values were non-detects and the other two gave values of 0.11 and 0.16 µg/l. The recent results show much lower BBP levels in the Wupper, indicating that levels of BBP are no longer of concern here.

The monitoring study of Vethaak et al. (2002) includes two samples taken from the North Sea and the estuary of the river Ems Dollard with measured concentrations of 1.8 µg/l and 1.0 µg/l.

Table 3.13 Measured BBP concentrations in surface water / seawater

Location	Concentration	Source
Netherlands, Rhine and Meuse 23 stations	0.01-0.6 µg/l (3 samples each station) median 0.08 and 0.06 µg/l	Vethaak et al. (2002)
Netherlands, North-Western areas, 11 stations, partly estuary	0.01-1.0 µg/l (3 samples each station) median 0.01 µg/l	Vethaak et al. (2002)
Netherlands, North Sea, 4 stations	0.01-1.8 µg/l (3 samples each station)	Vethaak et al. (2002)
Netherlands, surface water	< 0.010-1.8 µg/l, n=87, median 0.077 µg/l, n=83	Vethaak et al. (2002)
EU	Average 0.064 µg/l n=85	Compiled by CEFIC
Denmark Roskilde fjord Estuarine	Mean 2.9 ng/l, range 1.5-7.1 ng/l n=30	Vikelsøe et al. (2001)
Denmark stream and lake water	Mean 5.1 ng/l Range 1.9-13 ng/l n=19	Vikelsøe et al. (2001)
Norway freshwater lakes	ND or Traces < 60 ng/l	Braaten et al. (1996)
Norway fjords marine	ND	Braaten et al. (1996)
UK, Mersey Estuary	6-28 ng/l 100% of samples with BBP	Preston and Al-Omran (1986)
UK, Mersey Estuary	68-103 ng/l, 4 of 5 samples with detectable conc.	Preston and Al-Omran (1989)

Table 3.13 continued overleaf

Table 3.13 continued Measured BBP concentrations in surface water / seawater

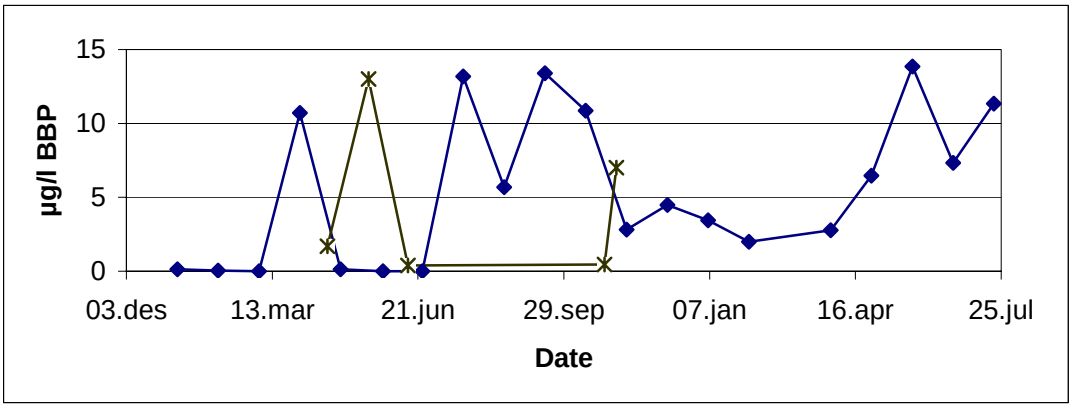
Location	Concentration	Source
Netherlands, surface water	< 0.1 µg/l 16 locations	Van der Velde et al. (1999)
Germany Rhine	0.04-3.4 µg/l, 25% of samples with BBP >0.04	Furtmann (1996)
Germany, Nordrhein- Westfalen, canals	0.07-0.93 µg/l, 60% of samples > 0.04 µg/l	Furtmann (1996)
Germany, Emscher	Mean 0.11 µg/l, max 2.0 µg/l, 19 of 21 samples with detectable conc.> 0.04	Furtmann (1996)
Gemany, rainwater ditches along roads	< 0.05-2.1 µg/l, 4 samples	Braun et al. (2000)
Germany tributaries of Rhine	12 samples from 4 locations with non detectable conc.	Braun et al. (2000)
Germany Rhine	< 0.05-0.68 µg/l, mean 0.22 µg/l, 8 samples 3 locations	Braun et al. (2000)
Germany Wupper (Feb-Dec 2003)	43 values, 41 non detects (det. lim. 0.063-0.080 µg/l), two samples 0.11-0.16 µg/l	Nord Rhine Westfalian Authorities (2003)
Germany Wupper(June 1999)	0.09-1.1 µg/l (5 stations)	Braun et al. (2000)
Germany Wupper (Oct. 1999)	0.18-1.6 µg/l (9 stations) 0.10-2.9 µg/l (6 stations)	Braun et al. (2000)
Germany Wupper (April-Nov 1999)	1.7-13.0 µg/l ,5 samples	Braun et al. (2000)
Germany, Wupper	Mean 3.45µg/l, 0,04-13.9 µg/l 15 of 21 samples with detectable conc.> 0.04	Furtmann (1996)
Germany, seep from landfill	0.04-13.2 µg/l	Furtmann (1996)
Sweden, lakes and rivers urban and remote	0.048 µg/l in 1 of 8 samples detection limit 0.006 µg/l	Parkman and Remberger (1996)
Germany, Rhine Germany, Lake Yessel	0.1 µg/l average < 0.01 µg/l average	Ritsma et al. (1989)
USA, Delaware r.	0.4-1 µg/l 5 of 11 samples winter 0.3-0.3 µg/l 11 of 11 samples, Summer	Sheldon and Hites (1978)
USA, L. Michigan	1 µg/l 1 sample	Konasewich et al. (1978)
USA, L. Michigan	2-4 µg/l in 2 of 13 samples	Pierce et al. (1980)
USA, 11 locations	0.2-2.4 µg/l range	Gledhill et al. (1980)
USA, STORET database 1220 samples	< 10 µg/l average	Staples et al. (1985)
USA, data compiled	0.35 µg/l average	Verschueren (1983)

Table 3.13 continued overleaf

Table 3.13 continued Measured BBP concentrations in surface water / seawater

Location	Concentration	Source
USA, Lake Pontchartrain	0.3 µg/l average	McFall et al. (1985)
USA	< 0.5 µg/l geometric mean 15 detects of 204 samples	Michael et al. (1984)
Canada, Drinking water reservoirs	< 5 µg/l	Hargesheimer and Lewis (1987)

Figure 3.1 Measured BBP concentrations in the Wupper (Germany; Furtmann, 1996) (-♦-) and from the Braun et al. (2000) study (–x-) at the same station (Opladen). Results of monitoring in 2003 not shown (out of 43 values, 41 values are below detection limit and two values of 0.11 and 0.16 µg/l).



3.1.5.3 Measured concentrations of BBP in the sediment

A summary of the BBP concentration in sediment and suspended matter is given in **Table 3.14**. The absolute highest concentration was recorded from Sweden with 190 mg/kg dwt (dry weight) in the sediment of a shallow lake (Golder, 2000). High concentrations of DBP (1.2 mg/kg dwt) and DEHP (< 0.1 – 100 mg/kg dwt) were also found in this sample. The sample locations of the Golder study has been resampled and analysed resulting in BBP concentrations in the range of < 10-25 µg/kg dwt, indicating that the former high concentration may be due to contamination during sampling or analysis. A recent survey from Braun et al. (2000) showed a high BBP concentration of 12 mg/kg dwt in sediment from rainwater ditches along German motorways. A DEHP concentration up to 1,300 mg/kg was also measured in the same samples. Maximum measured sediment concentrations elsewhere in Europe are from an industrial port basin along the Rhine in North Germany (0.31mg/kg wwt) and Rhine sediment with up to 0.32 mg/kg wwt (wet weight) (Furtmann, 1996).

In a monitoring study from Denmark (Boutrup et al., 1998) data from urban estuaries showed values of 0.1 mg/kg wwt while one sample out of 8 from a marine urban port had up to 1.3 mg/kg dwt. This high value will not be used further in the risk characterisation because more recent marine monitoring data from the Netherlands (Vethaak et al., 2002) and Denmark (Vikelsøe et al., 2001) are available showing much lower BBP levels in the range of 3-60 µg/kg dwt.

Rhine sediment showed in general high concentrations with an average of 178 µg/kg wwt (5 samples) and this might represent a regional case typical for a heavily industrialised area as the samples were taken along a more than 200 km stretch of the river. Other industrial areas of Germany show lower concentrations with only 2 of 31 samples having detectable BBP

concentrations. Recent work, commissioned by the European Council of Plastics Industries, showed levels of BBP in fresh water sediment in the Netherlands in the range < 4-78 µg/kg dwt from 27 different sites (Alcontrol/ECPI, 1999). Other data in **Table 3.14** from the Netherlands, Norway, Sweden and Denmark showed lower concentrations but indicate a general widespread exposure of BBP to the environment.

Table 3.14 Measured concentrations of BBP in freshwater and marine sediment

Location	Concentration	Source
Netherlands, 16 stations Sediment	< 4.5-60 µg/kg dwt (3 samples each station)	Vethaak et al. (2002)
Netherlands, North Sea 3 stations, Sediment	< 4.5-20µg/kg dwt (3 samples each station)	Vethaak et al. (2002)
Netherlands, 19 stations In suspended matter	< 0.004-3.0 mg/kg dwt (3 samples each station)	Vethaak et al. (2002)
EU	< 20 µg/kg dwt n=4 sites 94 µg/kg dwt n=1 site	RIVM/CEFIC data
Denmark Roskilde fjord Estuarine	Mean 6 µg/kg dwt, range 2.7-7.0 µg/kg n=36	Vikelsøe et al. (2001)
Denmark stream and lake water	Mean 2.6 µg/kg dwt Range 1.4-5.5 µg/kg n=14	Vikelsøe et al. (2001)
Netherlands, freshwater	< 4-78 µg/kg dwt	Alcontrol/ECPI (1999)
Netherlands 3 locations	< 10-60 µg/kg dwt	Van der Velde et al. (1999)
Netherlands, L. Ketelmeer	69 µg/kg wwt, top slice of sediment core	Remberger and Okengren (1997)
Germany, rainwater ditches along roads	12 mg/kg dwt, 1of 3 samples	Braun et al. (2000)
Germany, Rhine, harbour sed.	< 0.01-0.27 mg/kg dwt, 6 samples	Braun et al. (2000)
Germany, Rhine	110-320 µg/kg wwt 5 of 7 samples, mean 178 µg/kg	Furtmann (1996)
Germany, port basins along river Rhine	30-310 µg/kg wwt 2 of 9 samples	Furtmann (1996)
Germany, River Weser	ND in 10 samples	Furtmann (1996)
Germany, Nordrhein-Westfalen, Canals	ND in 12 samples	Furtmann (1996)
Sweden, close to cities	2-27 µg/kg dwt surface 0-2 cm ND-10 µg/kg dwt subsurf. 6-16 cm	Parkman and Remberger (1996)
Resampling of the Golder (2000) study, Sweden, small suburban lakes	< 10-25 µg/kg dwt, 5 samples	Venhuizen (2004)
Sweden, small suburban lakes	0.8, 2.2 and 190 mg/kg dwt	Golder (2000)
Sweden, west coast	60-170 µg/kg dwt, 4 of 33 samples	Neste Oxo (1996)
Denmark, marine urban port	1,300 µg/kg dwt 1 of 8 samples	Boutrup et al. (1998)
Denmark, urban estuaries	35, 130 µg/kg dwt, 2 of 6 samples	Boutrup et al. (1998)
Norway, urban estuary	20-112 µg/kg dwt 6 of 11 samples	Braaten et al. (1996)
Norway, freshwater lakes	23, 38 µg/kg dwt 2 of 6	Braaten et al. (1996)

Table 3.14 continued overleaf

Table 3.14 continued Measured concentrations of BBP in freshwater and marine sediment

Location	Concentration	Source
Denmark, lakes	27-44 µg/kg dwt 2 of 5 samples	Boutrup et al. (1998)
UK, Mersey estuary	3.4-47 µg/kg dwt range	Preston and Al-Omran (1986)
UK, Mersey estuary	ND-23 µg/kg dwt 2 samples	Preston and Al-Omran (1989)
USA	130-630 µg/kg dwt 9 of 122 samples	Michael et al. (1984)
USA, 11 locations	< 100-567 µg/kg 2 of 28 with detects	Gledhill et al. (1980)
USA, STORET database	< 500 µg/kg median 6% with detects 392 samples	Staples et al. (1985)

3.1.5.4 Measured concentrations of BBP in soil and sludge

Measurements of BBP in soil and in sludge are presented in **Table 3.15**. BBP in sludge becomes relevant to soil due to sludge application. Available studies of soil concentration are in the range of < 0.0001-0.4 mg/kg dwt. The highest concentrations (> 0.1 mg/kg dwt) are found close to BBP emitting sites and waste sites. Heavily sludge amended soil in Denmark had BBP concentrations in the range of 0.007-0.051 mg/kg dwt (Vikelsøe, 1999). The recent study in Netherlands (Alcontrol/ECPI, 1999), reports only 5 detects of BBP in 34 samples from 33 sites in the range < 0.004-0.009 mg/kg dwt. Another recent study from Denmark (Vikelsøe, 1999), with a detection limit of 0.0001 mg/kg dwt, found 7 detects in 30 samples in the range of 0.0001-0.001 mg/kg dwt.

Sludge concentrations from municipal STPs receiving effluent from households in Norway showed BBP concentrations in the range of 0.14-1.4 mg/kg dwt (Braaten et al., 1996). Activated sludge from a German STP receiving mostly industrial effluent (Furtmann, 1996) had no measurable BBP concentration (< 0.14 mg/kg dwt) while dewatered sludge from the same STP had 0.52 mg/kg dwt.

Table 3.15 Measured BBP concentrations in sludge/soil

Location	Concentration	Source
EU soil	Median 44 µg/kg dwt Range < 30-169 µg/kg dwt	RIVM/CEFIC
Netherlands, soil	< 0.004-0.009 mg/kg dwt, 5 of 34 samples with detects	Alcontrol/ECPI (1999)
Germany, industrial WWTP	ND – 2.7 mg/kg dwt	Furtmann (1996)
Germany, household WWTP	0.6 – 3.5 mg/kg dwt	Furtmann (1996)
Germany, sludge WWTP	< 0.05-0.7 mg/kg dwt	Zurmühl (1990)
Germany, soil 3 phthalate emitting plant sites	0.1 mg/kg dwt max mean 0.03 mg/kg one site	Müller and Kordel (1993)

Table 3.15 continued overleaf

Table 3.15 continued Measured BBP concentrations in sludge/soil

Location	Concentration	Source
Denmark, soil	< 0.0001-0.001 mg/kg dwt agricultural soils, 6 depths 7 of 30 samples with detects 0.0007-0.051 mg/kg dwt Sludge amended soil	Vikelsøe (1999)
Denmark, sludge 4 WWTP	0.001-0.037 mg/kg dwt average 0.053 mg/kg dwt.	Vikelsøe (1999)
Denmark, 6 WWTP	0.1-0.41 mg/kg dwt, 5 of 6 with detects (> 0.05 mg/kg dwt)	Boutrup et al.(1998)
Denmark, sludge winter/summer, 3 locations	< 0.5 mg/kg dwt, no detects	Kjølholt et al. (1995)
Spain soil waste site	0.4 mg/kg dwt 1 of 5 samples	Navarro et al. (1991)
Norway sludge 3 WWTP	140-1,400 µg/kg dwt	Braaten et al. (1996)
USA, sludge WWTP	40 mg/kg dwt in 1 of 3 samples	Strachan et al. (1983)
USA, sludge WWTP	0.52-210 mg/kg dwt(mean 15 mg/kg), 15 samples	Wild and Jones (1992)
USA, sludge WWTP	Max conc. 45 mg/l dwt 43% of samples had conc.> 2 µg/kg dwt and 17% has conc. > 160 µg/kg dwt	US EPA (1982)

3.1.5.5 Measured concentrations of BBP in biota

In oysters from Delaware (Orville et al., 1981) one of 17 samples had trace amounts of BBP (less than 0.15 mg/kg) and one of 6 oyster samples from St. Louis had a concentration of 0.5 mg/kg BBP.

Staples et al. (1985) identified that BBP was detectable in 3% of tested samples in the STORET database; this implied that 5 samples had detectable concentrations.

In a poster presented at a SETAC meeting van der Velde et al. (1999) presented results for biota in the range of < 0.01 to 1.7 mg/kg dwt. No information is available regarding the number of samples or species analysed in this study and without more information this data cannot be assessed further. Additional data of BBP in biota are presented in **Table 3.16**.

Table 3.16 Measured BBP concentrations in biota

Organism	Levels [µg/kg dwt]	Reference
Molluscs	< 1 µg/kg n=1 site 5-22 µg/kg n=2 sites	RIVM/RIC/CEFIC
Fish	< 1 µg/kg n=24 sampels 2 µg/kg n=2 samples	RIVM/RIC/CEFIC
Invertebrates	42-63 µg/kg n=3 sites	RIVM/RIC/CEFIC

3.1.5.6 Comparison of measured data and calculated PECs

A risk assessment should utilise the most realistic data when evaluating possible concentration in different compartments. With respect to measured data there are two important evaluations to be made: In the first place, if the quality is acceptable and in the second place to assign the data to its proper level (local, regional, continental).

Surface water

Estimated local PECs for all use categories are presented in **Table 3.2** with respect to 2004 estimated use volumes.

The regional PEC for surface water presented in **Table 3.5** is estimated by EUSES to be 0.17 µg/l. Measured concentrations in surface water, (see **Table 3.13**) show several values above this. This may either indicate that EUSES is underestimating regional levels or that the sampling sites are directly exposed to local sources. The average value compiled by CEFIC is 0.064 µg/l and therefore significantly lower than the regional PEC for surface water, but may not be representative. The regional PEC is supposed to be representative for a region that includes the highest density of industry with release to the environment. The Rhine, which flows through heavily industrialised areas, may be considered to represent such an area. Calculations based on the more recent monitoring values for the lower Rhine (Braun et al., 2000 and Vethaak et al., 2002) gave an average concentration of 0.083 µg/l for 32 samples (includes non detects at sample detection level). This value is quite close to the median of 0.077 µg/l, n=83 for surface water in the Netherlands (Vethaak et al., 2002). Although these values are higher than the CEFIC value, this is below the EUSES calculated regional PEC value. The EUSES estimated regional PEC will be used for the risk characterisation at a regional scale.

Several of the measured values are in the same range as local PECs calculated for different use categories (see **Table 3.2**). However, without knowledge of the actual sites a direct comparison is not possible. Therefore the EUSES calculated values for local PECs are used for the risk characterisation. Some high values have been measured in the Wupper (see **Table 3.13**; **Figure 3.1**), but a recent monitoring study from 2003 carried out by Nord Rhine Westfalian Authorities showed very low BBP levels, which were below the regional PEC.

Sediment

The regional PECsediment (see **Table 3.5**) as estimated by EUSES is 0.068 mg/kg wet weight. For converting data from wwt to dwt a factor 2.6 is used, resulting in a PECsediment 0.18 mg/kg dwt. Comparing this value with the values in **Table 3.14** we find a number of samples from different locations in Europe that exceed this value. The relatively high average value of 178 µg/kg wet weight found in the Rhine in the study by Furthman (1996) may warrant using this value as a basis for the regional PECsediment. Except for certain high values that are assumed to represent local sources, other monitoring data from Germany, the Netherlands, Sweden, Denmark and Norway, show values that are lower than the calculated regional PECsediment. The EUSES calculated value will therefore be used for the risk characterisation at a regional scale.

There are no sediment monitoring data that can be assigned to certain life cycle stages of BBP and the risk assessment will therefore be performed using the EUSES calculated PEC value with respect to local risk assessment. Very high concentrations were found in freshwater sediment of suburban lakes in Sweden, but these data were set aside by new results from the same location showing much lower BBP concentrations, which were also below the regional PEC sediment. In

one sediment sample from a rainwater ditch along a motorway in Germany a high BBP concentration (12 mg/kg dwt) was found. However, the road technosphere is not considered as an assessment endpoint for the purpose of the existing substances regulation.

Soil

The regional PECsoil (see **Table 3.5**) as estimated by EUSES is 3.02 µg/kg wet weight (3.41 µg/kg dwt). Only a few values have been measured above this. The number of measurements of BBP in soil are few and range widely, the older studies include some quite high values (400, 169, 100 µg/kg dwt, see **Table 3.15**) while more recent studies indicate lower values. Values above 3.02 µg/kg wwt may indicate that there might be a local contamination source. The available monitoring values do not give a reason to deviate from the EUSES calculated PEC. Hence, this will be used both with respect to the local and regional estimates for risk assessment purposes.

Air

The regional PECair (see **Table 3.5**) as estimated by EUSES is 0.63 ng/m^3. General monitoring data are in the range of 0.25-8.0 ng/m^3 and include the estimated regional PECair. The number of measurements is too few to specify a regional estimate and the EUSES estimated regional PEC is kept for risk characterisation.

With respect to BBP used in sealants, the monitoring values are much lower than the estimated value, however, these samples may not be considered representative for the actual emission situation (see Section 3.1.5.1).

A specific air monitoring of BBP levels at two flooring plants gave a maximum observed air concentration of about 0.4 µg/m^3. Comparison of this value with the EUSES estimated PEC values in **Table 3.2** show that the estimated PECs air for flooring are about 5-6 times higher. However, no information is available on the size of the sites and on air purification technologies in place at plant 2. Moreover, sampling at plant 1, where air treatment is in place, may not be representative (see Section 3.1.5.1). It is therefore unclear whether these two sites can be considered characteristic for the actual emission situation at flooring sites in general. Hence, the generic scenarios taken from the ESD "Plastics" seem sufficiently conservative in order to estimate worst case air concentrations in the vicinity of both an assumed large and a small site.

Biota

There are only few data available. The total range of these data are between < 1 and 1,700 µg/kg dwt. EUSES has estimated a regional concentration in fish of 120 µg/kg wwt using a BCF of 449, while available fish data are in the range of < 1-2 µg/kg dwt. Some of the sample analysis are old and may be overestimates. The high value of 1,700 µg/kg dwt cannot be assessed further due to lack of information. Other more recent data in **Table 3.16** are all below the EUSES estimated regional PEC. The EUSES estimates for biota will be used in the risk assessment.

3.1.6 Environmental Fate and Distribution

3.1.6.1 Degradation in the Environment

3.1.6.1.1 Abiotic degradation

Hydrolysis

The dark controls of the sunlight photolysis studies of Monsanto Report (1979, 1980) and Gledhill et al. (1980) show no significant hydrolysis in aqueous solutions of BBP after 28 days. These results are in line with the RIVM-conclusion (RIVM, 1991) that the contribution of hydrolysis to the overall environmental degradation of phthalate esters is expected to be insignificant.

Photolysis in water

Three studies of sunlight photodegradation of aqueous solutions of BBP are included in the HEDSET. An early Monsanto Report (1979) reported no photodegradation (0%) after 10 days, but 43% after 28 days. In 1980, two studies (Monsanto Report, 1980; Gledhill et al., 1980), both of good quality, showed < 5% photodegradation after 28 days and $t_{1/2}$ > 100 days. Based on these results, photodegradation is expected to be insignificant.

Photodegradation in air

BBP has an estimated half-life of 1.5 days (GEMS, 1984) in the atmosphere. Photooxidation by OH radicals is reported to be the main contributor to the elimination of BBP from the atmosphere. The first order degradation rate constant was also estimated with the AOPWIN model. A half life of 17 hours was estimated using the TGD default OH radical concentration. As no information exists of which model should be preferred the longer half life of 1.5 days is used in the EUSES calculations.

3.1.6.1.2 Biotic degradation

Several studies have been submitted regarding the biodegradation of BBP. All the studies are summarised in **Table 3.17**, **Table 3.18** and **Table 3.19**. They cover different aspects and these studies are presented according to their relevant endpoints for the risk assessment as follows.

Ready biodegradation tests

Two of the submitted reports are tests on ready biodegradation. Bayer AG (1989) performed a study, which in all essential parts is identical with OECD 301 F (Manometric Respirometry Test). 100 mg/l BBP were inoculated with activated sludge derived from a laboratory scale sewage treatment plant and oxygen uptake was measured over 28 days. A degradation of 88% of BBP (BOD = Biochemical Oxygen Demand) within 28 days was determined. In addition CSCL Japan (1992) has reported BBP to undergo 81% (BOD) degradation after 14 days in a modified MITI I test (OECD 301 C). The tests are presented in **Table 3.17**. These studies indicate that BBP is readily biodegradable meeting the 10-day time window.

Table 3.17 Readily biodegradation tests with BBP

Inoculum	Conc. BBP [mg/l]	Degradation	Test Method	Remark	Reference
Domestic Sewage	100 mg/l	86% after 14 days 88% after 28 days	OECD 301F	Not GLP	Bayer AG (1989)
Domestic Sewage	100 mg/l	81% after 14 days	MITI I	Not GLP	CSCL Japan (1992)

Inherent biodegradation tests

Information regarding inherent biodegradability have been submitted both as tests and as various articles related to the biodegradation of phthalates. They all show that BBP degrades rapidly in adapted systems.

A SCAS (Semi Continuous Activate Sludge) test was performed by Monsanto (Report AC-73-SS 20). The results from this study were later published by Saeger and Tucker (1976) and referenced in O'Grady et al. (1985). Primary degradation was investigated with a high (200 mg/24 hours) addition rate and a low (5 mg/24 hours) addition rate of BBP; the test temperature is not reported. Primary degradation of BBP was 93% and 99%. Parallel tests with monobutylphthalate and phthalic acid, the main metabolites of BBP both gave 99% primary degradation.

Surface water

In a river die-away test (Monsanto 85-9167; 1979) the primary degradation of 50 and 500 µg/l BBP with water from Mississippi was examined in the dark at 24°C for 5 days. Primary degradation was followed by analysing BBP. In unfiltered water BBP had a half-life of 1-2 days, while in filtered water (0.2 µm pore size) BBP had a half-life of 2-3 days. No primary degradation of BBP was observed in autoclaved water.

The primary biodegradation of 1 mg/l BBP was tested in river die-away tests with natural inoculum from the Mississippi and Meramec River (Monsanto MO 85-9173, 1978 Monsanto MO 77-0538 and MO 77-0551 both 1976). The results indicate a primary biodegradation of 50-99% of BBP after 2 days with a short half-life (0.5-2 days). Phthalic acid was run in a parallel system and gave primary degradation half-life of 11-19 days.

Tabak et al. (1981) studied primary degradation of several organic priority pollutants in a modified closed bottle test inoculated with domestic wastewater. No BBP was detectable after a 7-day incubation period.

Sugatt et al. (1984) investigated biodegradation of BBP in a procedure similar to OECD 302B. In addition to measuring CO_2 evolution also disappearance of BBP was followed. Primary degradation after 28 days was 77% and ultimate biodegradation was 43% with non-adapted inoculum and 97% and 88% with BBP adapted inoculum.

Petrasek et al. (1983) investigated the removal of BBP from the water phase in a pilot STP. The influent concentration used was 33.5 µg BBP/l. Initial concentration in sludge was 8.1 µg BBP/kg, but this was reduced to 7 µg BBP/kg after secondary treatment. The effluent concentration of BBP was < 1.3 µg/l, indicating 96% retention.

Table 3.18 Inherent biodegradation tests

Inoculum	Conc. BBP [mg/l]	Degradation	Test Method	Remark	Reference
Activated sludge	5 and 200 mg/24 hours	99% and 93%	OECD 302A SCAS	Only primary biodegradation	Monsanto (report AC-73-SS 20), 1970
Mississippi river	50-500 µg/l	DT½=1-3 days	River die-away	Only primary biodegradation	Monsanto (85-9167) 1979
Mississippi Meramec river	1 mg/l	DT½= 0.5-2 days	River die-away	Only primary biodegradation	Monsanto 85-9173 (1978) and 77-538-551 (1976)
Mississippi Meramec river	1 mg/l Phthalic acid	DT½= 11-19 days	River die-away	Only primary biodegradation	Monsanto 85-9173 and 77-538-551
Domestic sewage	5-10 mg/l	100% after 7 days	Closed bottle	Only primary biodegradation	Tabak et al. (1981)
Domestic adapted sewage	?	78% after 28 days 43% after 28 days DT½=15 days	OECD302B	Primary and ultimate degradation	Sugatt et al. (1984)
Domestic sewage	33.5 µg/l	96% removal	Pilot STP	Only primary biodegradaton	Petrasek et al., (1983)

Microcosm study

A lake water - sediment microcosm study was performed to investigate the environmental fate of BBP (Monsanto ES-82-SS53; 1982). Several tests were carried out with different concentrations in the range of 12-1,000 µg/l incubated for up to 41 days. Variable conditions with respect to oxygen saturation and light were used. Light and oxygen saturation did not prove to have any effect on the primary degradation rate and the mean half-life of BBP was 1.4 days. Total degradation to CO_2 was also measured and half-lives in the range of 8-13 days were found. Extractable intact BBP was < 0.3% at the end of the study. Sterilisation completely inhibited the biodegradation to CO_2.

In a freshwater microcosm study, the biodegradability of BBP in the course of 30 days was analysed (Monsanto MO-88-9228, also reported by Adams et al., 1989). The design was meant to simulate the conditions of Illinois River, including flow rate with magnetic stirrer and sediment. ^{14}C-labelled BBP was added to give a concentration of 10 or 100 µg/l of BBP, three times a week an additional half of initial dose was added. Primary degradation half-life of BBP in this system was 2 days or less. Complete mineralisation was observed to be 10.4% of added BBP after 30 days, less than 2% of the added compound was found in the sediment. The metabolites monobutyl phthalate, monobenzyl phthalate and phthalic acid were detected but not quantified.

Anaerobic biodegradation

Ziogou et al. (1989) investigated the primary degradation rate and kinetics of BBP in anaerobic municipal sewage sludge. The degradation rate was investigated in sludge spiked with BBP (approximately 4 mg/l) and was incubated without shaking at 37°C for 32 days under anaerobic conditions. Primary degradation of BBP was 90% after 8 days with a half-life of 4.5 days. The kinetics were studied in a sludge spiked with the following BBP concentrations: 10.1 and 0.5 mg/l (about 30-600 mg/kg dw). The samples were incubated anaerobically at 37°C over

32 days. The resulting primary degradation of BBP fitted first order kinetics. BBP in samples were analysed with a gas chromatograph equipped with electron capture detector after extraction with dichloromethane.

Horowitz et al. (1982) studied the anaerobic degradation of BBP after inoculation from two municipal sewage sludge's at 35°C. To a synthetic medium 10% inoculum and 50ppm carbon of BBP (= 68 mg/l) was added. Ultimate anaerobic biodegradation was monitored weekly by methane and CO_2 production for up to 8 weeks. Ultimate anaerobic biodegradation of BBP was only observed in one of the municipal sludge's (24% after 4 weeks).

Shelton et al. (1984) studied the anaerobic degradation of ^{14}C-labelled BBP in the same sludge that gave positive degradation in the study of Horowitz et al. (1982). Also here a 10% sludge inoculum in an anaerobic mineral salt medium was used. Nominal test concentration was 20 mg/l plus 3.6 µCi of BB [^{14}C]P. The test bottles were incubated at 35°C for 10 weeks, and samples were withdrawn periodically. Dried samples of sludge were extracted with dichloromethane and hexane prior to analysis with GC-FID. For the detection of intermediates samples were withdrawn, frozen, extracted with methanol and analysed by HPLC with UV detection. Mass spectrometry was used to identify monobutylphthalate. A small build-up (< 10%) of monobutylphthalate was observed as a transient peak after 3 days. Phthalic acid reached a peak of close to 100% of added after 10 days and was undetectable after 24 days. ^{14}C-labelled CO_2 continued to increase in a close to linear fashion right to the end after 10 weeks. Methane production indicated that ultimate degradation of BBP was 100% after 40 days.

Painter and Jones (1990) studied anaerobic biodegradation of BBP using inocula from four distinct anaerobic environments; freshwater lake sediment, salt marsh sediment, municipal digester sludge and anaerobic leachate from a lab-scale landfill digester. 10% of the four different inoculates were added to anoxic sterile medium, 5 ml aliquots of the mixture distributed to several culture tubes and 20 µM or 100 µM BBP were added. All cultures were incubated at 30°C without shaking for 365 days. Duplicate culture tubes were sacrificed at specific times. BBP was extracted with hexane and analysed with GC-FID. Freshwater, salt marsh, municipal sludge and landfill digester inocula gave respective primary degradation half-lives of about 15 days, 10 days, 63 days and no degradation observed.

Ejlertsson et al. (1996) studied the biodegradation of BBP in a model of a municipal solid waste landfill. A two stage reactor was run in continuous reflux of leachate. BBP was added at a concentration of 87 mg/l and the system run for 278 days. Methane production was monitored continuously. Incubation temperature was not given. After 278 days primary degradation of BBP was 77% and ultimate degradation was 11%.

Sediment

The effect of temperature and sediment microbial population on the degradation of BBP was studied in Norway by Rike et al. (1999). Sediments from Svalbard, Aalesund and Oslo were used. At the Aalesund site, 4,000 l DEHP had been spilled from a storage tank in April 1995. The selected sediment inoculums were acclimatised for 7 days with 0.25 g/l of BBP prior to testing. New sediment was added 1% inoculum of the acclimatised sediment. The sediments were incubated with BBP at 8-10°C and 20°C. Both primary aerobic and anaerobic degradation was studied following the reduction of BBP after addition of 0.25 g/l for 14 days and 90 days.

After 14 days, aerobic degradation was much faster at 20°C (96-100%) than at 8-10°C (0-30%). For anaerobic degradation the effect of temperature was less clear. Sediment from Aalesund had a content of BBP of 29 mg/kg upon collection. This sediment had a significant higher anaerobic

as well as aerobic degradation rate both at high and low temperature compared to the other two sediments. There was no significant difference between the sediment from Svalbard and Oslo with respects to degradation. The results from the aerobic experiments indicated that the degradation activity was considerably reduced at low temperature in microbial populations adapted to cold climate environments. A potential for bioremediation is present in all the cold climate sediments examined in the study. The report concluded that since the degradation activity is low, also at summer temperatures measured at the sites, phthalates from spill and diffuse sources will probably persist longer in sub-arctic and arctic regions.

Biodegradation in soil

Two studies of BBP removal/degradation in soil-sludge mixtures and composts are included in the HEDSET. Shell Environmental Group (1982) monitored the degradation of 500 mg/kg BBP in an artificial compost mixture over 7 and 30 days at a soil temperature of 60°C. The primary biodegradation was measured to 75 and 65%. The discrepancy in the results is not explained in the report. Kincannon and Lin (1985) monitored degradation of 117 mg/kg BBP in soil, which was added two different concentrations of wood preserving sludge and found half-lives of 59.2 and 178.2 days for BBP. The longer half-lives of BBP in these soils may be related to the potentially antimicrobial nature of the inoculum material, from which the sludge was derived (wood preservative).

Table 3.19 Other aerobic and anaerobic biodegradation tests with BBP

Inoculum	Conc. BBP	Degradation	Test Method	Remark	Reference
Lake or River	12-1,000 µg/l	Prim.degr. DT½=1.4 days Ultim. degr. DT½=8-13 days	Microcosm study	Tests performed under variable conditions	Monsanto ES-82-SS53).
Illinois river	10-100 µg/l	Prim. degr. DT½ =< 2 days Ultim. degr.= 10% after 30 days	Microcosm study	Simulated river conditions	Monsanto MO-88-9228
Anaerobic domestic sludge	0.5-10 mg/l	Prim. degr. DT½=4.5days 90% after 8 days	Anaerobic	Sludge adapted to BBP	Ziogou et al. (1989)
Anaerobic domestic sludge	68 mg/l	Sludge 1: none Sludge 2: Ultim. degr. 24% after 28 days	Anaerobic	Unadapted sludge	Horowitz et al. (1982)
Anaerobic domestic sludge	20 mg/l	Ult. degr. 100% after 40 days	Anaerobic	Same as sludge 2, Horowitz et al., 1982	Shelton et al. (1984)
4 different anaerobic environments	6,2 -31 mg/l	Prim. degr. DT½=10, 15, 63 and ∞	Anaerobic	Unadapted sludge	Painter and Jones (1990)
Landfill inoculum	87 mg/l	Prim. degr. 77% after 278 days Ultim. degr. 11% after 278 days	Anaerobic	Simulation landfill reactor	Ejlertsson et al. (1996)
Adapted sediment	250 mg/l	Prim. aerobic degr. after 14 days 8-10°C: 0-30% 20°C:96-100%	Anaerobic and aerobic	Large variations in degradation properties	Rike and Borresen, (1999)

Table 3.19 continued overleaf

Table 3.19 continued Other aerobic and anaerobic biodegradation tests with BBP

Inoculum	Conc. BBP	Degradation	Test Method	Remark	Reference
Artificial compost	500 mg/kg	Prim. degr. 75% after 7 days 65% after 30 days	Aerobic		Shell Environ-mental Group (1982)
Soil + wood preservation sludge	117mg/kg	Prim. degr. DT½=59-178 days	Aerobic	Degradation inhibited	Kincannon and Lin (1985)

Degradation pathways

The metabolic pathway of aerobic and anaerobic biodegradation of benzylbuthyl phthalate (BBP) appears to be as follows: BBP → monobutyl/monobenzyl phthalate → phthalic acid → 4,5 dihydroxyphthalic acid→ oxalic acid → formic acid → CO_2 (Adams et al., 1989 and Shelton et al., 1984).

Conclusion

Based on the available data on aerobic biodegradation, BBP must be considered readily biodegradable meeting the 10-day window criterion. Anaerobic degradation studies of BBP show that primary biodegradation of BBP takes place, however, with variable half lives and with a possible build-up of metabolites, mainly monoesters and phthalic acid. The results also suggest that BBP may undergo ultimate biodegradation under anaerobic conditions in sludge and sediment. However, the soil and sediment studies are not thought to be performed in a representative manner.Therefore sediment degradation rates for use in this risk assessment are derived by estimation from the ready biodegradation test as described in the TGD. The rate constants are presented in the **Table 3.20**.

Table 3.20 Degradation rate constants used in EUSES

	[d^{-1}] at 120°C
Total rate constant for degradation in STP	24
Total rate constant for degradation in surface water	0.0462
Total rate constant for degradation in sediment	0.00023
Total rate constant for degradation in soil	0.023

3.1.6.2 Distribution in the environment

Distribution in wastewater treatment plants

The distribution of BBP in a STP is obtained with EUSES, (Simple Treat).

Table 3.21 Distribution of BBP in STP

Compartment	% of BBP
Air	0.04
Sludge	41.9
Biodegraded	49.9
Water (effluent)	8.2

3.1.6.2.1 Adsorption/desorption

BBP is reported to have an adsorptive character to soil and sludge. A soil adsorption coefficient (K_d) of 68-350 and a sludge concentration factor of 244 have been reported by Gledhill et al. (1980) and Petrasek et al. (1983). The latter authors also observed 96% removal of BBP in a STP simulation test and attributed this to sludge adsorption (elimination). Shelton et al. (1984) reported that BBP was found in different digested sludges, indicating that complete degradation may not occur within the retention times of some municipal sludge digesters.

The high log K_{ow} and relatively low water solubility of BBP indicates a relatively low mobility in soil. However, binding of BBP to colloidal matter and humic substances may enhance subsurface transport through cracks and macropores in soils.

Table 3.22 summarises parameters relevant for estimating mobility. Comparing the values with those calculated by EUSES indicate that the EUSES estimated values are intermediate of those measured. Therefore EUSES calculated Koc value of 10,500 l/kg and Kd of 210 have been used in this RA.

Table 3.22 Parameters to assess the environmental mobility of BBP

Test parameters	Test result	Reference
Octanol /water partition coefficient (log K_{ow})	4.84	Mean value of RA
Organic carbon/water part. coefficient (K_{oc}) [l/kg]	9,000 17,000 10,500	Staples et al. (1997) Russel and McDuffie (1986) EUSES (used in this RA)
Soil adsorption coefficient (K_d)	68-350 210	Measured, Gledhill et al. (1980) EUSES (used in this RA)
Sludge concentration factor[1]	244	Petrasek et al. (1983)
Concentration factor [2]	172	Patterson and Kodukala (1981)

1) Concentration measured in the sludge samples [μg/l] divided by the influent concentration
2) Sludge (wet weight) pollutant concentration divided by influent wastewater pollutant concentration [μg/l].

3.1.6.2.2 Volatilisation

BBP has a vapour pressure of 0.00112 Pa (20°C) which indicates a low evaporation rate in its pure state. However, at the temperatures used at the stage of production and formulation of BBP (maximum 160°C), volatility is high. This may offer a mechanism for release of BBP to the atmosphere.

The calculated Henry's Law constant of 0.176 Pa · m^3 · mol^{-1} indicates that BBP is not likely to volatilise from surface water.

Distribution and air transport

BBP is expected to be deposited from the atmosphere by wet and dry deposition. Several studies have evaluated the presence and fate of BBP in the atmosphere (Aceves and Grimault, 1993 and Furtmann, 1993). Low BBP concentrations (0.25 – 8.0 ng/m^3) have been measured on coarse and fine particles of urban air in Barcelona. In addition, BBP has been measured at low concentrations (0.03 – 0.08 ng/l) in rainwater falling in industrial regions of Germany. These observations are in agreement with BBP's expected potential for wet and dry transport. Long distance transport is unlikely due to low volatility at ambient temperatures and a short half-life in the atmosphere.

Distribution to soil

The main contamination routes of BBP to soil are expected to be via atmospheric deposition and via STP sludge used as fertilizer. Information on atmospheric deposition of BBP in urban/industrial areas was discussed in Section 3.1.5.1 and 3.1.5.4. There are several available reports for water concentrations from WWTPs, but very limited information is available for municipal STP sludges. Some values are given in **Table 3.15**. Concentrations of BBP in sewage sludge vary from below the detection limits up to 210 mg/kg. A recent Danish study showed concentrations of BBP in sewage sludge from < 50 µg/kg to 410 µg/kg (Boutrop et al., 1998), while a similar study of Norwegian sewage sludge showed BBP concentrations from 140-1,400 µg/kg (Braaten et al., 1996).

3.1.6.3 Bioaccumulation

BBP is considered to have a high potential for bioaccumulation, based on a log KOW of 4.84 and a molecular weight < 700. Calculating the BCF according to the QSAR relationship suggested in the TGD (logBCF=0.85 · logKow-0.7) gives a value of 2,594.

Four bioconcentration tests are available, three fish studies and one study with mussels. The tests are described below.

Heidolph and Gledhill (internal Monsanto report no. 85-0170, 1979) measured a mean $BCF_{whole\ fish}$ of 188 l/kg, with a BCF_{muscle} of 28 l/kg and $BCF_{viscera}$ of 1,693 l/kg. The BCF values are measured using ^{14}C radiolabelled BBP, and thus measures BBP plus all metabolites. The exposure period was 17 days and the test temperature was 22°C, using the test species bluegill sunfish (*Lepomis macrochirus*). Short term studies (7 days) performed with exposure concentrations of 22 and 222 µg/l, yielded in BCFs of 71 and 70 l/kg. The mean BBP exposure concentration in the definite test was 2.96 µg/l. Some initial problems were encountered with the exposure concentration in the definite study, being too high on day 0, 1 and 2 (around 6-7 µg/l BBP) then dropping to 0.7 µg/l on day 4. Therefore day 4 was used as zero time in the computer modelling of the results. During this initial phase the BCF whole fish reached 143 on day 1 and 163 on day 2. After the initial 4 days exposure period the $BCF_{whole\ fish}$ varied in the range of 111-232. Depuration was rapid with a halftime of less than 1 day.

A follow-up of the study by Heidolph and Gledhill (1979) was performed by Carr et al. (internal Monsanto report no. 92-9760, 1992, published in 1997). This test was run for 3 days at 22°C. The study used a ring ^{14}C-labelled BBP. Results differ when using ^{14}C-labelled compound from

results gained when measuring only the parent compound BBP as not only BBP but also the monoester benzylphtalate will be recorded. A compound specific analysis of parent BBP was also performed both with respect to exposure concentration and concentration within fish. The study gave a $BCF_{whole\ fish}$ value of 255 l/kg measured as BBP equivalents based on total radioactivity (BCF_{muscle} = 25.5(45) l/kg; $BCF_{viscera}$=387(684) l/kg). The study gave a parent compound $BCF_{whole\ fish}$ of 12 (1 and 19 for muscle and viscera). Chemical analysis of the exposure medium showed that parent compound exposure was 34 µg/l while total radioactivity equalled an exposure of 60 µg/l BBP equivalents. Consequently, calculating the $BCF_{whole\ fish}$ based on the parent compound exposure rather than total radioactivity exposure gave a $BCF_{whole\ fish}$ of 449 (see Equation 1 below).

$$BCF = \frac{(BBP + metabolites)_{fish}}{BBP_{water}} \qquad \text{Equation 1}$$

The study by Carr et al. (1992) did not confirm that steady-state was reached as analysis of the fish was performed after 3.27 days only. However, based on the previous study by Heidolph and Gledhill (1979) it was assumed that equilibrium was obtained. The main purpose of the Carr study was to investigate the fate of the parent compound BBP in fish. The BCF values of Heidolph and Gledhill (1979) and Carr et al. (1992) of 188 l/kg and 255 l/kg are in good agreement. However, there are some differences between the two studies that should be commented. The $BCF_{viscera}/BCF_{muscle}$ ratio was 60.4 in the Heidolph and Gledhill study, while it was 15.1 in Carr et al. (1997). It is reasonable to assume that the same species of fish (*Lepomis macrochirus*) of the same age group (less than 1 year) would show more or less the same bioconcentration ratio of muscle to viscera. The reason for the discrepancy between the studies is probably the difference in the definition of "viscera". Heidolph and Gledhill (1979) define viscera as only the internal organs, while Carr et al. (1992) define viscera as all non-edible parts of the fish (head, tail and internal organs). A higher fat content would also result in a higher BCF. The fish used in Carr et al. (1992) had a mean weight of 9.7 g, a factor 10 greater than in Heidolph and Gledhill (1979) (0.92 g).

A study published as an article by Barrows et al. (1980) gave a $BCF_{whole\ fish}$ of 663 l/kg. The test was performed according to US EPA methods, and the same species as described above (*Lepomis macrochirus*) was used. Test temperature was 16°C and exposure period was 21 days. Mean exposure concentration was 9.73 µg/l. The test was performed at a temperature below that recommended for the species by OECD (20-25°C). The paper does not cite the measured tissue residues and no information is available with regard to variation during the equilibrium period or with respect to concentration in fillet versus viscera. The study also included BCF determinations for a number of other organic chemicals. The results have been evaluated to be valid and were refered to in the risk assessment of DEHP, acrylonitril, 1,4-dichlorbenzene, tetra- and trichloroethylene.

The same study reported in the paper by Barrows et al. (1980), was also reported as an article by Veith et al. (1980) submitted for a symposium, but here with a BCF of 772 for BBP (the results for the other organic compounds are the same in both papers). Veith confirmed by personal communication that the paper by Barrows et al should be used for evaluation.

The BCF of BBP in Eastern Oysters (*Crassostrea virginica*) was measured to be 135 l/kg by Springborn Laboratories (1986). This test was run over 11 days at 19.5°C, and included a depuration phase following exposure.

Table 3.23 Bioconcentration data for BBP

Species	Conc. [mg/l]	Exposure conditions	BCF [l/kg]	Test method	Remark	Reference
Lepomis macrochirus	0.00296	17 days 22°C	BCF_{wh}=188 Parent compound and metabolites		GLP=no data BCF_{muskle}= 28 $BCF_{viscera}$= 1,693 Uptake =143 d^{-1} Depuration $t_{1/2}$= 0.75 days	Heidolph and Gledhill (1979)
Lepomis macrochirus	0.034 parent compound	3 days 22°C	BCF_{wh}= 449 ** Parent compound and metabolites (12)*	EPA 560/6-82-002	GLP=yes Total (^{14}C)metabolite BCF_{muskle}=45 (1)* $BCF_{viscera}$=684 (19)*	Carr (1992)
Lepomis macrochirus	0.00973	21 days 16°C	BCF_{wh}=663 Parent compound and metabolites	Mount and Brungs (1967)	GLP=no data Depuration $t_{1/2}$= 1-2 days	Barrows et al. (1980)
Crassostrea virginica	0.012	11 days 19.5°C	BCF_{wh}=135 Parent compound and metabolites	Mount and Brungs (1967)	GLP=yes Depuration $t_{1/2}$= 1-2 days	Springborn Laboratories (1986)

BCF Bioconcentration factor
BCF_{wh} Bioconcentration factor for whole fish
* Results in parentheses are BCF for parent compound (BBP)
** See previous page for calculation (equation 1)

Discussion of the bioconcentration studies

The QSAR estimated BCF for BBP is 2,594 l/kg based on a logKow of 4.84. The experimental studies show that measured BCFs are much lower. Measured values for fish based on total radioactivity are in the range 188 to 663 l/kg and based on intact BBP tissue residues. In one of the studies) a BCF value of 12 l/kg has been measured. There are several differences in the test conditions between the different studies. When comparing BCF values it is important to look at variables like length of exposure, temperature and percent lipid of the species. The impact of differences in exposure concentration must also be considered.

For a substance like BBP it is important to make certain that the measurement was at or near steady-state. The Carr et al. (1992, 1997) study did not confirm that steady-state was reached, however, based on the previous study (Heidolph and Gledhill, internal Monsanto report, 1979) it was assumed that equilibrium had been reached within 3 days.

The study published by Barrows et al. (1980) was conducted at 16^{o}C. The temperature recommended for the bluegill sunfish according to the OECD guideline is 20-25^{o}C. The BCF-value is expected to increase with decreasing temperature due to lowered metabolisation in organisms, although one test on another phthalate (DEHP) has shown an increase of BCF with temperature (Barron et al., 1987).

The study of Carr et al. (1992) showed that less than 3% of the accumulated material remains in the form of BBP, the rest is transformed to other metabolites. The metabolisation rate may however differ significantly between different species/taxa although the BCF for oyster of 135 l/kg is comparable to the fish data.

Specific analysis during BCF-tests shows that the same metabolites occur in water and biological tissue after 1-3 days (Carr, 1992). Evaluation of these results indicates that BBP has a high uptake rate in biological organisms. The metabolites are reported to be readily excreted by the

organisms during the depuration phase of the BCF tests (Carr, 1992; Barrows et al., 1980; Springborn Laboratories, 1986). High water solubility of monoesters would indicate that it is mainly parent BBP that is taken up in fish and metabolites that are excreted. It should also be noted that the logKow of the mono-ester mono-butyl phthalate (MBuP) is around 2.8 (EU RAR for DBP).

Mammalian distribution tests have shown that BBP is rapidly hydrolysed to the monoesters after ingestion and that the hydrolysation starts in the intestine before absorption into the organism (see Section 4.1.1). The half-life of the monoesters in mammalian studies has been measured to be 5.9 hours after administration of a single dose (Eigenberg et al., 1986), indicating that the monoesters do not accumulate significantly in mammalian tissues. Several tests have shown that monoester phthalate glucuronidates are excreted through the bile duct, deglucuronised and the monoesters are then reabsorbed through the intestine. Complete biotransformation to phthalic acid has not been observed in any of the tests, which indicates that the main biotransformation step is hydrolysation to the monoester metabolites. Reproductive toxicity tests performed on mammals show that BBP and the corresponding mono-esters monobutyl phthalate (MBuP) and monobenzyl phthalate (MBeP) have similar effects and threshold values with regard to developmental toxicity, suggesting that the mono-esters may be responsible for the reproductive toxicity. No information is available concerning chronic toxicity of the mono-esters to aquatic organisms. However, the data from mammalian studies indicate that the mono-esters are responsible for or at least may play an important part in the reproductive toxicity of phthalates.

Conclusion bioaccumulation

The measured bioconcentration factors (BCF) based on total radioactivity are in the range 135-663 l/kg (see **Table 3.23**).

The BCF-value of 12 l/kg, taking only into account the accumulation of the parent compound, would mean that BBP is not considered to biomagnify. Based on the data from Section 4 it cannot be excluded that the metabolites can give endocrine/reproductive toxicity effects to other species like birds, fish etc, as they do to mammals. However, no information on such effects in these species is currently available. Therefore the BCF-value used should cover the BCF of the parent compound (BBP) and the accumulation of the two monoester metabolites (MBuP and MBeP).

Since none of the BCF tests performed include analysis of the monoesters, the values given in total ^{14}C-labelled BCF-values will probably be an overestimation of the true BCF for BBP and the accumulation of the toxic mono-esters. The GLP study performed by Carr (1992) shows that BBP is rapidly metabolised and excreted after exposure of fish at 22°C. However chronic exposure would lead to chronic levels of monoesters that may have harmful effects. This risk assessment should also cover this risk. Based on the evaluation of the BCF-tests regarding ^{14}C-method, the experimental BCF-value of 449 l/kg for fish will be used for estimating secondary poisoning in EUSES.

3.1.7 Secondary poisoning

BBP has a Log Kow of 4.84 and measured BCF values in the range 135 – 663 l/kg. An assessment of exposure through the food chain therefore becomes relevant.

The predicted environmental concentration in food ($PEC_{oral,\ predator}$) can be calculated according to the formula:

$$(PEC_{oral,\ predator}) = PEC_{water} \cdot BCF_{fish} \cdot BMF$$

According to TGD, two secondary poisoning scenarios may be considered. These are the aquatic foodchain "water - fish – fish eating birds or mammals" and the terrestrial foodchain "soil – earthworm – worm-eating birds or mammals". For fish eating birds and mammals, the PEC (oral, fish) can be calculated from PEC_{water} and a fish bioconcentration factor. A BCF value of 449 for fish has been chosen for the estimation of secondary poisoning (see Section 3.1.6.3). The BMF (biomagnification factor) used is 1 (default).

For the terrestrial food chain a bioconcentration factor for earthworm is needed but no measured BCF value for earthworm was available for BBP. The BCFearthworm can be calculated by the partitioning method as given in the TGD and calculation by EUSES gives a BCF for worms of 14.9 l/kg. The TGD allows the assessment of secondary poisoning via the terrestrial food chain by estimating a $PEC_{oral\ worm}$. With few actual measurements in biota an assessment will be made based on concentrations as estimated by EUSES (see **Table 3.24**).

Table 3.24 Local oral PECs for fish and worm with respect to secondary poisoning using 2004 tonnage

	Local PECoral [mg/kg]	
Life cycle	fish	worm
I Site A I Site B I Site C	0.19 0.09 0.13	Not relevant as sludge is incinerated
IIIa-1 PVC flooring, Large site	5.05	15.4
IIIa-2 PVC flooring, Small site	7.84	24
IIIb-1 Formulation PVC sealants	0.12	0.15
IIIb-2 Processing PVC sealants	0.08	0.009
IIIc PVC textiles	1.20	3.47
IIId Polymer films	0.75	2.08
IIIe-1 Formulation. general PVC	0.14	0.22
IIIe-2 Processing general PVC	0.18	1.44
IIIf-1 Formulation paints and inks	0.61	1.67
IIIf-2 Processing paints and inks	0.08	0.12
IIIg-1 Formulation Adhesives	0.35	0.84
IIIg-2 Processing Adhesives	0.15	0.58
IIIh-1 Formulation Confidential	1.84	5.47

3.2 EFFECTS ASSESSMENT

There are several tests performed for each group with respect to BBP. In the paragraphs below a fairly detailed description has been made of the more recent studies, while for the others the results are only summarised in tables. Taking into account that BBP seems to have general toxic effects with small intraspecies differences, derivations of aquatic PNEC will be performed using test data both with freshwater and marine species.

3.2.1 Aquatic compartment

3.2.1.1 Toxicity to fish

3.2.1.1.1 Acute studies

Several studies have been performed on fish. The high quality studies are described in some detail where information is available, while older data are only included in summary **Table 3.25**.

Freshwater tests

A test with rainbow trout (*Oncorhynchus mykiss)* performed in 1983 gave an LC_{50} of 0.82 mg/l after 96 hours under flow through conditions (EG&G Bionomics, BW-83-3-1373). This is a GLP study where BBP is one of 14 phthalates tested. There were 20 fish at each concentration. Test concentrations were measured at initiation and after 96 hours. Nominal test concentrations were 0.22, 0.45, 0.9, 1.8 and 3.6 mg/l. Test temperature was 12°C. The LC_{50} value was calculated based on the mean measured concentration at the start and after 96 hours, as recommended in the OECD guideline. The critical nominal test concentration of 0.9 mg/l showed the largest drop, with a 67% loss compared to the initial concentration. This strongly influences the estimated LC_{50}. The mean measured concentrations were 0.17, 0.28, 0.48, 1.4 and 3.1 mg/l. All fish died at 1.4 mg/l and one fish died at 0.48 mg/l within 24 hours. Later (72 hours) another fish died at 0.48 mg/l. The report estimated the LC_{50} to be 0.82 mg/l and the NOEC to be 0.28 mg/l. The reported LC_{50} value of 0.82 mg/l does not incorporate the mortality occurring at 0.48 mg/l. Including the mortality at 0.48 mg/l gives a LC_{50} of 0.76 mg/l which is not significantly different.

An acute static toxicity study with BBP with bluegill (*Lepomis macrochirus)* was performed by EG & G Bionomics in 1979 according to US EPA (1975) guidelines and according to GLP (Monsanto 82-0015). Test concentrations were in the range of 0.36-7.8 mg/l. Stock solutions were prepared in acetone. All of the solutions were either cloudy, had a surface film or both. The 96-hour LC_{50} nominal was 1.7 mg/l. The results should be used with caution.

An acute toxicity study with BBP (among others) with bluegill (*Lepomis macrochirus)* was performed according to US EPA (1975) guidelines (Buccafusco et al., 1981). Test concentrations are unknown and not verified. The 96-hour LC_{50} was 43 mg/l, which is above the water solubility of BBP. The results should not be used further in the report.

An acute static toxicity study with BBP towards rainbow trout (*Salmo gairdneri)* was performed by EG & G Bionomics in 1979 according to US EPA (1975) guidelines and according to GLP (Monsanto 82-0014). Test concentrations were in the range of 0.36-4.6 mg/l. Stock solutions were prepared in acetone. All of the solutions except the lowest were either cloudy, had a surface film or both. 96-hour LC_{50} was 3.3 mg/l, which is above the solubility of BBP in the water phase. Sub acute effects (fish at surface, dark coloration) were observed at all concentrations. The NOEC is therefore < 0.36 mg/l. However should the results not be used further in the report

An acute static toxicity study with BBP towards fathead minnow (*Pimephales promelas)* was performed by EG & G Bionomics in 1979 according to US EPA (1975) guidelines and according to GLP (Monsanto 82-0013). Test concentrations were in the range of 0.6-7.8 mg/l. Stock solutions were made in acetone. All of the solutions including controls were either cloudy, had a surface film or both. 96-hour LC_{50} was 2.1 mg/l. Sub acute effects (fish at surface, dark

colouration, lethargy) were not seen at the two lowest concentrations and the NOEC is therefore 1.0 mg/l. The results should be used with caution.

An acute static toxicity study with BBP towards fathead minnows (*Pimephales promelas)* was performed by EG & G Bionomics in 1979 according to US EPA (1975) guidelines and according to GLP (Monsanto 82-0016). The test was performed with hard water ($CaCO_3$=20-30 mg/l, pH = 6.7-7.4). The test concentrations were in the range of 0.78-13 mg/l. Stock solutions were prepared in acetone. All of the solutions were either cloudy, had a surface film or both. 96-hour LC_{50} was 10 mg/l, exceeding BBP's water solubility. Sub acute effects (fish at surface, dark coloration, lethargy) were not seen at the three lowest concentrations. The NOEC is therefore 2.2 mg/l. These results should not be used further in the report.

In an article by Adams et al. (1995) fathead minnows (*Pimephales promelas)*, rainbow trout (*Salmo mykiss)* and sheepshead minnow *(Cyprinodon variagatus)* were tested under either static or flow through test conditions according to US EPA guidelines and GLP. The diluent water used for the test had the following water qualities ($CaCO_3$=25-50 mg/l, pH = 7.6-7.9, temperature 22^{o}C). Oxygen, temperature and pH were measured at all observation periods. Concentrations were verified analytically by gas chromatography. In flow through studies concentrations remained constant and the LC_{50} values were calculated using mean measured test concentrations. In the static studies final test concentrations showed up to 50% reduction of the initial concentrations probably due to adsorption. The 96-hour LC_{50} for *Pimephales promelas* in a static test was > 0.78 mg/l; in a flow through test with *Pimephales promelas* 96-hour LC_{50} was 1.50 mg/l. The 96-hour LC_{50} in a flow through test for *Salmo mykiss* was reported to be 0.82 mg/l and a flow through test for *Cyprinodon variagatus* showed a 96-hour LC_{50} of 0.68 mg /l. The results are considered valid for risk assessment purposes.

Marine and estuarine tests

Ozretich et al. (1983) performed two 96-hour and one 8-day flow trough toxicity study with shiner perch *(Cymatogaster aggregate)* according to ASTM (American Society for Testing and Materials) guidelines. The tests were performed at 12°C, a salinity of between 30-34% and nominal test concentrations were 0.32, 0.42, 0.56, 0.75 and 1.0 mg/l. Test concentrations were confirmed by chemical analysis and the test was repeated. The measured concentrations were between 71-83% of the nominal concentrations (mean 76%). Stock solutions were prepared in ethanol resulting in 340 mg/l of ethanol in the test water. This is higher than the recommended maximum concentration of 100 mg/l. There were 20 fish per test concentration. The 96-hour LC_{50} values were 0.51 mg/l (95% conf.int. 0.46-0.56) of BBP in both acute tests. The 8-day LC_{50} was 0.49 mg/l. Sub lethal effects were also observed, schooling behaviour was affected at concentrations down to 0.27 mg/l after 3 hours and down to 0.08 mg/l after 96 hours. Higher concentrations gave coughing and reduced activity in addition. Two brain catecholamines were also monitored, however, no dose dependent change was observed. Darkening of skin was seen in all fish at 0.3 mg/l and above.

Randall et al. (1983) conducted both static and flow through BBP toxicity studies with English sole (*Paraphrys vetulus)*. The tests were performed at a salinity of 25-32% according to ASTM guidelines. Nominal test concentrations were 0.130, 0.216, 0.6, 1.0 and 1.5 mg/l in the static test and 0.130, 0.216, 0.36, 0.6 and 1.0 mg/l in the flow through test. Stock test solutions were made up in ethanol. Ethanol concentrations did not exceed 0.340 mg/l in test chambers. Test concentrations were confirmed by chemical analysis. However, the results of these analyses are not reported, and LC_{50} values reported were based on nominal concentrations. The article does indicate that measured concentrations were reduced to 48% of nominal concentrations after 24 hours. The 96-hour LC_{50} in the static test was 0.66 mg/l (95% conf. interv. 0.53-0.84) and in

the flow through test 0.55 mg/l (95% conf. interv. 0.48-0.64). The NOEC for mortality was 0.3 mg/l in both tests, however sub lethal effects (reduced swimming, loss of equilibrium) were observed at all test concentrations. Actual mortality was not reported. As the endpoints are based on nominal concentrations the results of this study should be used with caution.

An acute static toxicity test with BBP on sheepshead minnow *(Cyprinodon variegatus)* was performed according to US EPA (1975) guidelines and according to GLP (EG & G Bionomics 79-4-39, 1979). Salinity was 8%, test temperature 21°C and nominal test concentrations were in the range of 1-7.5 mg/l. Mortality was observed down to 1.6 mg/l but not at 1.0 mg/l. The 96-hour LC_{50} was reported to be 3.0 mg/l and the NOEC was 1.0 mg/l. Concentrations were not verified analytically and the results should not be used further in the report, because the LC_{50} is exceeding the water solubility of BBP.

Table 3.25 Summary table of acute toxicity studies of BBP to fish. Tests evaluated to be not valid lack documentation on test conditions

Species	Duration [h]	Test method	Static/Flow	Temp [°C]	Hardness [mg/l]	LC_{50} [mg/l]	Quality	Reference
Freshwater tests								
Pimephales promelas	96	US EPA	static	22	24-50	> 0.78 m	valid	Adams et al. (1995)
Pimephales promelas	96	US EPA	flow	22	24-50	1.5 m	valid	Adams et al. (1995)
Cyprinodon varigatus	96	US EPA	static	22	24-50	> 0.68 m	valid	Adams et al. (1995)
Oncorhynchus mykiss	96	US EPA	flow	22	24-50	0.82 m	valid	E&G Bionomics (1983)
Pimephales promelas	96	US EPA	static	22	28-44	2.1 n	valid	Monsanto 82-0013
Salmo gairdneri	96	US EPA	static	12	28-44	3.3 n	not valid	Monsanto 82-0014
Lepomis macrochirus	96	US EPA	static	22	28-44	1.7 n	valid	Monsanto 82-0015
Pimephales promelas	96	US EPA	static	22	28-44	5.3 n	not valid	Monsanto 82-0016
Oncorhynchus mykiss	96	other	static	12		1-10 n	not valid	Monsanto 74-0053
Lepomis macrochirus	96	other	static	18		1-10 n	not valid	Monsanto 74-0053
Marine tests								
Cyprinodon variegatus	96	US EPA	static	21	8	3.0 n	not valid	Monsanto 82-0017,
Paraphrys vetulus.	96	ASTM	static	12	25	0.66 m	valid	Randall et al. (1983)
Paraphrys vetulus.	96	ASTM	flow	12	32	0.55 m	valid	Randall et al. (1983)
Cymatogaster aggregata	96	ASTM	flow	12	31-32	0.51 m	valid	Ozretich et al. (1983)
C. aggregata	96	ASTM	flow	12	31-32	0.51 m	valid	Ozretich et al. (1983)
C. aggregata	8d	ASTM	flow	12	31-32	0.49 m	valid	Ozretich et al. (1983)

m Measured concentration
n Nominal concentration
Static Static conditions
Flow Flow through conditions
1 Indicates insufficient mortality observed at the highest test concentration (value shown) to calculate acute toxicity value

3.2.1.1.2 Subacute study

Pfuderer and Francis (1975) investigated the effect of BBP on heartbeat of goldfish and carp (species not specified). Significant heartbeat depression was observed after exposure to 200 mg/l BBP but not to 100 mg/l BBP. Solutions were established by sonification.

Chronic toxicity (14-day exposure) of fathead minnow (*Pimephales promelas)* to BBP was studied in a flow through system performed according to US EPA (1975) (Monsanto -78-0344, 1978). The nominal test concentrations were 0.96, 1.37, 1.96, 2.8 and 4.0 mg/l. The diluent water used for the test had the following water qualities (temperature of 21.4^{o}C, water hardness of 297 mg/l, pH 8.08-8.25). Mean measured concentrations were 0.74, 1.06, 2.1, 2.77 and 3.2 mg/l. Stock solutions were prepared in dimethylformamide; the maximum solvent concentration was 0.33 ml/l. No sub acute effects were noted at the lowest tested concentration. 14-day LC_{50} was calculated to be 2.25 mg/l. The NOEC was 0.74 mg/l with respect to mortality and observed sub lethal effects. The study is also published in Gledhill et al. (1980).

3.2.1.1.3 Chronic tests

There are two chronic tests performed on fish, these are presented below and summarised in **Table 3.26**.

A 30-day early life stage test with fathead minnow (*Pimephales promelas)* is included in the article of Gledhill et al. (1980) (EG&G Bionomics, 1981). Five concentrations were tested; 0.02, 0.03, 0.07, 0.14 and 0.36 mg/l (mean measured concentrations) in a flow through test system. A total of 120 embryos were tested at each concentration. The test temperature was 25^{o}C. There were no significant effects on hatching, length of fish or survival at any of the tested concentrations. A 14% reduced weight at the highest concentration resulted in a MATC value of 0.14-0.36 mg/l and a NOEC of 0.14 mg/l. The study is not documented to be a GLP study and the methodology and results of the BBP chemical analysis have not been submitted. Although some information is lacking, there is nothing in the available documentation that discredits the results.

An early life stage test in a flow through system with rainbow trout (*Oncorhynchus mykiss)* covering a 124-day period (109-day post hatch) was performed according to US EPA-TSCA guidelines and according to GLP (Rhodes et al., 1995). The water hardness was 275 mg/l, temperature was 12^{o}C. Mean measured test concentrations were 0.012, 0.021, 0.044, 0.095 and 0.2 mg/l. No effects were reported at any tested concentration. The NOEC is therefore ≥ 0.2 mg/l.

Table 3.26 Summary of chronic and sub acute fish tests. All test were performed as flow through tests with measurements of exposure concentrations

Species	NOEC [mg/l]	Duration [d]	Temp. [^{o}C]	Quality	Endpoint	Reference
Freshwater						
Pimephales promelas	0.74	14	21.4	Valid	Mortality	Monsanto -78-0344
Pimephales promelas	0.14	30	25	Valid	Weight gain	EG&G Bionomics (1981)
Oncorhynchus mykiss	≥ 0.2	124	12	Valid	Early life	Rhodes et al. (1995)

3.2.1.1.4 Conclusion for fish toxicity

The lowest acute LC_{50} is 0.51 mg/l for *Cymatogaster aggregata* (Ozretich et al., 1983). This value is relevant for classification purposes. With respect to chronic tests the NOEC value of 0.14 mg/l from the study with *Pimephales promelas* is the lowest value (EG&G Bionomics, 1981). One should also note that as a sub lethal effect in the acute test of *C. aggregata* schooling behaviour was affected at concentrations down to 0.27 mg/l after 3 hours and down to 0.08 mg/l after 96 hours.

3.2.1.2 Endocrine disruption

As a group, phthalates have been suspected to cause endocrine disruption in wildlife. One of the phthalates for which endocrine disrupting effects have been shown in experimental systems is BBP. There have been suggestions of estrogenic and anti-androgenic effects caused by this phthalate in fish. Following recent development of draft testing protocols for endocrine disruption in fish, more data has become available. The following section summarises the currently available information on possible endocrine disruption effects in aquatic organisms and especially in fish.

Estrogenicity

In an early study BBP was found to be weakly estrogenic (Jobling et al., 1995). BBP at a concentration of approximately 0.01 – 10 mg/l reduced *in vitro* binding of natural estrogen, 17β–estradiol, to the rainbow trout estrogen receptor by about 40-60%.

Christiansen et al. (2000) injected rainbow trout intraperitoneally with different compounds. BBP was found to cause significant induction of vitellogenin *in vivo*, but was a weaker inducer than e.g. bisphenol A. In further injection studies with juvenile rainbow trout, no induction of the estrogen receptor was observed at an exposure concentration of 50 mg/kg BBP, but both 5 and 50 mg/kg significantly lowered plasma levels of eggshell proteins (Knudsen et al., 1998), indicating an inhibition of synthesis of these proteins.

Similarly, Knudsen and Pottinger (1998) found that BBP would bind to the estrogen receptor, but only at high concentrations. There was no apparent binding of BBP to corticosteroid or testosterone receptors in that study. Work performed by Gimeno (at this time at TNO, The Netherlands) on the possible estrogenic effects of BBP on sexual differentiation in all-male carp did not indicate any effects of BBP (Gimeno, pers.comm.). Results for estrogenicity indicate that BBP may be estrogenic at high concentrations.

Harries et al. (2000) exposed fish to BBP (100 µg/l) and nonylphenol (1, 10 and 100 µg/l). Breeding pairs were exposed for 3 weeks during which reproductive performance was monitored. Endpoints were number of spawnings, number of eggs and size of eggs. At the end of the exposure period plasma vitellogenin and gonadosomatic index was determined. In addition, secondary sexual characteristics in male were quantified (growth of tubercles and thickness of dorsal fat pad). While nonylphenol caused dose-dependent estrogenic effects down to 10 µg/l, no effects were seen following BBP exposure.

BBP was included among other 11 substances in a screening study by Japanese authorities using a fish partial life-cycle protocol with Japanese medaka for a test period of 60 days. This test involves exposure from hatching to maturation. A summary of the protocol for the study and the results is available at the website of the Japanese Ministry of the Environment

(http://www.env.go.jp/en/topic/edcs/approach/2002.html#note1). The summary table from the website is presented as **Table 3.27** below.

As it could be seen from **Table 3.27** there seem to be some surprising results. For example, in the time to hatching results, the time 12.1 +/- 0.7 at 28.6 µg/l is marked as significantly different to the control, but 14.2 +/- 1.1 is not marked as significantly different. It is also surprising that the effect on vitellogenen observed on day 14 completely disappeared on day 21. There might be some asterisks missing indicating significant results in this table. This might be the case with the endpoint "Hepatosomatic Index".

As details on the Japanese study are missing and the results presented are not univocal no firm conclusions can be drawn.

Anti-androgenicity

Suggestions that BBP may be anti-androgenic have been derived from both mammalian studies (see Section 4.1.2.9 of this RAR) and the *in vitro* study by Sohoni and Sumpter (1998). Where specifically addressed, BBP has been found to be an as potent anti-androgen as the model substance used for that purpose, flutamide. Unfortunately there is no established model to ascertain anti-androgenicity in fish.

The Harries et al. (2000) study was not specifically designed to detect anti-androgenicity (e.g. through concomitant exposure to anti-androgens). Therefore, the lack of response following BBP exposure in this study does not disprove earlier suggestions that this phthalate may be anti-androgenic to wildlife. Recently, Ankley et al. (2004) provided *in vitro* support for using fathead minnow to identify anti-androgenic effects, but it is still necessary to establish such effects *in vivo*.

Table 3.27 Summary of the Japanese study

Butylbenzyl phthalate

1.Vitellogenin Assay

Table 1Results

Treatment	Vitellogenin (ng/mg liver)		Hepatpspmatic Index (%)	
	14 days	21 days	14 days	21 days
Control	0.6±0.1	1.5±0.2	2.08±0.56	1.87±0.16
14.0 (μg/L)	0.6±0.1	1.2±0.2	2.35±0.13	1.67±0.18
26.7 (μg/L)	0.7± 0.1	1.3±0.1	1.93±0.08	2.00±0.11
69.7 (μg/L)	1.1± 0.2	1.5± 0.1	1.93±0.11	1.72±0.12
337.1 (μg/L)	0.8±0.2	1.3± 0.1	2.37±0.16	2.12±0.26
1,045.4 (μg/L)	2.6±0.5**	1.5± 0.1	2.46±0.23	2.24±0.22

Statistically significant differences from control group(**indicates $p<0.01$, *indicates $p<0.05$)

2.Partial Life Cycle Test

Table 2-A Results

Treatment	Hatchability (%)	Time to hatching (Day)	Mortality (%)	Total length (mm)	Body weight (mg)
Control	98	12.7 ± 1.0	16.3	20.1 ± 0.2	129.9 ± 3.6
0.7 (μg/L)	94	11.1 ± 0.7	17.0	20.3 ± 0.2	137.8 ± 3.9
2.7 (μg/L)	89	14.9 ± 1.1**	25.8	21.4 ± 0.2**	162.9 ± 4.3**
11.5 (μg/L)	99	15.4 ± 1.1**	31.3	21.4 ± 0.2**	154.7 ± 3.8**
28.6 (μg/L)	96	12.1 ± 0.7**	11.5	20.1 ± 0.2	131.9 ± 3.1
99.5 (μg/L)	86	14.2 ± 1.1	30.2	22.0 ± 0.2**	179.4 ± 4.6**

Table 2-B Results (Continued)

Treatment	Gonadosomatic Index (%)		No. of fishes	No. of males with testis-ova/No. of males	Hepatosomatic Index (%)		Vitellogenin (ng/mg liver)	
	male	female			male	female	male	female
Control	0.83±0.07	7.40±0.26	20	0/10	2.14±0.15	2.52±0.19	1.12 ± 0.10	375.1± 200.6
0.7 (μg/L)	0.96±0.11	7.60±0.21	20	0/10	2.07±0.22	2.55±0.18	1.47 ± 0.36	457.7± 164.6
2.7 (μg/L)	1.09±0.08	7.63±0.19	20	0/10	2.68±0.29	2.99±0.24	1.43 ± 0.24	142.3± 96.7
11.5 (μg/L)	1.12±0.08	7.43±0.28	20	0/10	2.45±0.31	3.62±0.38	1.58 ± 0.23	90.9± 28.4
28.6 (μg/L)	1.16±0.09	7.52±0.23	20	0/10	2.81±0.37**	3.21±0.26	1.86 ± 0.40	330.4± 136.6
99.5 (μg/L)	1.17±0.07	7.55±0.31	20	0/10	3.30±0.57	4.25±0.46	1.47 ± 0.35	129.3± 69.9

Statistically significant differences from control group(**indicates $p<0.01$, *indicates $p<0.05$)

There is work ongoing within the OECD to establish appropriate test systems for endocrine disrupting effects. The test systems are expected to include a study in fish that will include endpoints relevant to estrogenic, androgenic, anti-estrogenic and anti-androgenic effects.

The tests performed with BBP referred to above have not incorporated the aspect of transfer from parent to offspring included in the current test requirements for BBP.

An agreement was reached with Industry to perform a partial life cycle study that was a combination of the two OECD recommended "tier 2 tests" for assessing possible endocrine disruption effects of chemicals in fish. The test is a combination of the so-called pair-breeding test in which egg production and hatchability of eggs from a breeding pair of *Fathead minnow* is assessed and an enhanced early life stage test in which exposure of the eggs from the pair breeding test is continued for sufficient time to enable sexual differentiation of the offspring. Exposure concentrations should be 25 and 100 µg/l.

After some pre-dosing trials a definite study with the pair breeding phase was attempted in March 2002. This study was aborted on day 47 because measured BBP concentrations in the exposure vessels were always below the nominal concentrations and appeared to decline throughout the study. In the nominal 25 µg/l exposure tanks mean concentration on day 32 was 20 µg/l and by day 47 the mean concentration had declined to 14 µg/l (56% of nominal). In the nominal 100 µg/l exposure tanks mean concentration on day 32 was 72 µg/l and by day 47 the mean concentration had declined to 59 µg/l (59% of nominal). Industry has performed a number of additional trials in order to investigate the optimal conditions for performing a chronic fish test. The trials included variable use of solvent, variable delivery systems and variable flow rates. These trials have indicated that it will not be possible to obtain a stable test concentration > 80% of the nominal BBP concentration as required according to the test guidelines. Apparently a plateau level could not be obtained either. Very high flow rates were also tested (residence time 1-1.15 hour) without attaining the required concentration level, although it seemed that a fairly stable level was achieved at about 50% of nominal.

The industry report concluded that the reason for these problems was the biodegradation of BBP to its primary biodegradation products (monobutyl-phthalate and monobenzyl-phtalate) and that these metabolites were also rapidly degraded within this test system. It should however be pointed out that other factors cannot be excluded like adsorption to the test system and that some solubility problems as reported may also be part of the problem.

The technical difficulties described above have been reported to the Technical Meeting. However, the TM (cf. minutes of TM II `03) "generally supported the request to industry to perform the endocrine effect test. The TM realised that the results would not meet the ideal test requirements, but would accept that in this particular case.

3.2.1.3 Toxicity to invertebrates

There are several acute tests available performed on freshwater and marine invertebrates. All acute studies are presented in **Table 3.28**. The most recent data seem to indicate a 48-hour EC_{50} of 1.0-2.0 mg/l (*Daphnia magna*). The data also indicate that there are small variations in sensitivity between species of invertebrates. There are also some tests available for the 3 main metabolites (phthalic acid, monobutyl phthalate and monobenzyl phthalate) of BBP. They are presented below in this section.

3.2.1.3.1 Acute tests

Freshwater tests

Barera and Adams (1981) investigated the acute toxicity of BBP towards water flea (*Daphnia magna)* using different solvents and different feeding regimes during the test. The test method followed ASTM guidelines as far as practically possible. Solvents tested were ethanol, dimethylformamide, acetone and triethylene glycol. There is no significant difference in the test results with regard to the used solvents. Feeding during test gave reduced toxicity at all levels tested (range 0.5 mg/l to 3.0 mg/l) both when alga or a food mixture was added. The effect is assumed related to the high Koc of BBP leading to adsorption of BBP onto feeding stuff. Most of the results are of acceptable quality but some EC_{50} values are exceeding the water solubility of BBP and are therefore assumed not to be valid.

An acute test on *Daphnia magna* was performed where the daphnia were exposed to freshly prepared solutions and to 7-day old solutions (Monsanto 85-9171, 1978). Triethylenglycol was used as solvent. The test method followed that of US EPA (1975). A small but not significant increase in EC_{50} from 1.7 mg/l in fresh solutions to 4.8 mg/l in 7-day old solutions was observed. The difference appeared to be caused by adsorption of BBP rather than biodegradation. There is a serious flaw in the reporting of this study whereby the results of the test after 7 days have been copied into the result table of the zero time tests. Also temperature, pH, oxygen saturation and water hardness were not reported. The results should therefore not be used further.An acute test with BBP on *Daphnia magna* and midge (*Chironomus tentans)* was performed according to ASTM guidelines (Monsanto 85-9180, 1984). Fairly hard water was used (alkalinity of 82-200 mg/l). EC_{50} values of 1.8 and 1.6 mg/l were found for the respective two organisms. No raw data are reported and it is assumed that the data may have been taken from other reports.

An acute test with BBP on *Chironomus tentans* was performed according to US EPA (1975) guidelines (Monsanto 85-9165, 1982). Fairly hard water was used (alkalinity of 288-302 mg/l). Test concentrations were not confirmed. A 48-hour EC_{50} of 1.6 mg/l was found.

An acute test with BBP on midges *Chironomus tentans* and *Paratanytarsus dissimilis* is reported in SRI LSC-1741. The method used is similar to OECD 202. Nominal test concentrations were 0.04-3.6 or 3.9 mg/l. The highest test concentration was measured analytically at the start of the test. The respective 48-hour LC_{50} was 3.6 mg/l and > 3.9 mg/l, which is exceeding the water solubility of BBP. Therefore the results are not further used in the report.

An acute test with BBP on the midge *Paratanytarsus parthenogenitica* was performed according to US EPA (1975) guidelines (Monsanto 83-x099, 1981). Fairly hard water was used (alkalinity of 303 mg/l). The test concentrations were not confirmed. A 48-hour EC_{50} of 7.2mg/l was found, which is not further used in the report.

An acute test with BBP on *Daphnia magna* with and without addition of fulvic acid was performed in order to investigate whether BBP would adsorb to this, resulting in a lower free concentration of BBP (Monsanto 78-0248, 1978). The results indicated that addition of fulvic acid resulted in an increased toxicity when compared with an earlier test, which gave an EC_{50} of 3.7 mg/l. However, there are many other studies with a comparable toxicity. Therefore the conclusion should be that fulvic acid does not influence the availability of BBP and its toxicity significantly.

Marine tests

An acute static test of BBP with mysid shrimp (*Mysidopsis bahia)* was performed according to US EPA (1975) testing guidelines without measurement of concentrations (EG&G Bionomics BP-79-4-38, 1979). Salinity of the test medium was 14% and the test temperature was 22°C. As this is a static test and BBP is quite unstable in water one may assume that the presented 96-hour LC_{50} of 0.9 mg/l is a minimum estimate. The results however are supported by a similar study performed with an acute flow through toxicity study.

An acute flow through toxicity study of BBP with *Mysidopsis bahia* was performed according to US EPA/OTS (1985) (Springborn 87-10-2525, 1988). Filtered seawater of 30% salinity was mixed with BBP dissolved in acetone. A flow rate of 13 volume exchanges per 24 hours was used. Nominal test concentrations were in the range of 0.13-2.0 mg/l, while mean measured concentrations during test were in the range of 0.041-0.74 mg/l (< 40% of nominal as average). Solubility of BBP in seawater was assumed to be approximately 1.0 mg/l. The highest tested concentration had a mortality of 35% after 96 hours. Therefore no LC_{50} could be estimated (LC_{50} > 0.74 mg/l). The NOEC was 0.041 mg/l, based on mortality.

An acute flow through toxicity study of ^{14}C-BBP *with* grass shrimp *(Paleomonetes vulgaris)* was performed according to ASTM (1985) and according to GLP (Springborn 86-7-2087). Filtered seawater of 32% salinity was mixed with BBP dissolved in acetone. A flow rate of 5.7 volume exchanges per 24 hours was used. Nominal test concentrations were in the range of 0.52-2.9 mg/l, while radiometric analysis of samples gave mean measured concentrations during test in the range of 63-93% of nominal. A film on the water surface was observed at concentrations of 0.78 mg/l and higher. No mortality was observed in the test. The LC_{50} and NOEC after 96 hours are therefore > 2.7 mg/l, which is very closed to waters solubility limit of BBP.

An acute flow through toxicity study of ^{14}C-BBP with eastern oyster *(Crassostrea virginica)* was performed according to US EPA (1985) guidelines and according to GLP (Springborn 86-7-2083, 1986). Filtered seawater of 32% salinity was mixed with BBP dissolved in acetone. A flow rate of 0.56 volume exchanges per 24 hours was used. Nominal test concentrations were in the range of 0.28-2.9 mg/l, while radiometric measurement of samples gave mean measured concentrations during test in the range of 42-68% of nominal. The highest concentration caused a 53% reduction in shell growth after 96 hours. The 96-hour EC_{50} was calculated to be 1.3 mg/l BBP. Zero inhibition was observed at 0.38 mg/l.

An acute flow through toxicity study of ^{14}C-BBP with a polychaete (*Nereis virens)* was performed according to ASTM (1980) guidelines and to GLP (Springborn 86-7-2094). Filtered seawater of 32% salinity was mixed with BBP dissolved in acetone. A flow rate of 0.65 volume exchanges per 24 hours was used. Nominal test concentrations were in the range of 0.52-2.9 mg/l, while radiometric determination of test concentrations was 55-129% of nominal. A surface film was observed at the three highest concentrations. No dose dependent mortality or sublethal effects were observed. The 96-hour LC_{50} was > 3.0 mg/l BBP and the result is not further used in the report.

An acute flow through toxicity study of ^{14}C-BBP with pink shrimp (*Penaeus duorarum*) was performed according to ASTM (1980) guidelines (Springborn 86-7-2093, 1986). The test was similar to Springborn 86-7-2094 (1986). Nominal test concentrations were in the range of 0.52-2.9 mg/l, while radiometric determination of test concentrations was 72-134% of nominal. No dose dependent mortality or sublethal effects was observed. The 96-hour LC_{50} was > 3.4 mg/l BBP and the result is not further used in the report.

Table 3.28 Summary table of acute toxicity studies of BBP towards freshwater and marine invertebrate species

Species	Duration [h]	Test method	Static/flow	Temp [°C]	Hardness [mg/l]	EC_{50} [mg/l]	Quality	Comment	Reference
Freshwater tests									
Daphnia magna	48	US EPA	static	22	241	1.0n	valid	No solvent	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	1.6n	valid	Ethanol	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	1.8n	valid	Dimthylform-amide	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	1.6n	valid	Acetone	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	2.2n	valid	Triethylene glycol	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	2.9n	not valid	fed during test	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	4.7n	not valid	fed during test	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	>10n	not valid	fed during test	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	2.1n	valid	fed during test	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	4.1n	not valid	fed during test	Barera and Adams (1981)
Daphnia magna	48	US EPA	static	22	241	> 10n	not valid	fed during test	Barera and Adams (1981)
Daphnia magna	48	US EPA	static			1.7n	not valid	bad reporting	Monsanto 85-9171
Daphnia magna	48	US EPA	static	22	136-262	1.8n	valid		Monsanto 85-9180
Chironimus tentans	48	US EPA	static	22	136-262	1.6n	valid		Monsanto 85-9180
Chironimus tentans	48	US EPA	static	24	288-302	1.6n	valid		Monsanto 85-9165
Daphnia magna	48	US EPA	static	24	90-105	1.9n	valid	fulvic acid added	Monsanto 78-0248
Daphnia magna	48	US EPA	static	24	90-105	2.4n	valid	river water	Monsanto 78-0248
Daphnia magna	48	US EPA	static	23.5	110-112	3.7n	not valid	well water	Monsanto 78-0247
Chironimus tentans	48	other	static	21.5	28	3.6m	not valid	above solub. of BBP	SRI LSC-1741

Table 3.28 continued overleaf

Table 3.28 continued Summary table of acute toxicity studies of BBP towards freshwater and marine invertebrate species

Species	Duration [h]	Test method	Static/flow	Temp [°C]	Hardness [mg/l]	EC_{50} [mg/l]		Comment	Reference
Paratanytarsus dissimilis	48	other	static	21.5	28	> 3.6m	not valid	above solubility of BBP	SRI LSC-1741 (1981)
Paratanytarsus parthenogenitica	48	other	static	21	244-276	7.2n	not valid	above solubility of BBP	Monsanto 83-x099
P. parthenogenitica	48	unknown				3.2n	not valid	review article	Staples et al. (1997)
Hexagonia sp	96	unknown				1.1m	not valid	review article	Staples et al. (1997)
Procambarus sp.	48					> 2.4	not valid	review article	Staples et al. (1997)
Marine tests									
Mysidopsis bahia	96	US EPA	static	22	14	0.9n	valid		EG&G Bionomics BP-79-4-38
Mysidopsis bahia	96	Fed. Reg. (1980)	flow	25	30	> 0.74m	valid		Springborn 87-10-2525
Mysidopsis bahia	96		static			9.6	not valid	No test details available	Sugatt and Foote (1981)
Paleomonetes vulgaris	96	ASTM(1980)	flow	20	32	> 2.7m	not valid	^{14}C-labelled BBP	Springborn 86-7-2087
Crassostrea virginica	96	US EPA	flow	20-22	32	1.3	valid	^{14}C-labelled BBP	Springborn 86-7-2083
Nereis virens	96	ASTM (1980)	flow	12-13	32	> 3.0	not valid	^{14}C-labelled BBP	Springborn 86-7-2094
Penaeus dourarum	96	ASTM	flow	22	32-33	> 3.4m	not valid	^{14}C-labelled BBP	Springborn 86-7-2093

m Measured concentration
n Nominal concentration
Static Static conditions
Flow Flow through conditions

3.2.1.3.2 Acute test of metabolites of BBP

An acute test with 3 metabolites of BBP on *Daphnia magna* was performed according to US EPA (1975) guidelines (Monsanto 88-9253, 1986). The metabolites tested were phthalic acid, monobutyl phthalate (MBuP) and monobenzyl phthalate (MBeP). The dilution water used was a mixture of distilled deionised water and well water and had the following water qualities (water hardness was 60-66 mg/l, pH 6.9-8.8, temp 20-23°C). MBuP and MBeP were dissolved in dimethylformamide. The nominal test concentrations were not verified analytically. 50% immobilisation of test organisms was not achieved for any of the compounds. EC_{50} values were greater than the highest tested concentration of 640 mg/l for phthalic acid, 320 mg/l for MBuP and 160 mg/l for MBeP. The solubility of MBuP and MBeP was exceeded at these concentrations. The NOECs were > 640, > 160 and > 40 mg/l indicating that the metabolites are a factor of 100 or more less acutely toxic to *D. magna* than the parent compound BBP. As a conservative approach the risk characterisation is based on BBP as the most acutely toxic compound.

Combined effect of BBP and DEHP

The acute toxicity of BBP and DEHP in combination to *Daphnia magna* was investigated according to US EPA (1975) guidelines (Monsanto 78-0276, 1978). BBP and DEHP were given in a 1:1 ratio at total concentrations from 0.56 to 4.22 mg/l. The combined 48-hour EC_{50} was 0.97 mg/l, which was less than the EC_{50} for BBP and DEHP alone (3.7 and 2.0 mg/l). The data suggest that DEHP and BBP are slightly more toxic when present together than when either of them is alone. However, the combined EC_{50} is only slightly different from the lowest EC_{50} value for BBP found for *Daphnia magna* (results see **Table 3.28**). A serious shortcoming of the study is that the test concentrations used considerably exceed the water solubility of DEHP and BBP. The very low water solubility of DEHP, approximately 3 µg/l (RAR DEHP) causes problems when testing toxicity to aquatic organisms and when interpreting the results.

3.2.1.3.3 Chronic tests

A 21-day chronic toxicity test (renewal) with BBP on *Daphnia magna* was performed according to Heidolph (1980); Environmental science method no. ES-80-M-42 (Monsanto 82-SS-103, 1983). The study was a GLP study. Nominal test concentrations were in the range of 0.12 to 2.0 mg/l. Measurement of new and old solutions gave mean exposure measurements, which were in the range of 70-100% of nominal. Very low (< 0.06 mg/l) exposure levels were observed for all levels during the last 3-5 days. Endpoints were based on mean measured levels. 21-day EC_{50} for survival was 0.7 mg/l. The 21-day NOEC for survival was 0.35 mg/l and the 21-day NOEC for reproduction was 0.22 mg/l.

A 28-day life cycle test with *Mysidopsis bahia* with BBP under flow through conditions was performed according to ASTM (1985) guidelines (Monsanto 86-7-2074). The study was performed according to GLP. Radiolabeled BBP was used and acetone as carrier solvent. Natural seawater with 32% salinity, 25 ± 1°C temperature and pH 7.9 ± 1 was used. Mean measured concentrations were 63-150% of nominal. Based on these results the mean measured exposure concentration range was calculated to be 0.024-0.75 mg/l over the 28-day test period. Endpoints were survival, growth and reproduction. The 28-day NOEC for survival was 0.17 mg/l based on measured concentrations. The 28-day NOEC for reproduction and growth was 0.075 mg/l.

A 21-day reproduction study in a flow through system was performed on *Daphnia magna* according to a protocol developed at Springborn laboratories (Rhodes et al., 1995). The study

was performed according to GLP. Mean measured test concentrations were 0.073, 0.21, 0.28, 1.4 and 2.4 mg/l. Both survival and reproduction were significantly reduced at the two highest test concentrations. No significant effects were observed at 0.28 mg/l. The 21-day NOEC for reproduction was 0.28 mg/l.

The review of Gledhill et al. (1980) includes a summary of the test conditions of a 42-day *Daphnia magna* two generation reproduction test. Mean measured test concentrations were 0.035, 0.058, 0.099, 0.26 and 0.76 mg/l. Reduced survival in the second generation was observed at 0.76 mg/l. No significant effects were observed at 0.26 mg/l. The 42-day NOEC for reproduction was 0.26 mg/l. The original study has not been submitted, however, as this study is reported in a peer reviewed journal it is assumed that it is of sufficient quality and can be regarded as valid.

Table 3.29 Summary of invertebrate reproduction tests

Species	NOEC [mg/l]	Duration [d]	Quality	Endpoint	Reference
Freshwater					
Daphnia magna	0.22	21	valid	reproduction	Monsanto 82-SS-103.
Daphnia magna	0.28	21	valid	reproduction	Rhodes et al. (1995)
Daphnia magna	0.26	42	valid	reproduction	Gledhill et al. (1980)
Mysidopsis bahia	0.075	28	valid	reproduction	Monsanto 86-7-2074

3.2.1.3.4 Conclusion with respect to invertebrate tests

The lowest acute LC_{50} in a good quality study is 0.9 mg/l for *Mysidopsis bahia* (EG&G Bionomics BP-79-4-38). The lowest chronic NOEC equal to 0.075 mg/l is that of the *Mysidopsis bahia* test of Monsanto 86-7-2074. This NOEC value will be used in the risk assessment to derive a $PNEC_{aquatic}$.

3.2.1.4 Toxicity to algae

Several algal tests have been performed and all the tests are presented in **Table 3.30**. Some of these studies should be used with caution because in these tests the relative cell number at the final day was used as an endpoint, (ASTM or U.S. EPA guidelines) rather than the area under the growth curve or the growth rate. In addition the test period was 96 hours rather than 72 hours. Hence, a direct comparison with test endpoints from the more acceptable OECD 201 method is not possible. Therefore, the focus has been put on the studies performed according to OECD 201. Recently, industry has submitted two additional tests on species that previously have shown EC_{50} < 1 mg/l (Huntingdon Life Science SLU 005/002302 and Huntingdon Life Science SLU 004/002301). These are discussed separately at the end of this section. Definition of EC_{50} in the context of algae toxicity is as follows: Concentration of test substance which results in a 50% reduction in either growth (E_bC_{50}) or growth rate (E_rC_{50}) relative to the control.

3.2.1.4.1 Freshwater tests

An acute toxicity study of BBP on several algae was presented by EG & G, Bionomics 78-9-148. The test method followed US EPA (1971) guidelines with respect to freshwater algae and US EPA (1974) guidelines with respect to marine algae. The EC_{50} was calculated from final cell

numbers after 96 hours compared to the control. Except for *M. aeruginosa*, which is a cyanophyte, the interspecies differences are small. Both the fact that a non standard method for the calculation of the EC_{50} (the method is not directly comparable with the E_bC_{50} or E_rC_{50} as defined in i.e. OECD 201 method) and a longer than usual test period was used, indicate that the results from this study should be used with caution.

The toxicity of BBP towards a diatom (*Navicula pelliculosa)* was performed according to OECD 201 and GLP (Carolina Ecotox 14-01-1, 1995). The range of nominal test concentrations was 0.075-2.4 mg/l. Initial measured concentrations were 85-101% of nominal; after 72 hours measured concentrations were 34-69% of the nominal. The EC_{50} was calculated using the initial measured concentration and the area under growth curve. The reported E_bC_{50} was 0.414 mg/l, and the NOEC was 0.064 mg/l. E_bC_{10} with respect to area under growth curve is 0.10 mg/l. Recalculation of data estimating E_rC_{50} from growth rate data gave an E_rC_{50} of 0.64 mg/l with an E_rC_{10} of 0.20 mg/l. Often EC_{10} is a better estimate of a no effect level than the NOEC value because it takes the whole set of results into account. The cells had a 24-hour lag phase before exponential growth occurred at all test concentrations and controls.

The toxicity of BBP towards a green algae (*Scenedesmus subspicatus)* was performed according to OECD 201 and GLP (Carolina Ecotox 14-01-2, 1995). The range of nominal test concentrations was 0.075-2.4 mg/l. Initial measured concentrations were 81-93% of nominal. No measurement of test concentration was made after 72 hours. The EC_{50} was calculated using the initial measured concentration and the area under growth curve. The reported E_bC_{50} was 0.33 mg/l and the NOEC was 0.26 mg/l. E_bC_{10} with respect to area under growth curve is 0.12 mg/l. Recalculation of data estimating E_rC_{50} from growth rate data gave an E_rC_{50} of 0.92 mg/l with an E_rC_{10} of 0.31 mg/l. EC_{10} is often a better estimate of a no effect level than the NOEC value because it takes the whole set of results into account.

A toxicity test with *Navicula pelliculosa* was performed according to OECD 201 and the EEC Method for "Algal Inhibition Test" (Huntingdon Life science SLU 002/992825). The test was performed according to GLP. Six concentrations were tested (0.046, 0.1, 0.22, 0.46, 1.0 and 2.2 mg/l) with three replicates at each concentration. Initial measured concentrations were (0.045, 0.1, 0.2, 0.46, 0.93 and 2.2 mg/l). Acetone was used as solvent. Both E_rC_{50} (2.1 mg/l) and E_bC_{50} (0.92 mg/l) were calculated based on initial measured concentrations. The NOEC was 0.46 mg/l for both endpoints.

The test is in general adequately performed and reported, however, with one important exception. The inoculum cell density was approximately a factor 10 higher than the recommended level in the OECD Guideline (10^4 cells ml^{-1}). A high inoculum density is inappropriate for several reasons. First of all it does not allow exponential growth in uninhibited cultures throughout the test duration (72 hours). When the cell density becomes too high, the growth rate is reduced because of self-shading, CO_2 or nutrient limitation. This may have the effect that cultures partly inhibited by the test substance can catch up with the control at the end of the test. Both ways of calculating EC values (E_rC or E_bC) depend heavily on the final cell density in the cultures. Therefore the toxic effect tends to be underestimated when density dependent growth limitation occurs in the control. A high inoculum density is also unfortunate because it gives a low test substance/biomass ratio. With test substances that adsorb to the algal cells, the "dose/cell" ratio becomes lower when the inoculum density is high. This will have the effect to reduce the sensitivity of the test. It was noted during the test that the presence of algal cells had an effect on the stability of the test concentrations during the exposure period, with the result that the concentration after 72 hours had dropped below the limit of detection. The OECD 201 guideline states that the test has to be performed on exponentially growing cultures of green algae. This does not seem to be the case in this test. A lag phase was observed during the first

day of exposure. The lack of exponential growth at the first and third day of the test is probably the reason why the validity criteria (increase of cell concentration with a factor 16 within 3 days) was not fulfilled in the *N. pelliculosa* test. It has to be stressed that the species *N. pelliculosa* is not listed in the guideline. In order to exemplify the importance of non-exponential growth during the final day in control cultures, the EC_{50s} were estimated after 48 hours using the reported cells counts in the report. For *N. pelliculosa* the reduction was from 2.13 mg/l (72 hours) to 1.43 mg/l (48 hours), estimated by the Probit method.

A toxicity test with *Scenedesmus subspicatus* was performed according to OECD 201 and GLP (Huntingdon Life science SLU 003/992087). Six concentrations were tested (0.1, 0.22, 0.46, 1.0, 2.2 and 4.6 mg/l) with three replicates at each concentration. Initial measured concentrations were 0.11, 0.24, 0.46, 1.03, 2.4 and 4.8 mg/l. Acetone was used as solvent. Both E_rC_{50} (4.6 mg/l) and E_bC_{50} (1.2 mg/l) were calculated based on initial measured concentrations. The NOEC is 0.46 mg/l with respect to biomass and 1.0 mg/l with respect to growth rate. The same evaluation applies here as for the test with *Navicula pelliculosa* but the effect of high initial inoculum is more pronounced for *S. subspicatus* because of its higher growth rate. In order to exemplify the importance of non-exponential growth during the final day in control cultures, the EC_{50} values were estimated after 48 hours using the reported cells counts in the report. With respect to *S. subspicatus* the E_rC_{50} was reduced from 4.60 mg/l (72 hours) to E_rC_{50} 1.46 mg/l (48 hours).

In the test protocol it was noted that the presence of algae cells had an effect on the stability of the test concentrations during the exposure period, with the result that the concentration after 72 hours had dropped below the limit of detection. This may be the reason for the recovery of *S. subspicatus* at concentrations above 0.1 mg/l. Declining concentrations of test substance are difficult to avoid in algae tests, but the problem gets more pronounced if the inoculum density is increased.

The deviation from the OECD and EEC protocols regarding inoculum density has not been indicated in the test reports. As specified above, this deviation has most probably had the effect to reduce the sensitivity of the tests significantly. Lack of exponential growth in control cultures towards the end of the test period also reduces sensitivity. The test results of both studies do not correspond in quality to the tests performed by Carolina Ecotox (*Navicula pelliculosa* 14-01-1 and *Scenedesmus subspicatus* 14-01-2) and they will not be taken into account in this risk assessment.

The studies Huntingdon Life Science SLU 003/992087 and Huntingdon Life Science SLU 002/992825 were repeated with lower initial inoculum and submitted as reports "Huntingdon Life Science SLU 005/002302" and "Huntingdon Life Science SLU 004/002301". These studies have been reviewed and concluded to be valid studies. The main results of these studies are given in **Table 3.30** and confirm the studies performed by Carolina Ecotox (Carolina Ecotox 14-01-1, Carolina Ecotox 14-01-2). The values reported for the repeated Huntingdon tests are NOEC values while those performed by Caroline Ecotox are EC_{10} values. Similar EC_{10} values calulated for the repeated Huntingdon tests are higher than those of Carolina Ecotox (EC_{10}=0.34 mg/l for *N. pelliculosa* and EC_{10}=0.33 mg/l for *S. subspicatus*).

The E_rC_{10} value = 0.20 mg/l based on growth rate for *Navicula pelliculosa (*Carolina Ecotox 14-01-1) remains the lowest valid EC_{10} value and will be used for the derivation of $PNEC_{aquatic}$.

3.2.1.4.2 Marine tests

The marine algae *Skeletonema costatum* a diatom was tested according to US EPA (1971) guidelines (EG&G Bionomics 78-9-148). Raw data are not submitted so it is not possible to evaluate the quality of the test directly. An EC_{50} was reported for 24 hours and 96 hours and was 0.9 mg/l and 0.6 mg/l. The first value is based on *in vivo* chlorophyll while the latter one is based on cell count. The EC_{50} value is based on decrease in cell count relative to the control (not growth rate decrease). A LOEC was determined as 0.1 mg/l (this was lowest tested concentration). The study results should be used with caution.

A study on marine algae *Skeletonema costatum* is presented in the review report of Sugatt and Foote (1981) with a NOEC < 0.03 mg/l. No more detailed test information is available. Industry has informed that the test seems to be fairly primitive compared to procedures used today. The quality is not considered to be adequate for the derivation on the PNEC as several studies are available of better quality.

3.2.1.4.3 Conclusions with respect to algal tests

The most sensitive species in a valid test are *Navicula pelliculosa* (Carolina Ecotox 14-01-1) with respect to both EC_{50} and EC_{10}. The E_rC_{10} value of 0.20 mg/l based on growth rate is used to derive the $PNEC_{aquatic}$. The EC_{50} value of 0.64 mg/l for *Navicula pelliculosa* may be used for classification purposes. A summary of all available studies is presented in **Table 3.30**.

Table 3.30 Summary of toxicity studies of BBP towards freshwater and marine algae

Species	E_rC_{50} [mg/l]	NOEC/EC_{10} [mg/l]	Quality	Test duration	Reference
Freshwater					
Navicula pelliculosa	0.66 m	0.17 m	Valid	72 hours	Huntingdon SLU 004/002301
Scenedesmus subspicatus	1.5 m	0.15 m	Valid	72 hours	Huntingdon SLU 005/002302
Navicula pelliculosa	2.1 m	0.46 m	Not valid[1]	72 hours	Huntingdon SLU 002/992825
Scenedesmus subspicatus	4.6m	1.0m	Not valid[1]	72 hours	Huntingdon SLU 003/992087
Navicula pelliculosa	0.64 m*	0.20 m*	Valid	72 hours	Carolina Ecotox 14-01-1
Scenedesmus subspicatus	0.92 m*	0.31 m*	Valid	72 hours	Carolina Ecotox 14-01-2
Selenastrum capricornutum	0.7 n	0.23 n	Not valid[2]	5 days	SRI 81-0252
Selenastrum capricornutum	0.5 n	< 0.02 n	Not valid[2]	14 days	SRI 81-0252
Selenastrum capricornutum	0.52 n	0.21 n	Used with caution[3]	96 hours, based on biomass	Monsanto 86-9076
Microcystis aeruginosa	> 1,000n	320n	Used with caution[3]	96 hours	EG&G Bionomics 78-9-148

Table 3.30 continued overleaf

Table 3.30 continued Summary of toxicity studies of BBP towards freshwater and marine algae

Species	E_rC_{50} [mg/l]	NOEC/EC_{10} [mg/l]	Quality	Test duration	Reference
Marine					
Selenastrum capricornutum	0.5n	0.10n	Used with caution[3]	96 hours	EG&G Bionomics 78-9-148
Navicula pelliculosa	0.6n	< 0.3n	Used with caution3	96 hours	EG&G Bionomics 78-9-148
Chlorella vulgaris	> 2.9	> 2.9	Not valid	72 hours	Carolina Ecotox 14-06-1
Skeletonema costatum	0.6 n	0.1 n	Used with caution[3]	96 hours	EG&G Bionomics 78-9-148
Skeletonema costatum	0.17	< 0.03	Not valid[4]	96 hours	Sugatt and Foote (1981)
Skeletonema costatum	0.19	--	Not valid[4]	96 hours	Sugatt and Foote (1981)
Dunaliella tertiolecta	1.0n	0.3 n	Used with caution[3]	96 hours	EG&G Bionomics 78-9-148

* Recalculated data according to the Probit method using growth rate data instead of area under the growth curve.

1) Not valid because inoculum was 10 · higher as recommended in OECD 201 guideline
2) Not valid because test period was > 96 hours
3) Used with caution because test period > 72 hours
4) Not valid, full test conditions are not available

3.2.1.5 Toxicity to microorganisms

In a respiration inhibition test (OECD 209) no effect was observed on respiratory activity in activated sludge at BBP's solubility limit of 2.8 mg/l (Volskay and Grady, 1988).

3.2.1.6 PNEC determination for the aquatic environment (including sediment)

There are valid long-term tests available representing the three trophic levels. The NOEC values available are:

Fish: 30-day early life stage test: NOEC (weight gain) = 0.14 mg/l
(*Pimephales promelas*, EG&G Bionomics, 1981)

Invertebrate: 28-day NOEC (reproduction) = 0.075 mg/l
(*Mysidopsis bahia* Monsanto 86-7-2074)

Alga: 72-hour EC_{10} (growth rate) = 0.20 mg/l
(*Navicula pelliculosa,* Carolina Ecotox 14-01-1)

An assessment factor of 10 should be applied to the lowest of the NOEC values, when there are three valid chronic studies available.

$$\mathbf{PNEC_{aquatic} = 0.075\ mg/l / 10 = 7.5\ \mu g/l}$$

However the $PNEC_{aquatic}$ is provisional as a fish reproduction study is ongoing in order to investigate possible endocrine effects of BBP.

For deriving a $PNEC_{marine}$ an assessment factor of 100 has to be applied because long term toxicity NOECs are available only from three freshwater or saltwater species for three trophic levels (algae, crustacean and fish). No effect data for additional taxonomic groups (e.g. molluscs, echinoderms) are available for BBP and lowering the assessment factor to 10 is therefore not justified.

$PNEC_{marine}$=0.07.5 mg/l/100 = 0.75 µg/l

No test is available for sediment dwelling organisms. According to TGD the equilibrium partitioning method may be used to estimate the PNECsediment. The following equations taken from the TGD are used to derive the PNECsediment from PNECaquatic and the PNEC marine sediment from PNEC saltwater. As the calculation of local PEC for sediment is based on the concentration in freshly deposited sediment (TGD) the properties of suspended matter have to be used. Therefore the partition coefficient for suspended matter –water and not the partition coefficient for sediment-water was used in the equation to calculate the PMEC sediment.

$$PNEC_{sediment} = \underline{K_{susp\text{-}water}} / RHO_{susp} \cdot PNEC_{aquatic} \cdot 1{,}000) \text{ (equation 70 in TGD)}$$

$PNEC_{sediment}$ = $\underline{263}$ /1150 l/kg · 0.0075 mg/kg · 1,000 = 1.72 mg/kg wwt

$$PNEC_{marine\ sediment} = \underline{K_{susp\text{-}water}} / RHO_{susp} \cdot PNEC_{saltwater} \cdot 1{,}000 \text{ (equation 88 in TGD)}$$

$PNEC_{marine\ sediment}$ = 263 /1,150 l/kg · 0.00075 mg/kg · 1,000= 0.172 mg/kg wwt

Where: $PNEC_{aquatic}$= 0.0075 mg/kg

$PNEC_{saltwater}$= 0.00075 mg/kg

RHO_{susp} = $Fsolid_{sus}$ · RHOsolid + $Fwater_{susp}$· RHOwater = **1,150 kg/m^3** (equation 18 in TGD)

$K_{susp\text{-}water}$ = $Fwater_{susp}$ + $Fsolid_{susp}$ · Kp_{susp} · RHOsolid/1,000 = **263** (equation 24 in TGD)

$Fwater_{susp}$ = 0.9 m^3/m^3 (Table 5 in TGD)

$Fsolid_{susp}$ = 0.1 m^3/m^3 (Table 5 in TGD)

RHOsolid = 2,500 kg/m^3 (Table 5 in TGD)

RHOwater = 1,000 kg/m^3 (Table 5 in TGD)

Kp_{susp} = Foc_{susp} · Koc = **1,050 l/kg** (equation 23 in TGD)

where Foc_{susp} = 0.1 (Table 5 in TGD) and Koc=10,500 l/kg (EUSES)

3.2.1.7 PNEC derivation for microorganisms

There is only one test available regarding toxicity to microorganisms. According to Volskay and Grady (1988) no effect was observed on respiratory activity in activated sludge at BBP's solubility limit of 2.8 mg/l. As no effects were observed at the highest concentration tested no PNEC could be derived.

3.2.2 Terrestrial compartment

3.2.2.1 Toxicity to soil invertebrates

The only submitted terrestrial test is an earthworm test (Huntingdon Life Science, SLU 001/983882, 1998). An acute toxicity test of BBP with earthworm (*Eisenia foetida*) was performed according to OECD 207 "Earthworm, acute toxicity test" and Directive 87/302/EEC part C "Toxicity for earthworm: artificial soil test". The study was a GLP study. The earthworm species *Eisenia fetida* (or the similar *E. andrei*) was exposed in artificial soil to BBP. Five concentrations were tested and the nominal concentrations were 95, 171, 309, 556 and 1,000 mg/kg dwt. There were four replicates with 10 worms in each replicate. Weight and survival were registered after 7 and 14 days. Neither mortality related to the chemical treatment nor differences between negative control and treatment were registered. A positive control showed an expected response. Determination of LC_{50} value is not possible due to 100% survival. The data generated in the study are considered to be valid.

3.2.2.2 PNEC derivation for the terrestrial compartment

As no negative effects were seen in the acute toxicity test with earthworm *(Eisenia foetida)* no $PNEC_{soil}$ could be derived.

$$\mathbf{PNEC_{soil} > 1{,}000/1{,}000/1.13 = > 0.89\ mg/kg\ wwt}$$

When only one terrestrial study is available the TGD recommends that PNECsoil should also be estimated according to the equilibrium partitioning method, using the PNECaquatic in the following equation:

$PNEC_{soil} = K_{soil\text{-}water} / RHO_{soil} \cdot PNEC_{aquatic} \cdot 1{,}000$ (equation 72 in TGD)

$\mathbf{PNEC_{soil}=315/1{,}700 \cdot 0.0075 \cdot 1{,}000 = 1.39\ mg/kg\ wwt}$

Where: $PNEC_{aquatic} = 0.0075$ mg/kg

$RHO_{soil} = Fsolid_{soil} \cdot RHOsolid + Fwater_{soil} \cdot RHOwater + Fair_{soil} \cdot RHOair =$ **1,700 kg/m^3** (equation 18 in TGD) $K_{soil\text{-}water} = Fair_{soil} \cdot K_{air\text{-}soil} + Fwater_{soil} + Fsolid_{soil} \cdot Kp_{soil} \cdot RHOsolid/1{,}000 =$ **315** (equation 24)

$K_{air\text{-}soil} = HENRY/8.314/285 =$ **7.42^{-5}** (equation 22 in TGD)

$Fwater_{soil} = 0.2\ m^3/m^3$ (Table 5 in TGD)

$Fsolid_{soil} = 0.6\ m^3/m^3$ (Table 5 in TGD)

$Fair_{soil} = 0.2\ m^3/m^3$ (Table 5 in TGD)

RHOsolid = 2,500 kg/m^3 (Table 5 in TGD)

RHOwater = 1,000 kg/m^3 (Table 5 in TGD)

RHOair = 1,3 kg/m^3 (Table 5 in TGD)

$Kp_{soil} = Foc_{soil} \cdot Koc =$ **210 l/kg** (equation 23)

where $Foc_{soil} = 0.02$ (Table 5) and Koc=10,500 l/kg (EUSES)

The PNECsoil of 1.39 mg/kg wwt estimated by the equilibrium partitioning method will be used with respect to the risk assessment of soil.

3.2.3 Atmosphere

Observations around phthalate production and manufacturing facilities indicate uptake by plants of phthalates through air (Müller and Kordel, 1993) (see also Section 3.2.4). Preliminary data on dibutylphthalate (DBP) have suggested potential for impact on plants. This potential has been substantiated in a recent short term and chronic fumigation study with plants representing European flora, indicating that *Brassica spec*. and *Trifolium spec*. show very distinct effects at concentrations relevant for the risk assessment. Industry has therefore performed a study to evaluate vapour phase exposure of BBP to plants.

Industry has submitted two test reports on a vapour phase phytotoxicity test. Both tests are performed as limit test with nominal concentration of either 1 or 10 µg/m^3 and with an exposure period of 21 days. The same three species mustard (*Sinapis alba* former known as *Brassica alba*), Chinese cabbage (*Brassica chinesis* former known as *Brassica camprestis var chinesis*) and white clover (*Trifolium repens*) were exposed in both test.

Method

The test was carried out following OECD 208 (growth test for terrestrial plants) and the updated OECD 208 draft document (July 2000) modified for gaseous substances. No standardised method for vapour phase phytotoxicity assessment is available at the moment. Vapour phase exposure was achieved by passing synthetic air from a gas cylinder through a vessel containing a stock solution of BBP. In the study with 1 µg/m^3 nominal test concentration, the BBP stock solution was cooled down to 10°C and then transported through a vessel with cotton wool and glass beads in order to minimize aerosol formation. The resulting air was diluted with pure air to give a nominal concentration of 1 µg/m^3. In the test with 10 µg/m^3 nominal test concentration the BBP stock solution was temperated to 27°C and then mixed directly with pure air to a nominal concentration of 10 µg/m^3.

In both tests the air stream was 2,000 l/hour equal to 0.145 air change per minute. Two control and two exposure chambers were used and only plants with equal growth developed first mature leaf were exposed to 1 and 10 µg/m^3. Plants were watered with a nutrient solution once a day.

For the study performed at 1 µg BBP/m^3 test conditions were as followed: Light intensity was 84 W/m^2 with a 16 hour light, 1 hour dawn/dusk and 6 hour night rythm. Humidity was 53-86% and the temperature range was 18-26°C over the whole period of the test.

For the study performed at 10 µg BBP/m^3 test conditions were as followed: Light intensity was 72 W/m^2 with a 16 hour light, 1 hour dawn/dusk and 6 hour night rythm. Humidity was 68-98% and the temperature range was 19-27°C (whole period).

BBP and DBP (which had been found as a background contaminant in initial trials) were measured by thermodesorption – GC-MS.

Endpoints measured were fresh/dry weight of the above ground plant parts after 21 days of exposure. Visual observations of macroscopic abnormalities during the test period should be carried out and at the end of the test observations were made concerning growth, abnormalities of above ground plant parts and roots, colour of roots.

Results

The exposure concentration was measured daily in the test chambers. In both tests the achieved test concentration was around 50% of the nominal BBP concentration (see **Table 3.31**). However, fairly good stability was achieved giving added confidence that observed test conditions are stable during the test period and that the total mean exposure concentration estimated is representative for the test. Measured DBP concentration in all four test chambers were below the limit of quantification of the analytical method (< 0.2 µg/m^3) during exposure period. **Table 3.32** shows results of dry weight measured of above ground plant parts after 21 days of exposure. A statistical evaluation according to Wilcoxon, Mann and Withney ($\alpha = 0.01$) showed no significant negative effects at any of the concentrations on the growth of any plant species. A similar evaluation was performed with respect to wet weight of above ground plant parts, showing no negative effects at any test concentration and any plant species. Both studies were valid showing at least 80% of the control seedlings exhibiting normal and healthy growth throughout the test. Although the reports state that plants were inspected also prior to the end of the test, these observations are not reported. Only visual observations confined to the end of the tests are reported. These observations did no indicate any exposure related negative effects on the growth of terrestrial plants.

Table 3.31 Test concentration in BBP exposed chambers during phytotoxicity test at 1 µg/m^3 and 10 µg/m^3

Exposure Time [Days]	BBP Concentrations [µg/m^3]			
	1 µg/m^3 Nominal Test		10 µg/m^3 Nominal Test	
	Chamber A	Chamber B	Chamber A	Chamber B
0.1	0.31	0.25	3.8	4.8
1	0.41	0.30	4.3	5.2
2	0.47	0.37	4.3	5.3
5	0.49	0.43	6.0	8.4
6	0.56	0.41	6.8	9.1
7	0.43	0.36	4.7	6.5
8	0.54	0.43	4.8	6.2
9	0.53	0.43	4.8	5.9
12	0.45	0.47	5.2	6.8
13	0.55	0.57	5.4	6.4
14	0.47	0.42	4.3	6.7
15	0.48	0.50	4.7	6.3
16	0.54	0.60	5.0	5.9

Table 3.31 continued overleaf

Table 3.31 continued Test concentration in BBP exposed during phytotoxicity test at 1 µg/m³ and 10 µg/m³

Exposure Time [Days]	BBP Concentrations [µg/m³]			
	1 µg/m³ Nominal Test		10 µg/m³ Nominal Test	
	Chamber A	Chamber B	Chamber A	Chamber B
19	0.61	0.64	5.3	6.8
20	0.55	0.54	4.2	5.4
21	0.27	0.48	4.4	6.0
Mean	0.48	0.45	4.9	6.4
Total Mean	0.47		5.7	

Table 3.32 Mean dry weight of above ground plant parts [g]. Nominal test concentration was either 1 or 10 µg/m³, mean measured concentration in test chambers was 0.47 or 5.7 µg/m³

Species	Nominal test concentration 1.0 µg/m³			
	Control A Dry Weight [g]	Control B Dry Weight [g]	Test Chamber A Dry Weight [g]	Test Chamber B Dry Weight [g]
Mustard	1.29	1.28	1.34	1.23
C. Cabbage	1.06	1.14	1.05	1.22
White Clover	0.54	0.56	0.49	0.56
	Nominal test concentration 10.0 µg/m³			
	Control A Dry Weight [g]	Control B Dry Weight [g]	Test Chamber A Dry Weight [g]	Test Chamber B Dry Weight [g]
Mustard	1.04	1.04	0.96	1.11
C. Cabbage	1.27	1.15	1.44	1.33
White Clover	0.77	0.77	0.82	0.85

Conclusion

5.7 µg/m^3 was the maximum achievable concentration in the vapour phase and no effects on plants could be observed at this concentration within 21 days. In the DBP test effects had been seen already after a short exposure period in the range of a few days. In contrary to DBP, which is phytotoxic via the gas phase, BBP did apparently not show that specific mechanism to affect plants via the gas phase and hence BBP can be considered to be not toxic to plants at the highest concentration tested.

Due to the absence of effects a reliable PNEC could not be derived. However, comparing the highest concentration tested (5.7 µg/m^3) with monitoring data it can be concluded that there is no concern for plant life. Monitoring air concentrations of BBP levels at flooring processing sites and sealant formulation sites are all below 0.4 µg/m^3.The highest PECs for air were estimated for flooring processing and sealant formulation with concentrations of BBP of 2.4 and 1.0 µg/m^3. These concentrations are also below the maximum concentration tested, indicating that there is no concern for plant life.

Without a reliable PNEC the risk assessment for air is only carried out in a qualitative manner.

3.2.4 Secondary poisoning – PNEC derivation

No information is available concerning the toxicity of BBP to higher organisms other than those related to human toxicology (see Section 4.1.2). Therefore the NOAEL of 50 mg/kg bw from a rat reproduction toxicity study will be used. The NOAEL is based on developmental effects: 50 mg/kg bw/day of BBP based on reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring (Tyl et al., 2004). PNEC for predators is derived as follows (conversion factor = 20, assessment factor = 30) which gives:

$$PNEC_{oral} = 50 \cdot 20/30 = 33.3 \text{ mg/kg in food}$$

A relevant flow of BBP in the food chain would be through uptake by plants from agricultural soil. Müller and Kordel (1993) investigated the uptake of several phthalates from soil into crops. Phthalate containing sludge was applied to soil and cultivated with maize, oats, and potatoes. After 1-2 months the plants were analysed for phthalates. The plants showed neither uptake of DEHP, DBP nor BBP. This is in accordance with the theoretical potential suggested by Wild and Jones (1992). Another relevant path of BBP is the air-plant-route. In the same study it was found that plants growing close to a phthalate emitting plant had DBP concentrations of up to 10 mg/kg dwt in maize leaves and up to 4 mg/kg dwt in the maize cobs. The origin of the phthalates from air was confirmed in a model experiment, which also includes BBP. BBP was also transferred from air to plant. Wild and Jones (1992) theorised that potential uptake from air would be high for compounds with a high Log Kow and low vapour pressure. More information is needed on the concentration-dependency of this uptake before a specific assessment of risk through this partway can be established.

3.3 RISK CHARACTERISATION

The risk characterisation presented below is performed using 2004 tonnages.

The following PNECs are used for risk characterisation:

$PNEC_{surfacewater} = 7.5$ µg/l
$PNEC_{marine} = 0.75$ µg/l
$PNEC_{sediment} = 1.72$ mg/kg wwt
$PNEC_{marine\ sediment} = 0.17$ mg/kg wwt
$PNEC_{soil} = 1.39$ mg/kg wwt
$PNEC_{oral} = 33.3$ mg/kg

For the risk characterisation these values are compared with the calculated PECs for all life cycle stages.

BBP is rapidly metabolised to its main metabolites monobutyl (MBuP) and monobenzyl phthalate (MBeP). The $PNEC_{surfacewater}$ based on BBP as the most acutely toxic substance is assumed conservative enough as the acute toxic effects of the metabolites MBuP, MBeP and phthalic acid are more than a factor of 100 less acutely toxic to *D. magna* than the parent compound. Furthermore the BCF-value used for the risk characterisation of secondary poisoning covers the BCF for both BBP and metabolites. This conservative approach for the estimation of the bioaccumulation potential is chosen because it cannot be excluded that the metabolites can give reprotoxic/endocrine effects to other species as they do to mammals. The potential reprotoxic/endocrine effects will be examined in the long term fish study.

Additive effects between DEHP and BBP cannot be excluded but a $PNEC_{surfacewater}$ addressing the combined effect of DEHP and BBP is not possible to derive. One acute toxicity study with *Daphnia magna* is available, but has serious shortcomings. Moreover it has not been considered suitable to specify a $PNEC_{water}$ for DEHP for exposure via water due to its very low water solubility of approximately 3 µg/l (RAR DEHP).

The $PNEC_{marine}$ is 0.75 µg/l. For the risk characterisation this value is compared with measured values. No $PNEC_{microorganism}$ could be deduced. Furthermore no $PNEC_{atmosphere}$ could be derived and only a qualitative assessment was conducted.

Life cycle I and II (Production and Distribution)

The local PEC/PNEC ratios for production sites for 1997 are presented in **Table 3.33**. As described in Section 3.1.1 release due to washing of tankers is assumed to occur at WWTPs at production sites. Sludge from production site WWTP is either deposited in landfill or incinerated. An assessment of local concentrations through sludge application to soil is therefore not considered relevant.

Table 3.33 Local PECs and PEC/PNEC for different compartments at production sites (1997 data)

Compartment	Site A		Site B		Site C	
	PEC	PEC/PNEC	PEC	PEC/PNEC	PEC	PEC/PNEC
Surface water [µg/l]	0.50	0.07	0.18	0.02	0.31	0.04
Sediment [mg/kg wwt]	0.14	0.08	0.07	0.04	0.10	0.06
Oral fish [mg/kg]	0.22	0.006	0.08	0.002	0.14	0.004

Life cycle III (Processing and Formulation)

All PEC/PNEC ratios for 2004 are compiled in **Table 3.35**. As sediment PEC and PNEC values are estimated using the partitioning equilibrium method they will be identical to PEC/PNEC for water and is therefore not included in the table.

Life cycle IV and V (Use and Disposal)

The local PECs of life cycle IV and V for 2004 are accessible in the EUSES report. All PEC/PNEC ratios are below 1.

Regional

All PEC/PNEC ratios are presented in the **Table 3.34**.

Table 3.34 Regional PEC/PNEC ratios using 2004 tonnages

Compartment	PEC	PEC/PNEC
Surface water [µg/l]	0.17	0.02
Sediment [mg/kg wwt]	0.07	0.04
Soil [mg/kg wwt]	3.0×10^{-3}	0.002

Table 3.35 Local PEC/PNECs for water, soil and secondary poisoning, and input parameters for life cycle stage III in 2004

Scenario	IIIa-1	IIIa-2	IIIb-1	IIIb-2	IIIc	IIId	IIIe-1	IIIe-2	IIIf-1	IIIf-2	IIIg-1	IIIg-2	IIIh
Type of use	Plastisol flooring Large site	Plastisol flooring Small site	Sealants		PVC coated textiles	PVC Films and sheet	General PVC		Paints and inks		Adhesives		Non polymer use
Industry and Use category	11 Polymer 47 Softener	11 Polymer 47 Softener	11 Polymer 47 Softener		13 Textiles 47 Softener	11 Polymer 47 Softener	11 Polymer 47 Softener		12 Pulp, paper, board 47 Softener		0 Others 47 Softener		0 Other 0 Other
Life cycle step	Processing	Processing	Formulation	Processing	Processing	Processing	Formulation	Processing	Formulation	Processing	Formulation	Processing	Formulation
Surface water	3.61	5.64	0.05	0.02	0.83	0.51	0.07	0.36	0.41	0.05	0.22	0.16	1.30
Soil	6.32	9.86	0.06	0.001	1.42	0.86	0.09	0.59	0.68	0.05	0.35	0.24	2.25
Fish	0.15	0.24	0.004	0.002	0.04	0.02	0.004	0.005	0.02	0.002	0.01	0.005	0.06
Worm	0.46	0.72	0.005	0.0003	0.10	0.06	0.007	0.04	0.05	0.004	0.03	0.02	0.16

3.3.1 Aquatic compartment (incl. sediment)

STP

No $PNEC_{microorganism}$ could be derived. However, predicted concentrations in STPs in all life cycle steps (see **Table 3.1** and **Table 3.2**) are well below the highest concentration tested (water solubility, 2.8 mg/l) which gave no adverse effect towards microorganisms. Therefore **Conclusion (ii)** is anticipated.

Surface water

Conclusion (i).

A long-term fish study on reproductive and endocrine effects has to be performed.

The PEC/PNEC ratios for all life cycle steps are presented in **Table 3.33** and **Table 3.35** and result in the following conclusions:

Production and Distribution (Life cycle stage I and II)

Conclusion (ii).

The exposure scenarios for the production sites are based on site specific information and on default values. The PEC/PNEC ratios for the aquatic compartment are below 1, thus a risk to the aquatic environment is not expected. However, **conclusion (ii)** has to be seen as provisional until possible endocrine effects in fish have been resolved.

Processing/Formulation (Life cycle stage III)

Conclusion (ii).

The exposure scenarios for processing of BBP are based on default parameters from the TGD or the ESD "Plastics". PEC/PNEC ratios for the aquatic compartment are below 1 for the use categories IIIb, IIIc, IIId, IIIe, IIIf and IIIg at life cycle stage III. Thus a risk to the aquatic environment is not expected. However, **conclusion (ii)** has to be seen as provisional until possible endocrine effects in fish have been resolved.

Conclusion (iii).

Two use categories show PEC/PNEC ratios > 1. These are the use categories IIIa (flooring large and small sites) and IIIh (formulation of confidential use). The exposure scenario for IIIa is based on the ESD "Plastics". The recently updated ESD "Plastics" has passed the OECD process and is based on best available information. Further site specific data have not been obtained. The exposure scenario for IIIh is based on information from Industry. The PEC/PNEC ratios for the aquatic compartment are above 1, thus a risk to the aquatic environment can be expected.

Flooring sites were split into large sites with air treatment facilities in place and small sites without air treatment (in accordance with the ESD on Plastics Additives from 2004). Industry stressed that the estimation of plant size on the basis of BBP consumption may be misleading because BBP is usually not used alone but in a mixture with other plasticisers. Hence, small sites with respect to BBP are not necessarily small sites in terms of plasticiser use and industry has confirmed that. However, information from industry has also shown that there are actually sites

without treatment and hence the worst case ESD-scenario for small sites, which do not have air treatment in place, could not be omitted even though the sites may not be small sites in terms of the definition of the ESD with respect to total plasticiser use.

According to industry the emissions to waste water are an overestimation, both for the large sites and for the small site scenario, but as no site specific emission data have become available emission factors are takes from the ESD.

Conclusion (iii) is based on BBP consumption data from 2004. For 2005 there are only two producers left and industry provided estimations of the expected use volume of BBP for all use categories. These figures are confidential as there are only two producers left.

The total BBP volume used for flooring in 2005 has been further reduced, but the scenarios used in the risk assessment are still relevant. In 2005 it is still valid to use the ESD emission factors for a small site since sites without air treatment have been identified.

Use and Disposal (Life cycle stages IV and V)

Conclusion (ii).

These exposure scenarios are based on several assumptions and on default parameters. The PEC/PNEC ratios for the aquatic compartment are below 1, thus a risk to the aquatic environment is not expected. However, **conclusion (ii)** has to be seen as provisional until possible endocrine effects in fish have been resolved.

Marine risk assessment

In the PBT assessment of the TGD the following criteria are used to decide if a substance should be regarded as a PBT substance.

- P (Persistence): Half-life > 60 days in marine water or > 40 days in freshwater or half-life > 180 days in marine sediment or > 120 days in freshwater sediment.
- B (Bioaccumulation): BCF > 2,000.
- T (Toxicity): Chronic NOEC < 0.01 mg/l or CMR or endocrine disrupting effects.

Substances are selected when they fulfil the criteria for all three inherent properties P, B and T. BBP is regarded as readily biodegradable meeting the 10 d window and the BCF is less than 2000, the B and P criteria are therefore not met.

The toxicity criterion is fulfilled when a substance is classified as carcinogenic (Category 1 and 2), mutagenic (Category 1 and 2) or toxic for reproduction (Category 1, 2 and 3) or when there is evidence of chronic toxicity, identified by the classification T, R45, R46, R48, R60 and R61 or Xn, R48, R62, R63 and R64. BBP is classified as toxic for reproduction (category 2 and 3) see also Section 1.4 and the T criterion is met. However, BBP can not be considered as a PBT-substance since only one out of three criteria is fulfilled.

The monitoring study of Vethaak et al. (2002) includes two sampling locations where the measured concentrations are above the $PNEC_{marine}$ (0.75 µg/l). For the North West region of the Netherlands one sampling location out of eleven different locations is exceeding the present $PNEC_{marine}$ with a measured concentration of 1.0 µg/l BBP. This location (Ems Dollard) is the estuary of the river Ems and several other canals and small streams. According to the TGD estuaries are assumed to be covered by either the inland or the marine risk assessment. Due to

the influence of freshwater this location can therefore be covered by the inland risk assessment resulting in a PEC/PNEC ratio below 1.

The other sampling location is situated in the North Sea 40 km north of the Wadden Sea, showing a measured concentration of 1.8 µg/l BBP. It is the only location out of four different locations in the North Sea exceeding the $PNEC_{marine}$. Two of the four sampling locations are near drilling platforms, where low BBP concentrations (0.20 µg/l) were found. Therefore the high measured concentration of BBP seems to be an outlier and no request for further data is considered necessary at this stage and **conclusion (ii)** seems to be adequate. However, a definite conclusion should await the results of the long-term fish study on reproductive and endocrine effects.

Sediment

The $PNEC_{sediment}$ is 1.72 mg/kg wwt. PEC and PNEC were calculated with the equilibrium partitioning method from the $PNEC_{aquatic}$ therefore the same conclusion as for water can be drawn.

3.3.2 Atmospheric compartment

A PNECair could not be derived from the vapour exposure plant study. Therefore only a qualitative risk assessment is performed. The maximum PECs for air were estimated for flooring at a small site and for sealant formulation with concentrations of BBP of 2.4 and 1.0 µg/m^3. These concentrations are below the highest tested average concentration of 5.7 µg/m^3, for which no effects were observed. Monitoring air concentrations of BBP levels at flooring processing sites and sealant formulation sites are all below 0.4 µg/m^3. A **conclusion (ii)** seems therefore adequate.

3.3.3 Terrestrial compartment

A PNECsoil could not be derived from the earthworm acute toxicity test. The PNECsoil of 1.39 mg/kg wwt was established using the equilibrium partitioning method. For the risk characterisation this value is compared with the PEC in agricultural soil for production, formulation and processing of BBP.

Production and Distribution (Life cycle stage I and II)

A risk assessment of local concentrations through sludge application to soil for life cycle step I and II is not considered necessary (see Section 3.1.2.1)

Processing/Formulation (Life cycle stage III)

Conclusion (ii).

The exposure scenarios for processing of BBP are based on default parameters from the TGD or the ESD “Plastics”. PEC/PNEC ratios for the terrestrial compartment are below 1 for the use categories IIIb, IIId, IIIe, IIIf and IIIg at life cycle stage III. Thus a risk to the terrestrial environment is not expected. However PNECsoil is calculated with the equilibrium partitioning method based on PNECaquatic. However, **conclusion (ii)** has to be seen as provisional until possible endocrine effects in fish have been resolved.

Conclusion (iii).

Three use categories show PEC/PNEC ratios > 1. These are the use categories IIIa (flooring large and small sites), IIIc (PVC coated textiles) and IIIh (formulation of confidential use). The exposure scenarios for IIIa and IIIc are based on the ESD "Plastics". The recently updated ESD "Plastics" has passed the OECD process and is based on the best available information. Further site specific data have not been obtained. The exposure scenario for IIIh is based on information from industry. The PEC/PNEC ratios for the terrestrial compartment are above 1, thus a risk to the terrestrial environment has to be expected.

Conclusion (iii) is based on BBP consumption data from 2004. For 2005 there are only two producers left and industry provided estimations of the expected use volume of BBP for all use categories. These figures are confidential as there are only two producers left.

The total BBP volume used for flooring in 2005 has been further reduced, but the scenarios used in the risk assessment are still relevant. In 2005 it is still valid to use the ESD emission factors for a small site since sites without air treatment have been identified.

Applying the expected use volumes for 2005 to "PVC coated textiles" (IIIc) no risk to soil is to be expected.

Use and Disposal (Life cycle stages IV and V)

Conclusion (ii).

These exposure scenarios are based on several assumptions and on default parameters. The PEC/PNEC ratios for the terrestrial compartment are below 1, thus a risk to the terrestrial environment is not expected. However PNECsoil is calculated with the equilibrium partitioning method based on PNECaquatic. **Conclusion (ii)** has to be seen as provisional until possible endocrine effects in fish have been resolved.

3.3.4 Secondary poisoning

The $PNEC_{oral}$ is determined to 33.3 mg/kg in food for birds and mammals. For the risk characterisation this value is compared with the PEC's in fish and worm for the various exposure scenarios. PEC_{fish} was determined using a BCF of 449 l/kg, while PEC_{worm} was determined using a BCF of 831 l/kg.

All life cycle stages show PEC/PNEC ratios < 1 indicating a **conclusion (ii)**.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

The human population can be exposed to BBP via the workplace, from the use of consumer products and indirectly via the environment.

BBP is present in a large number of end products some of which are available for consumer use. The vast majority of BBP use, > 90%, goes to plasticising of PVC or other polymers. Plasticisers are in general high boiling point compounds that, when incorporated into a polymer, cause a greater flexibility and workability of the material. The BBP plasticized polymeric material has consumer and industrial uses such as flooring, sealants, and paints. A relatively small but significant use is in the food wrap or food packaging area. Furthermore, BBP has been reported at low concentrations in baby equipment and children toys. However, in these products BBP probably occurs as byproduct/impurity and has not been added intentionally to the products (CSTEE, April, 1998).

Because BBP is not chemically bound to the matrix it can migrate from the polymeric material and become available for emissions to other matrices (environmental or biological). BBP can be released from polymer based products during its use or after disposal. The rate of emission is dependent on various factors, for example temperature and physical or mechanical handling of the product.

BBP has been identified in air, water and soil. Human exposure may therefore occur through contact with contaminated air, water, soil, and food. In recent studies urinary phthalate metabolites have been measured in human reference populations. These studies indicated that human exposure to phthalates including BBP is both higher and more common than previously suspected (Blount et al., 2000a; CDC 2001; CDC 2003; Hoppin et al., 2002; Koch et al., 2003; Brock et al., 2002) (see Section 4.1.1.4 for description).

4.1.1.2 Occupational exposure

The main routes of exposure for workers are expected to be by inhalation and dermal contact. Ingestion is not considered to be relevant for occupational exposure.

The exposure scenarios are described without taking into account the possible use and hence influence of personal protective equipment (PPE). PPE is usually intended for use during work operations entailing risk for increased exposure such as repair work, services and maintenance. Information about when and how PPE shall be used should be documented in instructions on safety and handling. If the results from the scenario assessments based on potential exposure indicate that there are risks associated with the handling and use of BBP, then the use of PPE may be one of several methods to decrease the actual risks. Other preferred methods may be organisational and/or technical.

Due to the very low vapour pressure of BBP, evaporation and hence the air concentration of BBP is very low at ambient temperatures. A large range of vapour pressures for BBP (0.00004-0.00799 Pa) is available in the literature. A vapour pressure within this interval corresponds to an air saturation concentration in the range of 0.005-1 mg/m^3. Thus the air concentration of BBP due to evaporation will not exceed 1 mg/m^3 at ambient temperatures. Exposure to BBP vapours via inhalation is expected to be much lower, except in processes where the temperature is elevated. However, if aerosols are generated, then the air concentration of BBP would be higher.

Data based on measurements are to be preferred when assessing exposure in the working atmosphere. However, few measurements regarding workers exposure to BBP have been found in the literature, and in several cases the measurement data are not presented with sufficient details to judge the relevance of the data. Due to the few measurements, all available data have been used, even when the information on the performance of the measurements has been scarce.

Some of the exposure scenarios described in this risk assessment report are lacking measured data. To be able to assess the risk at the workplace the EASE (Estimation and Assessment of Substance Exposure) model has been used to estimate the exposure. In the EASE calculations the parameter for saturated vapour pressure is set to 0.00112 Pa. No measured data are available for dermal exposure.

All values for BBP-concentrations are given in mg/m^3. Data originally reported in units of ppm, have been converted to the units of mg/m^3, by use of the conversion factor 1 ppm = 12.75 mg/m^3.

The following exposure scenarios are considered:

Scenario 1 Production of BBP.

Scenario 2 Industrial use of BBP containing products.

Scenario 3 Professional end use of semi- and end products containing BBP.

BBP may migrate from the finished plastic article, but those losses are limited due to the low vapour pressure and due to the very high compatibility of BBP with the polymers it is used with (PVC, PU, polysulphides, and acrylates). Because a plasticiser's purpose is to plasticise PVC and to do so over long periods of time these compounds are of very low volatility relative to many other commercially used products. BBP is a high priced plasticiser and this is the reason for its limited use.

Occupational exposure limits.

Only a few countries, shown in **Table 4.1**, have defined occupational exposure limits for BBP and/or phthalates.

Table 4.1 Occupationel exposure limits

Country	8-hour TWA	Source of information
United Kingdom	5 mg/m^3 (1)	Health and Safety Executive, UK, 2000 (EH40/2000)
Germany (MAK)		Deutsche Forschungsgemeinschaft (1999). List of MAK and BAT Values (1999). Phthalic acids (all isomers) are on the list of substances from which no MAK value can be established at present.
Sweden	3 mg/m^3 (3, 4)	Arbetarskyddsstyrelsens Författningssamling (1996)
Netherlands	Inhalable:10 mg/m^3 (2) Respirable:5 mg/m^3 (2)	Netherlands. National MAC List (1999)
Ireland	5 mg/m^3	Irish Occupational Exposure Limits (Code of Practice for the Safety, Health and Welfare at Work [Chemical Agents] Regulations, 1997)
Denmark	3 mg/m^3 (3)	Denmark. National Labour Inspectorate. Exposure Limit Values For Substances and Materials. Instruction No. 3.1.0.2. Dec 1996. [Arbejdstilsynet. Grænseværdier for Stoffer og Materialer. At-Anvisning. Nr. 3.1.0.2. Dec 1996.]
Norway	No occupational exposure limit for BBP. Under evaluation. The phthalates DBP and DEHP has an administrative norm of 3 mg/m^3.	Norwegian Labour Inspection Authority. Guidance on administrative norms for pollution of the working athmosphere. May 2001. [Veiledning om administrative normer for forurensning i arbeidsatmosfære. May 2001]

TWA) Time weighted average
1) OEL for BBP
2) OEL for all isomers of phthalates
3) OEL both for BBP and phthalates
4) The short-term exposure limit is set to 5 mg/m^3

4.1.1.2.1 Scenario 1: Production of BBP

Today there are three European plants that produce BBP (see Section 2). The average production days for two of these plants (Bayer and Solutia) are about 300 days/year. The third plant Lonza SPA has not submitted any occupational exposure data to the rapporteur. There is an annual maintenance shutdown, to maintain critical equipment. In addition there are change-overs, in which lines/kettles are emptied, and the reactor is periodically cleaned at regular intervals.

Several synthesis steps are required to produce BBP. The first step is estrification of phthalic anhydride at an elevated temperature (about 90^{o}C) which is done in a closed system where the vapours from the process being condensed and returned to the reactor. After virtually complete estrification the surplus alcohol is driven off under reduced pressure. The next step is neutralisation followed by washing and then filtering. These production steps are performed in closed systems. Purification of BBP is done by vacuum distillation at temperatures ranging from 150 to160^{o}C. The workers will not usually be exposed during the reaction step or during the vacuum distillation, unless the reaction vessel is opened (cleaning and maintenance).

The purified BBP is loaded in a closed system to a reservoir. From this reservoir, BBP is either pumped into tank trucks or in containers of 20 m^3 (distribution 9:1). A smaller portion of BBP is

pumped into smaller containers (1 m^3) and drums (size of 200 l). Road tankers are the major route of transportation, but some containers are transported by ship or railroad.

Exposure can occur during

A. Filling of tank trucks and rail tankers (pumped transfer from bulk storage vessels)

B. Drumming

C. Process sampling (manually sampling)

D. Maintenance and cleaning (e.g. cleaning the tanks in which BBP has been produced, stored or transported)

It is envisaged that both inhalation and dermal exposure can occur during the production of BBP. **Table 4.2** displays exposure data for different steps of the BBP-production.

Table 4.2 Exposure during the production of BBP

Activities/Events	Exposure level			Reference
	Air concentration given in mg/m³ (No. of samples)	Sampling time	Dermal contact mg/cm²/day	
Production	< 0.001-0.001 Mean:< 0.001 (3)	-		Monsanto Plant 7109 8/97-10/97
Truck Loading Loading 3-4 tank trucks	Range: < 0.1 Mean: < 0.1(2)	8-hour TWA		Solutia data plant 0013 11/95-12/95
Loading 1 tank truck	< 0.47-0.61 Mean: 0.54 (2)	40-50-minute samples		
Truck loading			0.1-1(1)	EASE
Sampling	0 –1.3(2)		0-0.1(1)	(E) EASE
Drumming	< 0.001- 0.004 Mean:0.002 (3)	Full shift samples		Monsanto data plant 7109 8/97-10/97
	Emptying the drum line 2.6 (1)	15-minute sample		
	0-1.3(2)2		0.1-1*1*	(E) EASE
Cleaning and maintenance	0-1.3(2)		0-0.1(3)	(E) EASE

1) Non-dispersive use, direct handling, intermittent contact level,
2) Non dispersive use, direct handling, dilute ventilation,
3) Non dispersive use, direct handling, incidental contact level

4.1.1.2.2 Scenario 1A: Filling of tank trucks and rail tankers

The majority of produced BBP is transported from the production site to the customers by tankers. On average about 4 trucks/day are loaded with BBP in a plant (Solutia communication 4/12/99). To fill the road tanker, the worker lowers a dip pipe into the compartment on the tanker, hooks another pipe to the exhaust outlet, and then remotely fills the tanker. This process is done outdoors. The worker waits for the tanker to be filled from an operator shed away from the tanker. Once the tanker is filled, the worker raises the dip pipe, unhooks the exhaust pipe on

the top of the tanker, and then closes the hatches on the tanker dome. This task takes 60-90 minutes (Bayer, 1998b).

Measurements from truck loading are given in **Table 4.2**. Inhalation and dermal exposure may occur during connecting a transfer line in order to pump the substance into the tanks or disconnecting the transfer line after ended work operation.

Inhalation

Evaporation of BBP may occur when handling the transfer lines e.g. when open surfaces appear. BBP has a low vapour pressure, and due to this the workers exposure by inhalation is considered to be low. Measurements are done for tank trucks only.

As seen from **Table 4.2**, two of the measurements were performed with personal sampling while the worker loads 3-4 tank trucks. These measurements are 8-hour TWA samples. The range and mean is < 0.1 mg/m^3. The other measurement has a range from < 0.47 to 0.61 mg/m^3, with a mean of 0.54 mg/m^3. These samples are 50 minutes and 40 minutes in duration. Only one tank truck was filled during this measurement in a very short period of time. It is important to have in mind that, as stated earlier, on average 4 trucks might be loaded during a day. Since the samples taken in this latter measurement were quite high we cannot dismiss the fact that the BBP exposure may be of this level.

Therefore, in lack of more representative data the highest measured mean value of 0.54 mg/m^3 is taken as a reasonable worst case value from this scenario.

Dermal

BBP-exposure of the hands can occur during connecting and/or disconnecting the transfer lines. Assuming non-dispersive use with direct handling and intermittent contact, EASE predicts an exposure of 0.1-1 mg/cm^2/ day (see **Table 4.2**). It is further assumed that palms of both hands will be exposed, which corresponds to an exposed area of 420 cm^2. This results in an exposure of 42 - 420 mg/day. Given the described scenario it is likely that the dermal exposure will be in the upper bound of the interval.

4.1.1.2.3 Scenario 1B: Drumming

An automated system feeds BBP from the reservoir and fills the drums or containers. The drums are on a conveyor belt that stops when an empty drum is on top of a scale to be filled. The filling station is made up of a filling lance which is connected to the BBP reservoir, and which is opened and shut automatically to fill the drums according to weight. Local exhaust ventilation is mounted over the drum or the operation takes place outdoors.

Drumming can be performed either about 8 times per month for a complete shift, or 30 minutes every day dependent on the plant layout and amount BBP produced. The outdoors filling system is done at ambient temperatures. The BBP filling line is a dedicated line, but the blend drum line can contain some BBP and is cleaned twice per month. The blend drum line is blown clean with air or nitrogen and the operator is not normally present in the building (personal communication Solutia 4/12/99).

Inhalation

The worker operating the drumming line can be exposed close to the filling station where BBP may evaporate and be inhaled. The drumming is divided in two different job tasks; the blending operator that drums BBP, and the worker who is emptying the drumming lines.

The measurement from the blending operation gave a mean exposure value of 0.002 mg/m^3 based on three full shift samples (range < 0.001-0.004 mg/m^3). The other task measurement was much higher; 2.6 mg/m^3. The latter measurement is one 15 minutes sample and is not regarded to be representative for an eight hour average for this scenario, but will be taken as a short time value. The EASE model, assuming non-dispersive use, direct handling and dilute ventilation, estimates an exposure level in the range of 0–1.3 mg/m^3.

Because of the small numbers of reported measurements and the spread in measured values, the EASE calculations are used for the determination of the exposure level. Aerosol formation is not expected from this working operation. Therefore 1.0 mg/m^3, the highest exposure level calculated from the vapour pressure at ambient temperature, is considered to be a reasonable worst case value.

Dermal

Handling transfer lines and drums may cause exposure to the hands of liquid BBP. Assuming non-dispersive use, direct handling and intermittent contact (15 minutes twice per month) the EASE estimates the exposure to be 0.1-1 mg /cm^2/day. The palm of both hands may be exposed, corresponding to an area of 420 cm^2, which results in a dermal exposure of 42-420 mg/day.

4.1.1.2.4 Scenario 1C: Process sampling (manual sampling)

For analysis during production, samples of BBP are taken from the production line. A sample bottle of 200 ml is screwed on to a sampling port on the reaction vessel, the valve is opened, and the bottle is filled with the reaction mixture, unscrewed, and capped. The sampling is usually done at ambient temperatures. On average the operator takes 3-5 samples per shift. It takes about 15 minutes or less to complete the sampling.The sampling procedures differ between the plants, but one worker usually performs the sampling.

Inhalation

The process sampling is done at ambient temperature, and different sampling procedures exist for the plants for example the number of samples taken, sampling method etc. There are no measured data for this job task.

The EASE model, assuming non-dispersive use, direct handling and dilute ventilation, estimates an exposure level in the range of 0–1.3 mg/m^3. As for scenario 1B, the highest value calculated from the vapour pressure at ambient temperature, 1.0 mg/m^3, is taken as a reasonable worst case value for this scenario.

Dermal

Dermal contact can occur if liquid BBP leaks during the sampling procedure either from the sampling port or from the sampler flask. By use of the EASE-model, assuming non-dispersive use, direct handling and intermittent contact level, dermal exposure is estimated to be 0.1-1.0 mg/cm^2/day. Assuming that palms of both hands (420 cm^2) may be exposed, this leads to

an exposure of 42-420 mg/day. The highest value is regarded representing a worst case exposure due to sampling.

4.1.1.2.5 Scenario 1D: Cleaning and maintenance

Cleaning

The tanks and vessels are cleaned using high-pressure water. Most vessels and tanks have attached cleaning heads for spraying the water used in cleaning. Some vessels may be cleaned from the outside through the manway. The worker cleaning the tanks can be exposed to residues of BBP, which can give rise to aerosols. The worker can also be potentially exposed to aerosols of BBP. The industry informs that before they start working inside a reaction vessel, the vessel will be cleaned carefully and the workers have to use special safety equipment (Bayer, 1998b). When dismantling the pipes for automatic cleaning, residues of BBP can still be inside the pipes. According to the industry drums are usually pumped empty using a drum pump, drained and sent off site to a drum recycling company.

Maintenance

The industry informs that maintenance (line breaking, pump repair) is done once a week. The line/pump is blown clean with air or nitrogen, and if possible flushed with water. If it is not possible to decontaminate the equipment it is drained and the BBP collected. The worker would perform the maintenance of tankers usually after the tanker has been washed. The risk for BBP contamination would then be if residues of BBP are left in the tank. According to industry the operator wears a chemical protective suit, face shield and chemical protective gloves during the maintenance tasks (Monsanto, 1999).

We do not have any measurements from either of these job tasks. The maintenance of the tank is done after the tank has been cleaned. The maintenance worker will, in worst case, be exposed for residues of BBP that escaped the cleaning process. The same arguments apply for service workers.

Inhalation

The cleaning of the equipment may create BBP containing aerosols, and the worker can inhale these aerosols. The amount of BBP-aerosols this task can give rise to is limited to residues of BBP in the tank. EASE is not suitable for estimating aerosol exposure, and hence no estimated value is given.

When estimating the inhalation exposure for the maintenance task, it is assumed non-dispersive use with direct handling and dilutes exhaust ventilation. This gives an EASE prediction of 0-1.3 mg/m^3. As for scenario 1A and B, the highest value calculated from the vapour pressure at ambient temperature, 1.0 mg/m^3 is taken as a reasonable worst case value for this scenario.

Dermal

During maintenance of the tanks it is assumed that both hands can be exposed. According to industry the operator wears special safety equipment during both the cleaning and maintenance tasks. Exposure of both hands corresponds to an exposed area of 840 cm^2. EASE estimation of this scenario given the parameters; non-dispersive use with direct handling and incidental contact, gives an exposure of 0-0.1 mg/cm^2/day, which results in a dermal exposure of

0-84 mg/day. The exposure value is a rather cautious approach since a dilution factor is not taken into consideration and the exposure is probably much lower than the highest EASE estimate.

4.1.1.2.6 Conclusion: production of BBP

In **Table 4.3** it is shown that the highest exposure occurs during drumming. Because of few measurements we will keep the EASE-value of 1.0 mg/m^3 as a reasonable worst case value, together with the measured value of 2.6 mg/m^3 as a short term value. The highest dermal exposure is considered to be 420 mg/day.

Table 4.3 Summary of exposure levels in the production of BBP

Workplace operation	Exposure by inhalation (mg/m^3)	Dermal exposure (mg/day)
Scenario 1A: Filling of road- and rail tankers	Reasonable worst case: 0.54	420 (E)
Scenario 1B: Drumming	Reasonable worst case value: 1.0 (E) Short term value: 2.6	420 (E)
Scenario 1C: Process sampling (manually sampling)	Reasonable worst case value: 1.0 (E)	420 (E)
Scenario 1D: Cleaning and maintenance	Reasonable worst case value: 1.0 (E)	84 (E)

E) Estimated by EASE

4.1.1.2.7 Scenario 2: Industrial use of BBP-containing products

BBP is used as plasticisers in various formulations: plastisol coating (flooring, floats), sealants, grouting agents, leather and cloth polymer, film polymer, coating and inks PVC polymer, adhesives polymer, general PVC, and other non polymer products (see Section 2). BBP has an impact on the flexibility property of the products. BBP is one of the highest priced phthalates and therefore other phthalates are used when possible. The main area where BBP is used as a plasticizer is in PVC products.

The worker may be exposed during manual handling of BBP, and this can occur when small amounts of BBP are added to the mixers in the polymer industry. When batches are being drummed, products containing a percentage of BBP may also be manually handled.

Two steps are identified for incorporating BBP in products: compounding or mixing of products containing BBP and processing of products containing BBP. Compounding or mixing is the process where additives are incorporated into plastics. There are several processes for the incorporation of BBP into polymers. These may be classified as closed (example planetary mixers, change-can mixers and dough mixers) or partially open processes (example two roll mills, extruders). Processing of products is when the BBP containing paste/compound is formed into products. These processes may also be classified as closed conversion processes (example extrusion, injection moulding, compression moulding) and partially open conversion process (example film extrusion, plasticol coating, calendering). Evaporation of the plasticizers can occur when the process is at its elevated temperatures, and aerosols may be formed when the process is open. The scenarios are divided according to the type of processing:

Scenario 2A: Plastisol coating
Scenario 2A1: Flooring with the plastisol spread coating process
Scenario 2A2: Processing of PVC floats

Scenario 2B: Processing of sealants

Scenario 2C: Calendering and extrusion of PVC polymers
Scenario 2C1: Flooring with the calendering process
Scenario 2C2: Films with the extrusion process

4.1.1.2.8 Scenario 2A: Plastisol coating

The major use of BBP (approximately 60%) is in the plastisol spread coating process as a plasticizer in the production of PVC coated sheet flooring. A small volume (< 5%) is used in the production of calendered floor tiles. The BBP content in plastisols is typically 25-35% of the total plasticiser (see Tucker and De Bie, 1998).

Exposure is possible during the mixing step of the process. Before the conversion or forming of the products can occur, the compound has to be fed from the mixer into the forming step. The transition can be done differently; either in an open or a closed transfer.

The exposure during the closed systems will be when sampling, cleaning and maintenance is being performed. When the process is operating on a normal basis, exposure to the worker is expected to be low. Also, BBP is typically pumped directly from the bulk storage tanks and metered in a closed system to shear mixers used to produce the plastisol. Normally there is no direct skin contact by operators (see Tucker and De Bie, 1998). Sources of exposure related to further compounding are: opening of the mixer, and the exit of the extruder.

4.1.1.2.9 Scenario 2A1: Flooring with the plastisol spread coating process

The layout of modern European PVC flooring production plants using the plastisol spread coating process is primarily the “discontinuous” type (Tucker and DeBie, 1998). BBP is pumped directly from the bulk storage tanks and metered into high shear mixers (closed) to produce the plastisol. Key process steps involve open coating with plastisol on the flooring substrate and heating of the coated “web” to gel and fully fuse the PVC. Plastisol temperature is at, or just above, ambient temperature.

The worker may be exposed during the coating process that is an open process. If the reeling of the flooring is not enclosed or the area is not efficiently ventilated, the worker can also be exposed. However, the low vapour pressure of BBP minimises airborne BBP in the working atmosphere.

Measured data (personal samples) from flooring production plants are given in **Table 4.4** (Solutia, 1999c; Tucker and De Bie, 1998), while stationary (area) samples are given in **Table 4.5** (Tucker and De Bie, 1998). The workplace exposure to BBP was measured during 1995-1997. The stationary samples were taken at locations chosen to represent typical crossection of the exposure to airborne BBP, including representative areas where low and high temperatures were applied, over large surfaces, and «open» processes along the production line.

Table 4.4 Exposure of BBP (personal samples) during plastisol coating (scenario 2A1) at 3 Flooring Plants (Solutia, 1999c; Tucker and DeBie, 1998)

Occupational task	Occupational Exposure Data full shift (No. of samples)	Absorbent/ desorbent	Comments	Reference
Pre-Coat Station	0.015 mg/m^3 (1)	Tenax/ Thermal	Pre-coat-, foaming-, oven- and glass fiber feed roller area	Solutia monitoring at Plant 1, September 1995
Clear-coat station	< 0.01 mg/m^3 (1)		100% of Shift Plant Zone Back Print/Clear Coat	
Oven Exit Reel-up Station	< 0.01 mg/m^3 (1)		50% of shift Control Panels Main and Reel-up 50% of shift PC Workstation Quality Check Area.	
Drumline	0.035 mg/m^3 (1) 0.053 mg/m^3 (1)	Tenax/ Thermal	Machine Operator Partial period sample	Solutia monitoring at Plant 2, November 1995
CFS Line	0.007-0.013 mg/m^3 Mean: 0.010 mg/m^3 (2)		Machine Operator	
Laboratory	0.011 mg/m^3 (1)		Lab Operator	
Compounding	< 0.0022-0.0057 mg/m^3 Mean: 0.004 mg/m^3 (2)	Tenax/ Hexane	Foam Mixer and Top Coat Operators	Solutia Monitoring at Plant 3, May 1997
Top Foam	< 0.002-0.0025 mg/m^3 Mean: 0.0023 mg/m^3 (3)		Knife Coating, Reverse Roll Coater and Roll Winder Operators	
Wear Layer and Backing	< 0.0022-0.0028 mg/m^3 Mean: 0.0023 mg/m^3 (4)		Reverse Roll Coater, Oven, Knife Coating, and End of Line Operators	

Table 4.5 Exposure to BBP (stationary samples) in flooring production plants (Tucker and DeBie, 1998)

Plant no.	Occupational Exposure Detain mg/m^3 (No of samples)	Sampling information		
		Absorbent/ desorbent	Aanalytical method	Sampling time
1	< 0.1-1.2 Mean: 0.24 (8)	Charcoal CS_2	GC/FID+ MS	not given
2	< 0.01-0.015 Mean: 0.01 (3)	Charcoal CS_2	GC/FID + MS	not given
3	0.0012-0.0070 Mean: 0.0024 (13)	Tenax tubes Hexane	GC/FID	8 hours

Inhalation

Forced ventilation of the compounding areas and extraction of vapour from the mixing vessels and vacuum de-aeration equipment is ducted to emissions control systems, reducing the level of worker atmospheric exposure. Personal and stationary samples were taken in these areas (see **Table 4.4** and **Table 4.5**).

Measured air concentrations given in **Table 4.4**, have been interpreted as a cross-section of the exposure, and hence the means are considered to represent the 8-hours TWA values. As the sampling has been conducted at sites of high and of low exposure, the results should include the sites of the highest exposures, as well. The highest measured stationary concentration is 1.2 mg/m^3, which is considered to represent the exposure for a reasonable worst case scenario, i.e. the exposure for 8 hour at the site of highest exposure. 8 area samples were taken. 7 of the measurements were < 0.1 mg/m^3, while 1 sample was 1.2 mg/m^3. The highest personal full shift measured value, 0.035 mg/m^3 was found at the drumline, which is taken as a typical value.

Dermal

No direct skin contact by operators was observed in this area of the process during any of the surveys (see Tucker and De Bie, 1998). However, the greatest possibility of operator contact with plastisol/plasticisers in the flooring production lines evaluated are at the knife coating head and the reverse roll-coater, where a significant area of plastisol surface is typically exposed. Automated on-line control of both the coating head and roll-coater, and automatic flow control for plastisol supply minimises the need for operator intervention. The plastisol is not sprayed, but poured. Finished flooring is cooled and put on rolls fully automatically.

Since BBP is incorporated in a matrix, it is not possible to use the EASE model to estimate the dermal exposure. The dermal exposure is expected to be low, but in case of dermal contact the palm of both hands can be exposed (420 cm^2).

4.1.1.2.10 Scenario 2A2: Processing of PVC floats

BBP is used in small PVC floating items; floats or ball floats, for fishing nets. The first step in the production is the mixing of BBP with the other raw materials. 10 litres of BBP is added manually, once a day, and the operation lasts for about 15 minutes at the Norwegian plant where the measurements are from. The BBP, PVC, and other ingredients are weighed openly, and emptied into the mixer. The mixer is then closed. This is a dispersion mixer or an intensive vortex action mixer.

The blend is then transferred in pipelines with pressure air to the compression moulding step. The moulding is performed by two workers standing on both ends of a carousel, one filling the mould with a filling pistol directly connected to the pipeline, and the other worker takes out the bowl-shaped PVC with tongs after it has passed the heating area. These workers will be prone to exposure, especially the worker filling the moulds. The carousel workers wear PPE.

The stacked bowls are put in a hydraulic press with heat in a closed process. This foaming process creates large cells which are crossbinded in the PVC. This is done 4-5 times per shift. The newly shaped PVC-balls are allowed to cool down for 2 hours before taken out, and cooled even further down in a water bath, which is fully self-contained to equalise the pressure.

After the setting period of 2 days the PVC-balls are shaped to their final shape by expansion in a boiling water bath for 2 hours. During the extrusion steam is emitted and can produce aerosols

with PVC residue, hence also BBP. After the extrusion the floating balls are taken out of the mould and packed for shipping.

Table 4.6 The exposure to BBP in a plant processing small PVC items (scenario 2A2). The data are from the National Institute of Occupational Health, Oslo, 1999

Activities/events	Exposure level	
	Air concentration in mg/m^3 mean (range) 1) [No of samples]	Dermal contact mg/cm^2/day
Mixing raw materials	0.00144 (0.00034-0.00255) [2]	0.1-1 *(E)*
Mixing raw materials/ filling/hardening area	0.00488 (0.00404-0.00572) [2]	0.1-1 *(E)*
Filling	0.00124 (0.00063-0.00178) [3]	0.1-1*(E)*
Filling moulds	0.00195 (0.00057-0.00292) [3]	0.1-1*(E)*
Filling/hardening area	0.00175 (0.00024-0.00384) [7]	2)
Filling/painting/hardening area	0.00141 (0.00141) [1]	2)
Filling/expanding area	0.00175 (0.00109-0.00242) [2]	2)
Packing	0.00078 (0.00071-0.00086) [2]	2)
Expanding area/packing	0.00134 (0.00038-0.00302) [8]	2)

All samples are personal.
E) Input to EASE: direct handling, intermittent contact and non dispersive use,
1) The absorption material used in the sampling was glass fibre and cellulose acetate, sampling time was 4 hours, and analytical method was GC.
2) The combined activities cannot be estimated with EASE because the time of distribution between the two is not known. In addition BBP is enclosed in a matrix.

Inhalation

The worker can be exposed during the manually weighing and loading of the mixer, and as seen from **Table 4.6** the highest measured value (> 0.005 mg/m^3) was found in the area for mixing raw materials, filling and hardening. The worker with the filling pistol filling the moulds on the carousel can also be exposed. In addition, the other worker at the carousel will be operating close to the heated area and hence be exposed to the emitting fumes. The heating press is a closed system, and the exposure to the worker performing this task is negligible.

The resting period to the PVC-bowls may give off gases, but this is stacked in a ventilated room. The only possible exposure is when the worker rolls the racks of PVC-bowls in or out of this area, and this exposure is thought to be negligible. The boiling water-bath gives off steam that can contain BBP as an aerosol. The worker handling this task can be exposed to BBP.

The typical exposure level was < 0.005 mg/m^3.

Dermal

Dermal contact with BBP is likely to occur when manually weighing and loading the mixer. The EASE estimate with direct handling, intermittent contact and non-dispersive use gave 0.1-1 mg/cm^2/day. Assuming both hands being exposed this leads to an exposure of 84-840 mg/day.

Dermal exposure for the combined activities (see **Table 4.6**) cannot be estimated with EASE because the time of distribution between the two is not known. In addition BBP is enclosed in a matrix in the second step of the production.

4.1.1.2.11 Scenario 2B: Processing of sealing compounds/fillers

Different types of sealants are produced with BBP; acrylic-based sealants, polyurethane foam sealants, and polysulfide sealants. About a quarter of known production volume of BBP is used as a plasticizer in polysulfide sealants. These types of sealants are used to provide airtight fittings of thermopane double glass windows. The BBP content in the polysulfide sealant is typically 15%. No detailed information/descriptions about the processing of the sealants have been available. The formulation of the sealant may be done with a closed or an open compounding process. In the open process, the worker may be exposed during the mixing of ingredients. Before the conversion or forming of the sealant can occur, the compound has to be fed from the mixer into the forming step. After mixing, the mixer is opened and the vessel is rolled to a press where the sealant is pushed to the packaging line (closed system). The sealant is packed in plastic cartridges for home and industrial use. Sealants are prepared as ready to use.

Measurements from sealant production plants are scarce. A workplace measurement from one plant has been done, including 4 samples of 20-40 minutes (3 stationary and 1 personal). The stationary samples were close to the reaction vessel, between the dissolver during addition of the substance, and close to the press.The samples taken showed a mean BBP air concentration of < 0.1 mg/m^3 (range < 0.1 mg/m^3) (Bayer, 1998a).

According to the industry one of the leading manufacturers of polysulphide sealants (Solutia, 1999e) sampled a worst case formulation and measured BBP at the blending machine where the sealant was being maintained in a closed system at an elevated temperature of 140°C and contained a 10% (weight) BBP. The exposure value was 0.47 mg/m^3 over an eight hour period with an exposure time of 4 hours for the person operating the blender. This application at elevated temperature is atypical since sealants are typically manufactured without the addition of heat. This formulation is no longer being produced, but still serves as a worst case reference for the company. The same manufacturer also sampled BBP at the drumming station that pumps the sealant into the lined drums in an open system. The exposure was measured to be < 0.1 mg/m^3.

Inhalation

Reported air concentrations are < 0.1 mg/m^3. According to industry the sealants are made using a low temperature process. It would therefore be expected that the exposure is less than the plastisol applications in flooring. The flooring data (Scenario 2A1: Flooring with the plastisol spread coating process) is therefore taken as a worst case for Scenario 2B.

Dermal

The formulation of sealants may be done with an open compounding process. Since there is no detailed description available, it seems reasonable to compare dermal exposure with the scenario of processing PVC floats (Scenario 2A2) where concentrated BBP is added manually during the mixing of ingredients. As a reasonable worst case an EASE estimate of dermal exposure of 84-840mg/day is assumed with the same parameters as given in Scenario 2A2.

4.1.1.2.12 Scenario 2C: Calendering and extrusion of PVC polymers

PVC polymers may undergo many different processing steps. Among other products, BBP is used in flooring and films with the calendering and extrusion processes.

4.1.1.2.13 Scenario 2C1: Flooring with the calendering process

The making of flooring commences with preblending and mixing. The mixing is done with intensive powder mixers under elevated temperatures. These types of mixers are closed mixtures. The resulting preheated compound is fed onto a calender, which is composed of a number of rolls. These rolls compress the melt into a thin layer, which make up the flooring. Adjusting the distance between the rolls, different gauges of sheeting become possible.

Calendering takes place at elevated temperatures, 150-180^{o}C. Sources of exposure during the flooring process may be related to opening of the processing vessel, film travelling over the reels, the exit of the oven, exit of the extruder, extrusion coating, when heat is applied to the extruded sheet, curing and above the calender mill.

Two air concentration measurements are published in the literature. Nielsen et al. (1985) measured exposure of different phthalates, mostly DIDP, DEHP and BBP. Here BBP was less then 10% of the sum of phthalic acid esters. Hagmar et al. (1990) also performed exposure measurements of different phthalates, mainly DEHP, DIDP and BBP. Measurements were taken every calender year 1945-1980. The exposure levels were stable during the whole study period.

ECPI (European Council for Plasticisers and Intermediates) has submitted a report from 1996 on phthalate esters used in plasticised PVC. The report points out the production of sheet and films by calendering or spread coating as the processing technique giving rise to the highest exposure levels. The evaporation of plasticisers may be visible as fumes, and these fumes are extracted by air exhaust systems in order to ensure that the plasticisers' concentration in the work place does not exceed an acceptable level of 5-10 mg/m^3. The report does not give any measurements on the level of exposure.

Measurements from US tile manufacturing using the calendering process (Solutia, 1999d) have been submitted. In addition, descriptions of the different job tasks have been given. The calender operator monitors the control panel for the water bath, waxing, and buffing portion of the line. The furnish mill operator monitors the control panel and cuts a periodic QC (quality control)

sample for the tile. The mill operator monitors the control panel and television monitors of the process. Most process changes can be made through the control panel. The mixer operator runs a motorised fork truck that takes premix from the rotary mixers and dumps it into the feed hopper for the calendering operation.

Measurements and the dermal exposure estimated with EASE are presented in **Table 4.7**.

Table 4.7 Exposure to BBP during processing PVC by the calendering process (Scenario 2C1)

Activities/events	Exposure level		Reference
	Air concentration mg/m^3 mean (range) (no of samples)	Dermal mg/cm^2/day	
Calendar operators	2,0 (1,0-2,8) (12)	-	Nielsen et al. (1985) [1)]
Calendar operators/ machine attendants	0.4 (0.1 - 0.8) (16)	-	The reported exposure levels are mixed plasticizer concentrations (DEHP, DIDP and BBP). The content of BBP was less than 10%.
Machine attendants	0.2 (0.1 - 0.2) (8)	-	
Repair men	0.3 (0.1 - 0.3) (8)	-	Sampling time: 2 hours
Mixing workers	0.02 (0.01- 0.02) (8)	-	
Others	0.1 (0.1-0.3) (44)	-	
Calendar operations	0.5-3 (Not known)	-	Hagmar et al. (1990) [2)]
Workers in the mixing departments and machine attendants	0.1-0,5 (Not known)	-	The reported exposure levels are mixed plasticizer concentrations (DEHP, DIDP and BBP).
Quality inspectors and packing personnel	$\leq$ 0.1 (Not known)	-	Number of samples and sampling time were not given.
Calender Operator	0.16 (0.031-0.42) [10]	-	Solutia (1999d)
Furnish Mill Operator	0.075 (0.073-0.078 [4]	-	TWA samples
Mill Operator	0.072 (0.053-0.091) [2]	-	
Mixer Operator	0.4 (0.19-0.61) [2]	-	
Calendering	0.29-0.33 (2) 2 hour sample		BG Chemie (1994) (cited in the Risk Assessment Report of Dibutyl phthalate)

Table 4.7 continued overleaf

Table 4.7 continued Exposure to BBP during processing PVC by the calendering process (Scenario 2C1)

Activities/events	Exposure level		Reference
	Air concentration mg/m³ mean (range) (No. of samples)	Dermal mg/cm²/day	
Mixing		0.1-1	*(E)*[3]
Cleaning, service and maintenance		0-0.1	*(E)*[3]

E) Estimated by use of EASE
1) Personal sampling, 2 hours of sampling time, on glassfiber filter
2) No information on type, duration or method of sampling
3) Direct handling, intermittent contact and non-dispersive use. EASE is not applicable and hence not used where BBP is locked in a matrix

Inhalation

The highest levels of BBP in the working atmosphere have been measured where the calendering and mixing takes place. Due to the elevated temperature and the more open process, the chance of exposure via inhalation is high. Local exhaust ventilation is provided to reduce exposure potential. The highest measured values reported (see **Table 4.7**) is 2.8 mg/m^3 (Nielsen et al. 1985), where the content of BBP was less than 10% of the total phthalates, and 3.0 mg/m^3 (Hagmar et al. 1990) for a mixture of phthalates. Solutia has measured a mean value of 0.4 mg/m^3 BBP from a tile manufacturing plant using the calendering process. This value was based on 2 personal samples from a mixer operator.

In lack of other measurements and since EASE is not suitable for aerosol-exposure, the value 3 mg/m^3 (Hagmar et al. 1990) is taken as a reasonable worst case, even if this is a mixed exposure of phthalates.There is not enough data available to underpin the distribution of BBP and other phthalates in the working atmosphere. A typical value of 0.4 mg/m^3 is set based on a mean value measured by Solutia (1990d). These values are carried forward for further evaluation. No short term values have been reported.

Dermal

According to the industry the charging of liquid BBP is done automatically with no manual handling for calendering production. Considering that dermal contact with BBP is likely to occur when manually weighing and loading the mixer, the EASE estimate with direct handling, intermittent contact and non-dispersive use gives an exposure of 0.1-1 mg/cm^2/day. Assuming palms of both hands being exposed this leads to exposure of 42-420 mg/day. BBP is enclosed in a matrix and hence EASE estimation is not possible for calendering and mill operators.

It is assumed that cleaning may cause exposure similar to the scenario of cleaning and maintenance for production i.e. 0-0.1 mg/cm^2/day. Furthermore, assuming exposure of both hands (80 cm^2) this results in a dermal exposure of 0-84 mg/day.

4.1.1.2.14 Scenario 2C2: Processing of films

The compounding process when making films takes place in the extruder. This is a partially open process. Blends of polymer, additives and/or master batch are mixed either in the hopper or in the tumblers and then fed into an extruder comprising one or two screws. These both shear the material and transport it through a heating regime. The resultant compound can be converted

directly into an extrudate as a film. The film is cooled by travelling upwards over a vertical bubble of air before being taken up onto reels (blown film). Film may also be extruded through a slit die; this approaches a closed process since the extrudate is quenched immediately after it leaves the die.

Volatile emissions may be produced and these are vented at various points in the extruder barrel. The worker may be exposed if the vents are opened, while the film is being blown with air, and during the reeling of the film. Some measurements from the film process are given in **Table 4.8**.

Table 4.8 The processing of film polymers containing BBP (scenario 2C2) at Solutia Monitoring plant 7289, June 1996 (Solutia, 1999c)

Job task	Occupational exposure data (mg/m³) Full shift (no. of samples)	Comments
Field man	< 0.03 (1) < 0.03 1) (1)	According to the industry the operator is responsible for running the overall extruder line.
Feeding Extruder	< 0.03 (1)	According to the industry this is a short-term task that lasts about 30 minutes when the extruder is starting up
Pulling film	< 0.03 Mean :< 0.03 (2)	According to the industry this is a short-term task < 30 minutes of manually pulling film during start-up of the extruder line.

1) Partial period sample.

Inhalation

From the submitted measurements, the exposure is below 0.03 mg/m^3.

Dermal

Since BBP is incorporated in a film, it is not possible to use the EASE model to estimate the dermal exposure. The dermal exposure is expected to be low, but in case of dermal contact the palm of both hands can be exposed (420 cm^2).

4.1.1.2.15 Conclusion: industrial use of BBP-containing products

As seen from the table below the calendering process (2C1) seems to represent the highest exposure level for inhalation. Dermal exposure may also be a problem during mixing of raw materials in 2A2. A reasonable worst case value of 3.0 mg/m^3 and a typical value of 0.4 mg/m^3 (Scenario 2C1) are taken forward to RC, together with a dermal exposure of 420 mg/day from the same scenario.

Table 4.9 Summary of exposure levels for the industrial use of BBP-containing products.

Workplace operation	Exposure by inhalation (mg/m^3)	Dermal exposure (mg/day)
2A1: Flooring with the plastisol spread coating process	Typical value: 0.035 Reasonable worst case: 1.2	–
2A2: Processing of PVC floats	Typical value: < 0.005	840 (E)
2B: Processing of sealants	< 0.1 The exposure under scenario 2B is less than in scenario 2A1	840 (E)
2C1: Flooring with the calendering process	Typical value: 0.4 *Reasonable worst case: 3.0*	420 (E)
2C2: Processing of films with the extrusion process	< 0.03	–

E) Estimated by EASE

4.1.1.2.16 Scenario 3: Professional end-use of products containing BBP

BBP is reported to be used in many types of products. Leather and cloth polymers are being produced with the same coating technology as flooring, and hence the data for plastisol coating can apply to e.g. handling of raw materials, mixing and processing. The use of leather and cloth polymer is very versatile, and hence no specific scenario is given. Another reported use of BBP is in coating and inks. From the Norwegian Product Registry it is reported that BBP is used in inks in the graphic industry, but in very small amounts. BBP is also known to be used in PVC polymer based car care products, dyes, paints and lacquers, grouting agents, paper and board. BBP is also used as a plasticiser in some adhesives polymers. Other material where BBP is used is in hard plastic and non-polymer products such as cosmetics.

Because of the low vapour pressure of BBP, the inhalation exposure will be negligible during non-aerosol forming activities (e.g. normal use of paint, sealants). On the other hand the exposure can be significant with the use of products that involves elevated temperature or spraying technique, which can generate aerosols. The exposure levels of aerosol forming processes during industrial use may be comparable with the exposure levels of aerosol forming end uses. We will in this document describe the occupational exposure for two scenarios for the professional end use of semi- and end products containing BBP, namely the use of sealants in glass insulation and grouting/filling agents.

4.1.1.2.17 Scenario 3A: Use of polysulfide sealants for glass insulation

The sealant is used for providing airtight fittings for thermo pane double glass windows.

The tightening process takes place at the window production sites. In the case of mono component sealants this is done immediately at ambient temperatures using either a pneumatic or hand operated “gun”. For two components, the final user has to mix the base and the catalyst components. Today this is very seldomly hand mixed. Usually it is mixed in line in the extruding “gun”.

Most of the sealant producers supply the sealants in standardised containers that are locked into presses, which pistons expel and meter the components to an in-line mixer, which feeds in an extrusion nozzle. The sealant is applied in the groove formed between two glass panels and an Aluminium spacer. It is extruded in place usually by a robot in open air at ambient temperature.

The two parts of the sealant have generally no contact with the workers other than unplugging of the extrusion nozzles. In the conditions of application no gases or aerosols are produced. An increase of Argon filled windows is produced, replacing dry air filled windows. Producing the Argon filled windows, the sealant is applied in a closed container, which is roof-vented when the operation is completed. In its final use, on the window, the sealant is out of reach of the user. BBP as the other ingredients remain compatible and do not vaporise or migrate anywhere. The contact the workers have with the sealant is therefore limited. There are no measurements for this scenario, but an EASE estimate has been performed.

Inhalation

The tightening occurs at ambient temperature. BBP has a low vapour pressure, the temperature is not elevated, and BBP is included in a matrix. Evaporation and airborne exposure to workers is therefore considered to be negligible.

Dermal

Assuming direct handling and incidental contact level, EASE estimates the skin exposure to be 0-0.1 mg/cm^2/day for the palm of both hands, corresponding to an area of 420 cm^2 which results in a dermal exposure of 0-42 mg/day.

4.1.1.2.18 Scenario 3B: Use of polyurethane sealants/fillers/grouting agent

A minor application of BBP is as a component in the curing agent of expanding polyurethane used for grouting operations in tunnels/rocks to prevent water leakage. In Norway the application of this grouting agent is very limited and only small volumes (batches of < 10 l) is handled each time. These types of polyurethane sealants are also used when filling cracks in construction concrete. It is not known to what extent these types of fillers are used, but it seems like the use is increasing. The use pattern for injecting into tunnels/rocks or into concrete is similar.

The sealant consists of a 1-component prepolymerized isocyanides including 10-20 weight% BBP and an accelerator containing selected amines and 60-100 weight% BBP. The quantity of accelerator added to the prepolymer is in the range of 1-10%, usually 1-2%. An amount of 1-2% of the accelerator implies a curing time of 20-30 minutes, whereas 10% implies a curing time of 1-2 minutes (according to information from the Norwegian supplier of the product).

The mixing process is manually performed, where the chemicals are poured into a mixing battery and typically stirred with a stick. When mixed, the hardening process is initiated, and the preparation is at once injected into cracks in the concrete/rock to restrict the flow of water through a structure. The injection is done with small electric pumps (of volume 1 - 10 l) at high pressure. BBP is incorporated in the polyurethane matrix after the reaction is finished (about 20-30 minutes). The redundant expanded polyurethane foam that oozes out of the wall is cut off. During this cutting process the friction of the cutting-knife through the hardened foam may create some evaporation including BBP.

Inhalation

This process does not create high temperatures, and due to the low vapour pressure of BBP, exposure to pure vapours is considered to be negligible.

Due to the short time required for the mixing of the monomer and the curing agent, it is considered that the exposure for this scenario is much lower than the exposure during the mixing of raw materials described under scenario 2A2: processing of PVC floats. The mean air concentration was for the PVC floats scenario measured to be < 0.005 mg/m^3.

During the mixing process, it is not likely that BBP aerosols will be generated. However, in the injection step aerosols can be produced when high-pressure "guns" are used. The temperature and the humidity when applying the sealant will also play a role in the amount of aerosols produced. The cutting of the redundant expanded polyurethane foam that oozes out of the wall may also create some evaporation including BBP due to the friction of the cutting-knife. It is not known to what extent these steps will give exposure to BBP.

Dermal

As for inhalation, dermal exposure is also probable during the mixing process (splash-hands), the injection (aerosol-face/hands), and cutting of redundant expanded polyurethane foam. The same uncertainty applies for dermal exposure.

In the mixing step the workers pour a volume of < 0.5 l of the curing agent (constituting of more than 60% BBP) into the monomer. This simple operation requires only a few seconds. Due to this, it is considered that the dermal exposure will be much lower than the value estimated by EASE (0.1-1 mg/cm^2/day) for the mixing process in the processing of PVC floats (Scenario 2A2) i.e. << 84-840 mg/day.

The hardening process initiates immediately after adding the curing agent to the monomer. As the absorption rate of BBP is low (0.15-0.3 µg/cm^2 · minute) (See the risk characterisation Section) there will be no time to any significant absorption of BBP when the skin is exposed to the liquid blend.

4.1.1.2.19 Conclusion: professional end-use of semi- and end products containing BBP

No measured exposure data are available for this scenario. For the use of polysufide sealants for glass insulation, dermal exposure has been estimated to 42 mg/day with the EASE model. The exposure level will most probably be low for handling end products, but aerosol-forming activities cannot be excluded. As the exposure levels of aerosol forming processes during industrial use may be comparable with the exposure levels of aerosol forming end uses, the same values given for Scenario 2 is taken forward to the risk characterisation section for Scenario 3.

Table 4.10 Summary of exposure levels for the professional end-use of semi- and endproducts containing BBP

Workplace operation	Inhalation (mg/m^3)	Dermal (mg/day)
Scenario 3 (the values are taken form scenario 2, see text above)	Less than for scenario 2C1: Typical value: < 0.4 Reasonable worst case: < 3.0	Less than for scenario 2C1: < 420 (E)
3A: Use of polysulfide sealants for glass insulation	Negligible	0-42 (E)

Table 4.10 continued overleaf

Table 4.10 continued Summary of exposure levels for the professional end-use of semi- and endproducts containing BBP

Workplace operation	Inhalation (mg/m³)	Dermal (mg/day)
3B: Use of polyurethane sealants/fillers/grouting agent	Typical value: < 0.005	<< 84-840 (E)*

E) Estimated by EASE,
* It is assumed that the dermal exposure in 3B is much lower than the exposure estimated for Scenario 2A2. i.e. << 84-840 mg/day (E)

4.1.1.2.20 Overall conclusion for all scenarios

Table 4.11 Summary of exposure levels for occupational exposure of BBP. The values taken forward to the risk characterisation are emphasised

Workplace operation	Inhalation (mg/m³)	Dermal (mg/day)
Scenario 1: Production of BBP		
1A: Filling of and rail tankers	Reasonable worst case: 0.54	420 *(E)*
1B: Drumming	Reasonable worst case: 1.0 Short term value: 2.6	420 *(E)*
1C: Process sampling (manually)	Reasonable worst case value: 1.0 (E)	420 *(E)*
1D: Cleaning and maintenance	Reasonable worst case value: 1.0 (E)	84 *(E)*
Scenario 2: Industrial use of BBP-containing products		
2A1: Flooring with the plastisol spread coating process	Typical value: 0.035 Reasonable worst case: 1.2	_
2A2: Processing of PVC floats	Typical value: < 0.005	840 *(E)*
2B: Processing of sealants	< 0.1 The exposure under scenario 2B is less than for scenario 2A1	840(E)
2C1: Flooring with the calendering process	Typical value: 0.4 Reasonable worst case: 3.0	420 *(E)*
2C2: Processing of films with the extrusion process	< 0.03	-
Scenario 3: Professional end use of semi- and end products containing BBP		
Scenario 3 (values taken from scenario 2)	Typical value: 0.4 Reasonable worst case: 3.0 From scenario 2C1	420 *(E)* From scenario 2C1 840 *(E)* From scenario 2A2
3A: Use of polysulfide sealants for glass insulation	Negligible	0-42 *(E)*
3B: Use of polyurethane sealants/fillers/grouting agent	Typical value: < 0.005	<< 84-840 *(E)*

E) Estimated by EASE

4.1.1.3 Consumer exposure

BBP is used in several products; see Section 4.1.1.1, some of which are available to consumers. BBP alone is not available to consumers as a product.

In a Norwegian Survey from 1996 BBP was found in 5 products available to consumers (sealing compounds, plastics, adhesives, car care products and cosmetics) accounting for about 12 tonnes/year. In 3 of the products, adhesives, plastics and sealants the concentration of BBP was 1-5%, 5 – 30% and 1-30% (SFT report 96:21, 1996). According to information from the Norwegian Food Control Authority BBP is no longer used in cosmetics in Norway. In Sweden BBP has been found in 79 products of which 12 are available to consumers (National Chemical Inspectorate, Sweden, 1997).

To cover the consumers' exposure to BBP via the use of consumer products containing BBP, the following scenarios are considered and include exposures through food, indoor air, and baby equipment/children toys.

Three exposure scenarios are considered referring to the above mentioned uses of BBP;

I: Food and food packaging

II: Indoor air

III: Baby equipment/children toys

4.1.1.3.1 Scenario I: Food and food packaging.

Plasticizers are substances that have been used to give flexibility to the food packing material, and one of the known plastisizer is BBP. The typical range for this application would be 23-28% BBP in the PVC. Consumer exposure to BBP may occur via the consumption of food to which BBP may have migrated from the packaging material or due to an indirect exposure to BBP in the food via the environment. Directive 2002/72 relating to plastic materials and articles intended to come into contact with foodstuffs, regulates certain monomers, other starting substances and additives. The directive does not set limits for the content of BBP. BBP has also been used in regenerated cellulosefilm. The use has decreased during the recent years and is no longer allowed. This is due to EU Directive 2004/14/EC amending Directive 93/10/EEC relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs. BBP may still used in other types of food packaging materials. However, the extent of use is unknown.

In a Norwegian investigation the content of BBP in plastic films was determined in 6 plastic films by extracting the plastic films in diethylether, and measuring the BBP content in the extract. The migration from plastic films to olive oil for the same 6 products was also measured. The amount of BBP from the extraction and migration study is presented in **Table 4.12** (SNT rapport No 1, 1996, Norway).

Table 4.12 Extraction and migration of BBP from plastic films

Plastic film No.	Extraction (μg/g)	Migration (μg/g)
1 Polypropylene	Nd.	1
2 Sacs for bread	Nd	3
3 For chocolate, oriented polypropylene	2752	968
4 For vegetables, polyethylene, ethylvinylacetate	Nd	14
5 Film, oriented polypropylene	Nd.	< 2
6 Cling film, PVC	Nd.	No measurement

a) Nd means not detected in the sample

Most of the food-samplings have been performed from 1987-1989, however since that time considerable changes in the levels of the plasticizers in the food-packaging material have occurred, as seen in **Table 4.13**.

Table 4.13 Concentrations of BBP in food

Food type	Concentrations of BBP mg/kg	References
Baked savouries	1.5	MAFF (1987)[a]
Meat pies	4.8	MAFF (1987)
Sandwiches	14.0	MAFF (1987)
Butter	3.1 - 47.8	Page and Lacroix (1992)
Margarine	5.1 – 16.1	Page and Lacroix (1992)
Yoghurt	0.60	Page and Lacroix (1995)[b]
Cheddar cheese	1.60	Page and Lacroix (1995)
Butter	0.64	Page and Lacroix (1995)
Crackers	0.48	Page and Lacroix (1995)
Carcass meat	0.09	MAFF (1996a)[c]
Poultry	0.03	MAFF (1996a)
Eggs	0.09	MAFF (1996a)
Milk	0.002	MAFF (1996a)

a) In the MAFF (1987) study the food were stored in their packing until their "best before" date
b) In the Page and Lacroix (1995) study BBP was detected in 4 out of 100 foodstuffs examined
c) In the MAFF (1996a) study BBP was detected in stored fatty foods, from a total diet study

In the Page and Lacroix (1992) study the samples with higher surface/weight ratios tended to have higher phthalate levels, samples taken progressively closer to the surface of the food gave the highest concentrations of phthalate(s), and analysis of the packaging material showed the same phthalate esters as in the contacted food. These findings demonstrated the wrapper to be the source of the phthalate contaminateion in this survey.

In the Page and Lacroix (1995) study BBP was detected at a very low frequency. Only in 4 out of 100 food samples from a total diet, significant amount of BBP was reported. The samples were from 1986 and the degree of migration increased with the lipid content of the food and the surface area and time of contact.

In the MAFF (1996a) study lower levels of BBP were detected in the foodstuffs compared to the earlier studies. In over half of the samples the concentrations of the phthalates at the core were about equal to or higher than the concentrations found at the surface. These results suggested that phthalates in food orginate at least partly from the general environmental contamination.

The estimated average intake of BBP based on total diet study was 0.008 mg/person/day and the high level estimate was 0.02 mg/person/day (MAFF, 1996a). For the risk assessment, the MAFF (1996a) estimate of 0.02 mg/person/day (0.0003 mg/kg bw/day if the weight is 70 kg) will be used as a worst case approach.

Infant formulae

The concentrations of BBP were also measured in 59 individual samples of 15 different brands, including casein or whey dominant products or soya-based products of infant formulae from retail outlets in five towns across the United Kingdom (MAFF 1996b). Significant levels of BBP were detected in all the 15 different brands, and the concentration of BBP in these samples ranged from < 0.004 to 0.25 mg/kg infant formula. This levels results in an intake of BBP at birth from 0.1 to 8.7 μg/kg bw/day, and at six month from 0.9 to 5.6 μg/kg bw/day. However, in a new identical study performed in 1998 where 39 samples were tested including casein or whey dominant products, BBP was detected in 27 of the samples. The levels of BBP in infant formula were from 0.003 to 0.015 mg/kg of infant formula (MAFF, 1998, No. 168). These levels resulted in an average intake of BBP at birth at 0.2 μg/kg bw/day, and at 6 month of age at 0.1 μg/kg bw/day. In the risk characterisation 0.015 mg/kg BBP in infant formula will be used (MAFF, 1998).

Table 4.14 Concentration of BBP in infant formula, and estimated daily intake (from MAFF, 1998)

Year	BBP mg/kg infant formula	BBP μg/kg Bw/day	
		Birth	6 month
1996	> 0.004 – 0.24	8.7	5.6
1998	> 0.003 – 0.015	0.2	0.1

Infants (8 kg weight) drink infant formula and eat more or less the same food as adults. When assuming that an infant drinks 0.1 kg of infant formula per day and eats three times less food than an adult this will result in a daily intake of BBP at 0.00102 mg/kg bw/day which is the value to be used in the risk characterisation (0.015 mg/kg of BBP in infant formula divided with 10 plus 0.02 mg/person/day of BBP from food divided with 3). The intake of BBP from infant formula for children (8 kg) is then 0.000187 mg/kg bw/day, and the intake via food is 0.00083 mg/kg bw/day (0.000187 + 0.00083 = 0.00102 mg/kg bw/day), and these are the values used in the risk characterisation.

4.1.1.3.2 Scenario II: Indoor air

The potential for inhalation of air containing BBP is greatest in enclosed areas containing BBP plasticizeds materials, and is therefore best presented by indoor air exposures. Typically BBP is used in both PVC and non-PVC polymeric materials found in the home. BBP in these products could have the potential for release into indoor air. These products would be flooring, fillers (caulks), sealants and adhesives. The maximal uses of BBP in such applications are 30-40% BBP in the product. The main route of release of BBP would be through the migration of BBP to the

polymeric surface and eventual off-gassing of BBP from the polymeric matrix. This is a slow process resulting in low rates of loss from the products. Actual data for these types of exposure has been gathered and is discussed below.

The indoor air concentrations of BBP in offices were measured at two different places in the U.S (Weschler, 1984). The concentrations of BBP were reported to be 1 and 20 ng BBP/m^3. The different levels of BBP may be due to different sampling time. 1 ng BBP/m^3 was detected in late winter and early spring, whereas 20 ng BBP/m^3 was detected in fall and early winter. In this study phthalate esters were assumed to be the most important class of compounds associated with the indoor dust. The BBP in indoor air is related to the use of BBP as a plastisizer, and in most indoor environments plastics are ubiquitous. The estimated daily intake of BBP in the two offices are calculated to be 0.00028 μg/kg bw/day or 0.0057 μg/kg bw/day when the ventilation rate for humans are assumed to be 20 m^3/day and weight 70 kilo (TGD values).

The California Environmental Protection Agency (1992) monitored the BBP levels in indoor air in 125 homes in Riverside, California. The median value for night-time sampling (35ng/m^3) was used in calculating the minimum estimate of exposure to BBP in indoor air. The 90th percentile for day-time sampling (140ng/m^3) was used in calculating the maximum estimate of indoor exposure. The estimated daily intake of BBP is presented in **Table 4.15**.

Table 4.15 Estimated daily intake of BBP (μg/kg bw/day) via indoor air

Exposure	0-6 month[a]	7 month-4 years[b]	5-11 years[c]	12-19 years[d]	20+ years[e]
Min. exp.	0.009	0.021	0.016	0.008	0.007
Max. exp.	0.035	0.083	0.063	0.032	0.026

a) Assumed to breathe 2.1 m^3 of air per day (Allan, 1995), and weight 7 kilo (Health Canada, 1994)
b) Assumed to breathe 9.3 m^3 of air per day (Allan, 1995), and weight 13 kilo (Health Canada, 1994).
c) Assumed to breathe 14.5 m^3 of air per day (Allan, 1995), and weight 27 kilo, (Health Canada 1994).
d) Assumed to breathe 15.8 m^3 of air per day (Allan 1995), and weight 57 kilo, (Health Canada, 1994).
e) Assumed to breathe 15.8 m^3 of air per day (Allan, 1995) and weight 70 kilo (Health Canada, 1994).

In addition to indoor exposure to BBP due to evaporation of BBP from PVC plasticized materials, inhalation exposure to BBP may also occur as aerosols of BBP adsorbed to suspended particulate matter. This latter form of exposure has been evaluated in a Norwegian study which examined the residential exposure of BBP from suspended particulate matter (Øye et al., 1997). The level of BBP exposure from totally respirable dust measured from suspended particulate matter was 14 ± 30 μg BBP/100 mg suspended particulate matter, and calculated to be 0.0036 ± 0.008 μg/kg bw/day when the ventilation rate in humans is assumed to be 20 m^3/day and weight 70 kg (TGD values), and exposure to particulate matter (PM_{10}) is estimated to be 90 μg/m^3 (Norwegian 8-hour average guideline, National Health Authority, 1990). The contribution of BBP adsorbed to sedimental dust and suspended particulate matter to the overall indoor air exposure to BBP is difficult to assess. In the Norwegian study (Øye et al., 1997) the number of samples analysed were small, and there was a high degree of variation in BBP content in the dust/suspended matter between the various samples.

More recent studies are available where the levels of BBP have been measured in indoor air and in indoor house dust (Fromme et al., 2004; Rudel et al., 2003; Bornehag et al., 2004). In the study by Fromme et al. (2004) the 95th percentile level of BBP in apartments (n = 59) was reported to be 75 ng/m^3 and in kindergartens (n = 18) 26 ng/m^3. The estimated daily intake of BBP for children in kindergartens is calculated to be 0.01 μg/kg bw/day, and the calculated daily intake for children in apartments is calculated to be 0.028 μg/kg bw/day when the

ventilation rate for children is assumed to be 5 m^3/day and the weight 13 kg. For adults the estimated daily intake of BBP in apartments is calculated to be 0.021 μg/kg bw/day when the ventilation rate for adults is assumed to be 20 m^3 and the weight 70 kg (TGD values). In the same study the level of BBP in house dust was also measured. In the kindergartens (n = 74) no BBP was measured in house dust, however, in apartments (n = 50) the 95th percentile of BBP in house dust was 218 μg/g house dust. The estimated intake of BBP for children from house dust in apartments is then calculated to be 0.007 μg/kg bw/day and for adults 0.0056 μg/kg bw/day when the ventilation rate for children is assumed to be 5 m^3/day and the weight 13 kg, and the ventilation rate for adults is assumed to be 20 m^3 and the weight 70 kg (TGD values), and exposure to particular matter (PM_{10}) is estimated to be 90 μg/m^3 (Norwegian 8-hour average guideline, National Health Authority, 1990). In the study by Rudel et al. (2003) the median level of BBP in house dust measured in 119 homes was 45.4 μg/g house dust. The estimated intake of BBP for children from house dust is then calculated to be 0.0016 μg/kg bw/day, and for adults 0.0012 μg/kg bw/day based on the same assumptions as described above. In the study by Bornehag et al. (2004) the level of BBP in house dust was measured in houses with cases of children with allergic symptomes (cases, n = 175) and in houses with no cases of children with allergic symptomes (controls, n = 177). The median concentrations of BBP in house dust among cases were 150 μg/g dust, and in house dust among controls 120 μg/g dust. The estimated daily intake of BBP from house dust among cases is calculated to be 0.0052 μg/kg bw/day, and among controls 0.0041 μg/kg bw/day based on the same assumptions as described above.These calculated BBP exposure values based on recent data on exposure to BBP from indoor air and house dust are shown to be in the same range as the studies described above (Weschler et al., 1984; California Environmental Protection Agency, 1992; Øye et al., 1997).

Exposure to BBP for consumers may also occur in indoor air via the use of sealants or adhesives. Sealants available to consumers include polyurethane foam sealants which may be used for window frame insulation. However, these products are only used occasionally by consumers; therefore no exposure scenario is estimated.

For the risk assessment the maximum exposure estimate of 0.083 μg/kg bw/day from the California Environmental Protection Agency (1992) will be used as a worst case approach.

4.1.1.3.3 Scenario III: Baby equipment, children toys

The occurrence of BBP as a plasticizer in baby equipment and children toys, which children of young age can put in their mouth, is documented by the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, Brussels, April, 1998; *Migration of phthalates from teethers*, Research notes from NERI No. 64, Danish Environmental Protection Agency, 1998). However, in contrast to other phthalates such as di-iso-nonyl phthalate (DINP) and di(2-ethylhexyl)phthalate (DEHP), BBP in these products has been detected in trace amounts and, probably occurs as byproduct/impurity and has not been added intentionally.

Children may be exposed to BBP from toys in different ways. The exposure via vapours in the air is probably small as the vapour pressure is low. However, BBP can be transferred to the skin via direct physical contact. However, for small children the oral exposure is probably the most important 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 salvia around the products will serve as an extraction procedure for BBP. Therefore, the oral exposure route is discussed below.

The amount of BBP in children toys has been assessed in various studies. In an investigation published by Greenpeace (1997) 71 toys were analysed. These toys were bought in 17 countries (9 from the EU), but at least half of the toys were produced in China. BBP was detected in 6 toys. In these toys BBP comprised equal or less than 0.02% of the weight of the toys. In a Danish study (Danish Environmental Protection Agency, 1998) the leaching of phthalates under static and dynamic experimental conditions in artificial saliva for 20 hours at 37^{o}C was studied in 14 teethers. BBP was detected in 12 of the products at migration levels ranging from 0.01 to 611 μg BBP/dm^2/24 hours.

In a Norwegian study the presence of phthalates in 15 toys for small children were analysed (SFT reference No. 98/1292-603.1). The analyses of phthalates in the toys were performed as described by Rastogi (1998). Twelve of the 15 toys were found to contain one or more phthalates, however, BBP was not detected in any of the toys analysed.

The highest emission value from the Danish study is used as a worst case scenario and is converted to a daily dose for a 8 kg infant mouthing 10 cm^2 of the material 3 hours/day (approximate maximal total mouthing time for 6/12 months old children, RIVM Report 613320 002, Sept. 1998, CSTEE/97/1-Add. 107). This gives a calculated maximum release of BBP corresponding to 0.95 μg/kg bw/day.

Calculations: 611 · 3/24= 76.4 => 76.4/8= 9.5 =>9.5/10= 0.95 μg/kg bw/day

This worst case scenario is warranted because more than one phthalate may occur in baby equipment or children toys. Possible use of BBP as a replacement of other phthalates in baby equipment and children toys has not been evaluated.

4.1.1.4 Indirect exposure via the environment

BBP is widely distributed in the environment as a consequence of its manufacture, use and disposal. BBP may be released to the environment through waste water and air effluents at the sites where it is produced, processed, formulated as well as after end use. These exposure routes are taken into account in Section 3.

For the local exposure assessment processing and formulation are considered (Life cycle III). These scenarios are based on the Emission Scenario Document on Plastic Additives of the OECD from 2004 and the EU Technical Guidance Document (TGD) and evaluated with EUSES 2.03. The estimated concentrations of BBP in leaf crops and root crops are results of exposure via air and via soil, as calculated by EUSES using default values. Translocation of BBP within the plant is probably of minor importance. There are indications that the uptake of BBP from soil is limited (see Section 3.2.4). Therefore exposure via roots may be overestimated using EUSES default values for uptake from soil. In the EUSES estimations the partition coefficient between plant tissue and water is set to 1. The calculated daily human intake of BBP [mg/kg bw/day] is presented in **Table 4.16** and **Table 4.17**. Life cycle steps II, IV and V show lower values and are not included in the table.

Table 4.16 Estimated daily human intake of BBP through environmental exposure

Scenarios[a]	Fraction of dose through wet fish	Total human intake [mg/kg bw/day]
IIIa large site	0.87	0.0189
IIIa small site	0.87	0.0295
IIIb-1	0.39	0.0007
IIIb-2	0.59	0.0002
IIIc	0.87	0.0043
IIId	0.87	0.0027
IIIe-1	0.91	0.0004
IIIe-2	0.74	0.0006
IIIf-1	0.88	0.0021
IIIf-2	0.60	0.0002
IIIg-1	0.89	0.0011
IIIg-2	0.86	0.0004
IIIh-1	0.88	0.0067

a) Referes to the scenarios described in Section 3.1

For regional BBP exposure assessment, production, processing/formulation, and distribution are considered. In **Table 4.17** the calculated daily human intake of BBP [mg/kg bw/day] from different compartments are presented.

Table 4.17 Estimated daily human intake of BBP at regional levels

Compartment	BBP Concentration [µg/kg]	Fraction of dose	Total human intake [mg/kg bw/day]
Air		0.0014	$1.8 \cdot 10^{-7}$
Root tissue	0.023	0.001	$1.3 \cdot 10^{-7}$
Leaves of plant	0.011	0.0015	$2.0 \cdot 10^{-7}$
Drinking water	0.086	0.019	$2.4 \cdot 10^{-6}$
Meat	0.012	0.0004	$5.2 \cdot 10^{-8}$
Fish	76.8	0.977	$1.3 \cdot 10^{-4}$
Milk	0.0038	0.00024	$3.1 \cdot 10^{-8}$
Total		1	$1.3 \cdot 10^{-4}$

For the risk characterisation the total daily human intake of BBP [mg/kg bw/day] from local (worst case) and regional release of BBP will be used.

There are recent biomonitoring data on the excretion of BBP-metabolites in urine of different populations including young children (1-2 years), children (6-11 years) and adults (Blount et al., 2000a; CDC 2001; CDC 2003; Hoppin et al., 2002; Koch et al., 2003 and Brock et al., 2002). In humans the measure of the level of monobenzyl phthalate (MBeP) in the urine is indicated to reflect exposure to BBP (Anderson et al., 2000). Based on the measured metabolite data and the knowledge of the fraction of BBP metabolites that is excreted in the urine from the study by

Anderson et al. (2000) the total daily intake of BBP can be calculated (see **Table 4.20**). By this approach, an estimate of the total regional exposure can be obtained. Theoretically, these measured data should give more reliable intake values than the EUSES model. Below the biomonitoring data is described.

4.1.1.4.1 Biomonitoring of BBP metabolites in urine as measure of the BBP intake

Monoester metabolites of commonly used phthalates [Di-(2-ethylhexyl)phthalate (DEHP), Dibutylphthalate (DBP), Benzyl butyl phthalate (BBP), Di-isononyl phthalate (DINP), Di-isodecyl phthalate (DIDP), and Diethyl phthalate (DEP)] were measured randomly in urine in a reference population of 289 adult humans (56% female) (Blount et al., 2000a). The samples were collected from 1988 – 1994, at different times during the day and were not first-morning voids. The population studied comprised noninstitutionalised adults aged 20-60 years. The racial distribution was Caucasian 39%, African American 30%, Mexican American 23%, and others 8%. The phthalate metabolites were measured suing HPLC-APCIMS/MS by a method described by Blount et al. (2000b). The urine samples were spiked with $^{13}C_4$-labeled phthalate monoesters and 4-methylumbelliferone glucorinide. The samples were then treated with β-glucuronidase to release the monoesters from its conjugated form. Precautions were taken to avoid contamination from phthalate-containing plastics, reagents, labware and from diester lipase activity in the glucoronidase enzyme preparation. Phthalate monoester levels in human urine varied videly. The geometric mean of mono benzyl phthalate was 22.6 ng/ml urine, the maximum value 1,020 ng/ml urine, the 95th percentile value 137 ng/ml urine, the 50th percentile value 21.2 ng/ml urine, and the minimum value 1.4 ng/ml urine. For mono butyl phthalate the geometric mean was 41.5 ng/ml urine, the maximum value 4,620 ng/ml urine, the 95th percentile value 294 ng/ml urine, the 50th percentile value 41.0 ng/ml urine, and the min value 2.2 ng/ml urine. However, creatinine adjustment reduced this variation. The highest urinary levels of phthalate monoester found were; monoethyl phthalate (MEP) 16,200 ppb, 6,790 μg/g creatinine, monobutyl phthalate (MBuP) 4,670 ppb, 2,760 μg/g creatinine and monobenzyl phthalate (MBeP) 1,020 ppb, 540 μg/g creatinine which reflected exposure to DEP, DBP, and BBP. The level of mono-2-ethylhexyl phthalate (MEHP) was 67 ppb, 192 μg/g creatinine. Women of childbearing age (20-40 years) had significantly higher urinary levels of MBuP (46.9 μg/g creatinine) than other sex/age groups (31.4 μg/g creatinine; $p = 0.0003$). Furthermore, six of the eight highest MBuP levels were found in these women. Ten subjects had urinary MBuP > 300 μg/g creatinine. DEHP and DINP are produced in larger quantities than BBP, DBP, and DEP, however, this study indicate a substantial internal human dose of BBP, DBP, and DEP.

Comments to the study by Blount et al. (2000) is available regarding exposure estimates to phthalate esters derived from the values of phthalate metabolites measured in the Blount et al. (2000) study (David, 2000 and Kohn et al., 2000).

David (2000) calculated the exposure to BBP by using values from a study where the molar ratio of phthalate ester metabolites were measured in the urine of human volunteers given known amounts of phthalate esters (Anderson et al., 2000). With this data the levels of phthalate metabolites in the Blount et al. (2000) study were converted to estimated intake levles of phthalates. In this calculation the 95th percentil value of daily intake of BBP was 3.34 μg/kg/day, and the highest value was 19.79 μg/kg/day, compared to the estimated intake at 6 μg/kg/day from the Agency for Toxic Substances and Disease Registry (ATSDR), International Programme of Chemical Safety (IPCS) or EU.

The Kohn et al. (2000) comment describes a calculation of the estimated total daily intake of phthalates that would result in the reported urinary concentrations of monoester metabolites in the Blount et al. (2000) study. Kohn and co-workers used published animal data for the measurement of excretion of metabolites of BBP (Nativelle et al., 1999 and Eigenberg et al., 1986). With this calculation the estimated minimum daily intake of BBP was 0.094 μg/kg/day, the median daily intake 0.88 μg/kg/day, the 95th percentile 4.0 μg/kg/day, and the maximum 29 μg/kg/day, compared to the value at 2 μg/kg/day from National Toxicology Program (NTP) Center for the Evaluation of Risk to Human Reproduction (CERHR).

Koo et al. (2002) used the same human reference population of 298 adult humans as used in the study by Blount et al. (2000a) to study possible demographic variables that might affect phthalate levels in individuals. Included as demographic variables were age, sex, ethnicity, residency, family income and education levels. Statistical tests were used to determine whether any of the demographic variables affected mean phthalate levels. The results of the study indicated that individuals with only a high school education had higher levels of DBP than individuals with education beyond high school. Subjects who had family income less than $ 1,500 in the month before sampling and/or only high school education had higher levels of BBP than other groupings. DEHP was higher in males and/or in urban populations and/or in people who had family income less than $ 1,500 per month.The authors of the study suggests that there may be significant demographic variations in exposure and/or metabolism of phthalates.

The levels of phthalate monoester were also measured on the Centers of Disease Control and Prevention, National Report on Human Exposure to Environmental Chemicals, Atlanta, Georgia, 2001 (CDC, 2001). The samples were from 1999, and the numbers of participants were 1,024, aged 6 years and older. In this study the median level of the monoesters when measured as μg/l urine was as following; mono benzyl phthalate (MBeP) 18.5 (minimum 2.2, maximum 101), mono butyl phthalate (MBuP) 27.5 (minimum4.6, maximum 122), mono ethylhexyl phthalate (MEHP) 3.3 (minimum 0, maximum 17.3), reflecting exposure to BBP, DBP and DEHP. When measured as μg/g creatinine, the median levels were for MBeP was 14.2 (minimum 3.9, maximum 72.7), for MBuP 22.0 (minimum 6.2, maximum 93.0) and for MEHP 2.8 (minimum 0, maximum 11.2).

The levels of phthalate monoesters were measured in a more recent study at the Centers for Disease Control and Prevention, January 2003 (Second National Report on Human Exposure to Environmental Chemicals). The samples were from 1999 – 2000, and were divided into four age groups. Group A 6-11 years, group B 12-19 years and group C 20 years and older, and finally a group D age 6 years and older (the total group), the same group as in the CDC 2001 study. The sample size and geometric mean of phthalate monoesters in the urine in ng/ml (95% conf. interval) for MBuP, MBeP and MEHP, reflecting exposure to DBP, BBP and DEHP is shown in **Table 4.18** below.

Table 4.18 The geometric mean (gm) and 95th percentile of the phthalate monoesters MBuP and MBeP in μg/l urine

Phthalate monoesters	Group A 6 to11 years	Group B 12 to 19 years	Group C 20 years and older	Group D 6 years and older
MBuP	*n* = 328 41.4 μg/l (*gm*) 163 μg/l (95th)	*n* = 752 36.0 μg/l (*gm*) 165 μg/l (95th)	*n* = 1461 21.6 μg/l (*gm*) 142 μg/l (95th)	*n* = 2541 24.6 μg/l (*gm*) 149 μg/l (95th)
MBeP	*n* = 328 39.4 μg/l (*gm*) 214 μg/l (95th)	*n* = 752 25.6 μg/l (*gm*) 125 μg/l (95th)	*n* = 1461 12.4 μg/l (*gm*) 86.3 μg/l (95th)	*n* = 2541 15.3 μg/l (*gm*) 103 μg/l (95th)

In a study by Hoppin et al. (2002) urinary phthalate metabolites were measured in first morning urine samples from 46 African-American women. The urine samples were taken between 1996 and 1997. The monoesters were measured in two consecutive first morning void urine samples on the second and third day of their menstrual cycle. The women were from a randomly selected group of members of a prepaid health plan in Washington, DC, and ranged in age from 35 to 49 years. The women selected for this analysis were not anticipated to have unique phthalate exposures. The phthalate monoesters were measured using high-pressure liquid chromatography followed by tandem mass spectrometry on a triple quadruple instrument using atmospheric pressure chemical ionization. The median level of the monoesters when measured as μg/l urine was as following; mono benzyl phthalate (MBeP) 31.5 (minimum 5.6, maximum 135.2), mono butyl phthalate (MBuP) 53.0 (minimum 0.7, maximum 251.3), mono ethylhexyl phthalate (MEHP) 7.3 (minimum 1.0, maximum 143.9). When measured as μg/g creatinine, the median levels were for MBeP was 21.6 (minimum 5.6, maximum 135.2), for MBuP 43.4 (minimum 0.4, maximum 157.3) and for MEHP 6.4 (minimum 0.4, maximum 77.3). The levels of phthalate monoesters measured in this study were similar to those reported in the study by Blount et al. (2000) and the CDC, 2001 study (described below). In the study by Hoppin et al. (2002) an extensive variation in the level of exposure to phthalates among individuals was indicated. However, there was a high degree of reliability in urine phthalate levels from one day to the next, suggesting that the women's phthalate exposure were relatively stable. There were no significant differences in phthalate monoester levels from one day to the next.

The phthalate metabolites have also been analysed in 85 urine samples of the general population in Germany (Koch et al., 2003). The urine samples were collected from 53 not occupationally exposed women (median age 29 years) and 32 not occupationally exposed men (median age 36 years). They measured the secondary metabolites of DEHP; mono(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP) and mono(2-ethyl-5-oxo-hexyl)phthalate (5oxo-MEHP) and the primary monoester metabolite of DEHP, di-n-octylphthalate (DnOP), di-n-butylphthalate (DBP) and butyl benzylphthalate (BBP) and diethylphthalate (DEP). Based on the internal exposure values (μg/l of metabolite in urine, data not shown) they calculated the daily intake of the parent phthalates using urinary metabolite excretion factors from the study by Anderson et al. (2000) see equation below. However, in the study by Anderson et al. (2000) only 7 volunteers/group were used which limit the value of this study due to the low number of volunteers per group. The low number per group leads to an uncertainty in the calculations of the exposure to the phthalates based on measured urinary monoester metabolites. For BBP the intake value at the 95th percentile was determined to be 2.5 μg/kg bw/day. For the other phthalates, DEHP, DBP, DnOP, and DEP the intake values at the 95th percentile were determined to be 52.1, 16.2, 0.41 and 22.1 μg/kg bw/day. The median intake value for BBP, DEHP, DBP, DEP and DnOP was determined to be 0.6, 13.8, 5.2, 2.32 and limit of quantification. For BBP the calculated levels were in accordance with the estmated levels given in Section 4.1.1.4 for regional exposure to BBP in the general poplation. In the concluison of the study the authors

indicated that the general population in Germany was exposed to DEHP to a much higher extent than previoulsy assumed.

In a pilot study the phthalate monoesters levels were measured in the urine of 19 young children 12 to 18 month of age (5 girls and 14 boys) by Brock et al. (2002). Urine samples were collected from the children during a clinical contact for vaccination, and again during a second visit about 4 weeks later. The analysis method was the same as used by Blount et al. (2000b). All urine samples had detectable levels of MEP, MBeP and MBuP, which suggests exposure to DEP, BBP and DBP. Eight urine samples from 6 children had detectable levels of MEHP suggesting exposure to DEHP. The mean levels of MBeP was 35.6 ± 44.8 ng/ml urine, MBuP 117.4 ± 287.6 ng/ml urine, MEP 184.1 ± 246.9 ng/ml urine, and MEHP 4.6 ± 6.4 ng/ml urine. The mean urinary MBuP, MBeP and MEHP levels for the children in this study were above the 50th percentile of the previous reported adult monoester levels by Blount et al. (2000).

The levels of phthalates in 48 hours personal air samples were measured from parallel cohorts of pregnant women in New York (*n*= 30) and Krakow (*n* = 30), and spot urine samples were collecteds during the same 48 hours period from the New York women (*n* = 25) (Adibi et al., 2003). The medium age of the New York women were 22 years and the Krakow women 27 years. The New York women were 70% Dominican or Dominican American and 30% African American. The Krakow women were 100% ethnically Polish. The following four phthalates and their metabolites were measured in both personal air samples and in urine: diethyl phthalate (DEP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP) and butyl benzyl phthalate (BBP). All phthalates were present in 100% of the air and urine samples. The ranges in personal air samples were: DEP (0.26-7.12 μg/m^3), DBP (0.11-14.76 μg/m^3), DEHP (0.05-1.08 μg/m^3) and BBP (0.00-0.63 μg/m^3). The mean personal air concentrations of DBP and DEHP were higher in Krakow, whereas the mean personal air concentration of DEP was higher in New York. The creatinin adjusted phthalate monoetster concentrations (mean ± SD in μg/g) were as following: monoethyl phthalate 690 ± 1.43 · 10^3, monobutyl phthalate 54.4 ± 24.5, monobenzyl phthalate 26.0 ± 28.2 and monoethylhexyl phthalate 40.5±98.4. Statisitcally significant correlations between personal air and urinary levels were found for DEP and monoethyl phthalate (*r* = 0.42) DBP and monobutyl phthalate (*r* = 0.58) and BBP and monobenzyl phthalate (*r* = 65). This study demontrate that pregnant women are exposed to phthalates during pregnancy, and indicate that inhalation is an important route of exposure. The levels of monobenzyl phthalate measured in urine in the CDC (2001) study and the Hoppin et al. (2002) study are in good agreement with the levels measured in the study by Adibi et al. (2003), 14.2 μg/g, 21.6 μg/g, and 12.1 μg/g.

Table 4.19 Urinary levels in ng/ml of phthalate monoesters from four different studies in USA and one from Germany. The levels are geometric mean (gm) and 95th percentile

Phthalate monoesters	Blount et al. (2001) (*n*=298)	CDC (2003) (*n*=2541)	Brock et al. (2002) (*n* = 19 children 1-2 years)	Hoppin et al. (2002) (*n*=46)
MBuP	41.5 (*gm*) 294 (95th)	24.6 (*gm*) 149(95th)	117.4 (*gm*) 2,540 (max value)	78.1 (*gm*) 251 (max value)
MBeP	22.6 (*gm*) 137 (95th)	15.3 (*gm*) 103 (95th)	35.6 (*gm*) 316 (max value)	39.4 (*gm*) 135.2 (max value)

The daily intake of BBP can be calculated as indicated in the study by Koch et al. (2003), see above. In **Table 4.20** the daily intake values for BBP are calculated from the measured level of MBeP in the urine for the studies by Blount et al. (2000); Hoppin et al. (2002); CDC (2003) for the two age groups; children 6 to 11 years, the total group (20 years and older) and from the

study by Brock et al. (2002) young children (1-2 years). The excretion value of the phthalate metabolite MBeP was converted to daily intake (DI) values for BBP when applying the following equation according to David et al. (2000):

DI (μg/kg bw/day) =

[UE(μg/g) multiplied with CE (mg/kg bw/day)] and divided with [F*ue* x 1,000 (mg/kg)]

This value is then multiplied with [MWd divided with MWm].

Where UE is the urinary excretion of MBeP in μg/g creatinine, CE is the creatinine excretion rate normalised by the body weight (18 mg/kg/day for women and 23 mg/kg /day for men (Harper et al., 1977; Kohn et al., 2000). F*ue* is the molar fraction of the urinary excreted MBeP which was 0.73 according to Anderson et al. (2000). However, there may be some uncertainty to this value due to low number of volunteers per group (7/group), Mwd is the molar weight of BBP (312) and Mwm is the molar weight of MBeP (256).

Table 4.20 The estimated daily intake values (μg/kg bw/day) from the study by Koch et al. (2003); Blount et al. (2000a); CDC (2003); Brock et al. (2002) and Hoppin et al. (2002) based on the urinary excretion of MBeP in μg/g creatinine in these studies

Studies	Geometric mean MBeP excretion μg/g creatinine	95th percentile excretion μg/g creatinine	Geometric mean estimated BBP intake values μg/kg bw/day	95th percentile estimated BBP intake values μg/kg bw/day
Koch et al. (2003)	Data not given	Data not given	0.60	2.52
Blount et al. (2000a)	20.2	91.9	0.78[a]	3.5[a]
CDC (2003) 6 to 11 years	40.0	142	1.54[a]	5.46[a]
CDC (2003) 20 years and older	11.8	57.2	0.45[a]	2.2[a]
Brock et al. (2002)	128.1	473.8 (maximum value)	4.9[a]	18.2[a] (maximum value)
Hoppin et al. (2002)	26.5	119.7 (maximum value)	0.8[b]	3.6[b] (maximum value)

a) CE at 23 mg/kg/day for men is used.
b) CE at 18 mg/kg/day for women is used.

The following estimated worst case exposures will be brought forward to the risk characterisation. The highest estimated intake level at 18.2 μg/kg bw/day for young children (1-2 years) from the study by Brock et al. (2002) the 95th percentile estimated intake level at 5.46 μg/kg bw/day for children 6-11 years from the CDC (2003) study, and the 95th percentile estimated intake level at 3.5 μg/kg bw/day for adults from the study by Blount et al. (2000a).

4.1.1.4.2 Combined exposure

Due to the use of BBP in formulation and processing of products, and the diffuse emission of BBP from these products, humans may be exposed to BBP from different sources. The combined exposure to BBP is the sum of all the specific sources (occupational exposure, consumer exposure, and indirect exposure via the environment), and by all routes of exposure. However, since occupational exposure values will totally dominate the exposure levels for adults, it is not considered relevant to make a separate calculation for combined exposure for adults including occupational exposure.

Children are potentially exposed via many products and sources, combined exposure values have been calculated.

Therefore, in this combined exposure assessment two combined exposures are evaluated (see **Table 4.21**)

I. Children exposure to BBP from toys, infant formula, indoor air and indirectly via the environment (air, water and food).

II. Adult exposure to BBP as a consumer and indirectly via the environment (air, water and food).

Table 4.21 Combined exposure to BBP

Exposures	Daily intake (μg/kg bw/day)	
	Child (0-2 years old)	Adult
Food and foodpackaging	0.83	0.3
Infant formula	0.187[a]	
Indoor air	0.083	0.032
Baby equipment and baby toys	0.95	
Indirectly via the environment (local)[b]	29.5[b]	29.5[b]
Indirectly via the environment (regional[d])	0.13[d]	0.13[d]
Calculated daily intake from urinary concentrations of BBP metabolites[e, f]	18.2[e]	3.5[f]
TOTAL (local)[b]	31.55[b]	29.83[b]
TOTAL (regional[d]),	2.18[d]	0.46[d]
(calculated from urinary excretion of BBP metabolites[e, f])	18.2[e]	3.5[f]

a) Mean value from birth and 6 month.
b) Worst case scenario from local exposure (scenario IIIa small site).
d) Regional exposure as estimated by EUSES
e) Regional exposure (maximum value) as measured by Brock et al. (2002)
f) Regional exposure (95th percentile) as measured by Blount et al. (2000a)

4.1.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

A number of studies on the toxicity of butyl benzyl phthalate (BBP) in experimental systems have been performed. Few studies have been performed according to current guidelines (OECD Test Guidelines or EU Annex V Guidelines) or in compliance with GLP. A number of studies are, however, comparable to guideline studies. When studies were performed in accordance with current guidelines and/or under GLP conditions, this information is included in the text.

4.1.2.1 Toxico-kinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

Oral

BBP is partially hydrolysed by intestinal esterases, primarily to monobutyl phthalate and benzyl alcohol with monobenzyl phthalate and n-butanol as minor products of hydrolysis. There is a preference for hydrolysis of the benzylester, resulting in a preponderance (approximately 3:1) of monobutyl phthalate in the urine (Agarwal et al., 1985).

Male Fischer-344 rats were dosed with ring-labelled [^{14}C]BBP at 2, 20, 200 or 2,000 mg/kg p.o. or 20 mg/kg i.v. to determine the rates and routes of excretion. In 24 hours, 61-74% of the dose was excreted in the urine and 13-19% in the faeces at 2-200 mg/kg. At the 2,000 mg/kg dose, 16% of the ^{14}C was excreted in the urine and 57% in the faeces. Increases in fecal elimination at 2,000 mg/kg may be due to incomplete absorption of administered BBP or BBP metabolites during the enterohepatic circulation. Urinary ^{14}C was composed of monophthalate derivatives (10-42% of the dose) and glucuronides of these monophthalate derivatives (2-12% of the dose). At 4 hours after i.v. administration of BBP (20 mg/kg), 53-58% of the dose was excreted in the bile. No parent compound was found in the bile, but monobutyl phthalate-glucuronide and monobenzyl phthalate-glucuronide (26% and 13% of the dose) and trace amounts of free monoesters (2% of the dose) and unidentified metabolites (14% of the dose) were present. Larger quantities of monobutyl phthalate than of monobenzyl phthalate were formed (monobutyl phthalate = 44% versus monobenzyl phthalate = 16%). The half-life of both parent BBP and the monoesters were approximately 6 hours in all tissues (Eigenberg et al., 1986).

A study has been conducted of the *in vitro* hydrolysis of butyl benzyl phtalate (BBP) by rat tissue preparations and the tissue distribution and excretion of the diester following the oral administration to rats of single and multiple doses. The hydrolysis of ring-labelled [^{14}C] BBP by hepatic and intestinal preparations from untreated male Sprague-Dawley rats was studied by modification of the method of Albro and Thomas (1973) for cholate-dispersive substrates. ^{14}C-BBP was hydrolysed by both rat hepatic and intestinal mucosal cell preparations. The rate of hydrolysis was proportional to both the tissue concentration and the incubation time employed. The rate of BBP hydrolysis by hepatic alkaline non-specific esterases was 45.3 μmole product formed per hour per g of liver (50% of previously reported rates for di-n-butyl phthalates). BBP was rapidly hydrolysed by intestinal mucosal cell homogenates, 1.64 μmoles per mg of intestinal mucosal cell protein/hour (3 and 15 times faster than previously reported data on di-n-butyl and di-(2-ethylhexyl) phthalates). Attempts to determine the hydrolysis products were hampered by poor separation. However, free phthalic acid appeared to be absent from both hepatic and

intestinal mucosal cell tissue incubate extracts indicating that BBP was being metabolised to either mono-*n*-butyl phthalate and/or mono-benzyl phthalate. To determine excretion and tissue distribution of [^{14}C]-BBP, rats were treated orally with [^{14}C]-BBP at dose levels of either 16, 160 or 1,600 mg/kg and the urine and faeces collected for 5 days after which the animals were killed and residual radioactivity in the tissues determined. The largest amounts of radioactivity remaining in the animals at sacrifice were located in the liver, kidneys, small intestine and total gut contents. However, the radioactive BBP residues present in any of the tissues were very small and there was no evidence to indicate tissue accumulation of BBP residues. The excretion in urine was rapid and appeared to be largely independent of the dose of BBP administered. More than 80% of the administered doses of BBP were excreted in the urine within 5 days; the bulk of the remainder being excreted by faeces (Lake et al., 1978 and BIBRA, 1978).

The metabolism of BBP was studied in female Wistar rats (180 – 200 g) (Nativelle et al., 1999). Four doses of BBP dissolved in corn oil were administered by gavage, 150, 475, 780 and 1,500 mg/kg bw/day for 3 consecutive days. Urine samples were collected 24 hours after each gavage. The metabolites recovered in the urine were analysed by gas chromatography-mass spectrometry (GC-MS) after 24, 48 and 72 hours. In this study six metabolites of BBP were identified. The parent compound, BBP was not recovered in the urine. Monobutyl phthalate (MBuP) and Monobenzyl phthalate (MBeP) represented 29-34% and 7-12% of the total recovered metabolites. Hippuric acid, the main metabolite of benzoic acid, represented the second major metabolite (51-56%). Phthalic acid and an ω-oxidised metabolite of MBuP were also recovered in the urine 2 – 3% and 1 – 2%. Very small quantities of Benzoic acid were also measured. The recovered metabolites in the urine represented 58%, 54%, 43% and 30% of the doses 150, 475, 780 and 1,500 mg/kg bw/day. After 24 hours the metabolites recovered in the urine were similar in the rats exposed to 150 and 475 mg/kg bw/day (except for MBuP). At 780 mg/kg bw/day hippuric acid was detected in a lower amount. At the highest dose all levels of metabolites decreased significantly compared to the levels measured at 150 and 475 mg/kg bw/day. After 48 hours the level of hippuric acid started to decrease significantly at 475 mg/kg bw/day, however, elimination of MBuP, MBeP was similar in the 150 and 475 mg/kg bw/day dose group. Elimination of MBuP and MBeP and hippuric acid was similar for the two highest doses but in a lower amount compared to the first doses. In contrast, after 72 hours the levels of metabolites excreted were constant with the dose of BBP. As regards the time dependency, treatment with 475 mg/kg bw/day resulted in a steady-state level of urinary excretion of metabolites within 72 hours. Multiple dosing with 1,500 mg/kg bw/day showed identical levels of excretion of all metabolites the first 48 hours, whereas after 72 hours, the levels of MBuP, MBeP and hippuric acid were increased 1.9, 2.4 and 1.4 fold.

Immature female Alpk:APfSD rats (20-22 days old, groups of five) were administered a single oral or subcutaneous dose of BBP (purity > 98.5%) at dose levels of 1, 10 and 100 mg/kg. Urine was collected for a period of 24 hours following dosing and blood was collected for plasma preparation at termination. All urine and plasma samples were frozen proir to analysis by GC-MS. The levels of BBP, monobutyl phthalate (MBuP) and monobenzyl phthalate (MBeP) were analysed in the plasma samples. Urine samples were analysed for the level of MBuP and MBeP. Twenty-four hours after BBP administration, plasma levels of BBP and MBeP were below the limit of detection (0.04 mg/l) in all dose groups after oral or subcutaneous administration. However, for MBuP an average of 0.14 mg/L was measured after oral exposure to 100 mg/kg BBP, and an average of 0.37 mg/L was measured after subcutaneous exposure to 100 mg/L BBP. In urine MBuP and MBeP, the major metabolites of BBP, were detected at all dose levels of BBP following either route of administration. Generally, MBuP was detected at higher concentrations than MBeP. The urinary excretion of MBuP after oral exposure to 1, 10 or 100 mg/kg BBP was 10.4, 2.5 or 1.8% and for MBeP 5.0, 0.5 or 0.5%. After subcutaneous

administration of 1, 10 or 100 mg/kg BBP the urinary excretion of MBuP was 10.5, 4.3 or 9.0% and for MBeP 2.6, 4.7 or 2.5%. The average level of MBuP in urine after oral exposure to 1, 10, and 100 mg/kg BBP was 1.87 mg/L, 6.60 mg/L and 34.45 mg/L. After subcutaneous administration the average level of MBuP in urine after exposure to 1, 10, and 100 mg/kg BBP was 1.89 mg/L, 8.15 mg/L and 238.45 mg/L. The average level of MBeP in urine after oral exposure to 1, 10 and 100 mg/kg BBP was 1.0 mg/L, 1.50 mg/L and 11.58 mg/L. The average level of MBeP in urine after subcutaneous administration of 1, 10 and 100 mg/kg BBP was 0.53 mg/L, 10.22 mg/L and 70.20 mg/L. Following oral administration of 1, 10 or 100 mg/kg the percentage of the dose recovered in the urine was 15.4, 3.0 and 2.3%. Following subcutaneous administration of the same doses the recovery in the urine was 13.1, 9.0 and 11.5%. At 1 mg BBP/kg bw similar systemic exposure (measured as monophthalates in the urine) was observed in the animals irrespectively of the route of administration (oral versus subcutaneous). At higher doses (i.e. 10 and 100 mg/kg) animals administered BBP subcutaneously exhibited greater systemic exposure relative to animals dosed by oral gavage (Monsanto, 1997a). The study indicated that the urinary excretion of MBuP is higher than MBeP after exposure to BBP in immature or adult rats. However, the percentage of excreted metabolites in adult rats was shown to be higher compared to immature rats.

Pharmacokinetic studies in four beagle dogs administered a divided oral dose equivalent to 5,000 mg BBP/kg bw over a period of 4 hours demonstrated recovery of 88% (male) and 91% (female) of unchanged BBP from the faeces; approximately 4% was excreted as urinary phthalic acid (Erickson, 1965).

Dermal

Dermal absorption of [^{14}C]-BBP was measured in male Fischer-344 rats. Hair from a skin area (1.3 cm in diameter) on the back was clipped, the [^{14}C]-BBP was applied in a dose of 157 μmol/kg, and the area of application was covered with a perforated cap. The applied dermal area dose was 30-40 mg/kg. This represents a dose to the skin of 5-8 mg/cm^2. The exposure duration was 7 days. Urine and faeces were collected every 24 hours. The percentage of ^{14}C excreted in the urine and feces, as well as that remaining in the body (aside from the skin area of application), was taken as an index of the percutaneous absorption. After 7 days, approximately 30% of the applied dose was excreted in the urine or faeces, 4.6% of the applied dose was found in the muscle, 0.5% was found in the brain, spinal cord and testis, and 45% was found at the skin area of application. This study indicates that BBP is extensively, but slowly absorbed by the dermal route. BBP is rapidly metabolised and the major route of excretion of metabolites is biliary (Elsisi et al., 1989).

The absorption rate of BBP over human skin is not known. However, due to the log-K_{ow} (4.84) and the molecular weight (298.3) these values do not indicate a high dermal absorption of BBP. From an *in vitro* study the absorption rate of DBP over skin from both rats and humans were examined, and it was concluded, that DBP was absorbed more slowly by human skin compared to rat skin. The absorption rate for DBP (fluid) over human skin was measured to be 1.9 μg/cm^2/hour with occlusion and 1.7 μg/cm^2/hour without occlusion and for rat skin 39.2 μg/cm^2/hour and 43.2 μg/cm^2/hour (Mint and Hotchkiss, 1993). DBP is very similar to BBP regarding molecular weight (278) and, length of the side chain, and lipophilicity, the log-K_w value for DBP is 4.57. From an *in vivo* study in rats (Elsisi et al., 1989) it was shown that approximately 5% of BBP was absorbed each day, leading to absorption of approximately 30% within 7 days. The dermal absorption rate for DBP in the Elsisi et al. (1989) study was approximately 10% each day, leading to a total absorption of approximately 70% within 7 days. However, results from *in vivo* dermal absorption studies can only be used for conclusions on

percutaneous absorption when the experimental exposure conditions resemble the estimated actual exposure conditions. In the study be Elsisi et al. (1989) a detailed description on the exposure conditions were not included. Considering the available data, dermal absorption is considered to be 5% as a worst-case estimate.

Inhalation

No information related to inhalation exposure was found in the literature.

4.1.2.1.2 Studies in humans

There are several recent biomonitoring studies on the excretion of BBP metabolites in urine of different human populations including young children (1-2 years) children (6-11 years) and adults (Blount et al., 2000a; CDC, 2001; CDC, 2003; Hoppin et al., 2002; Koch et al., 2003 and Brock et al., 2002). These studies are described in Section 4.1.1.4 indirect exposure via the environment under regional exposure. The only study included in this section is the study by Anderson et al. (2000). This study describes a quantitative biomarker method for correlating human urinary phthalate monoester elimination with known exposure to the corresponding diester. However, in this study only 7 volunteers /group were used, which limit the value of the study, and leads to an uncertainty in the calculations of the exposure to the phthalaes based on measured urinary monoester metabolite.

In the study by Anderson et al. (2000) volunteers (7/group) were administered stable isotope-labelled BBP (d_4-BBP) in a single dose, which was spiked into margarine and administered on a toast as breakfast. The amount of phthalate monoesters excreted in the urine was measured 1 day before the dose, 1 day after the dose, and subsequently 2 and 6 days after the dose. The control group received no dose of BBP, the low dose group received 168 to 255 μg of d_4-BBP and the high dose group received 336 to 510 μg of d_4-BBP. The excreted phthalate monoesters were measured by LC-MS following hydrolysis of conjugates. Background levels of unlabelled mono butyl phthalate (MBuP) and mono benzyl phthalate (MBeP) were detected in most of the urine samples 24 hours before dosing. The majority of the labelled phthalate monoesters were excreted in the first 24 hours period following the dose administration. The formation and excretion of MBeP was high after 24 hours, 67% and 78% on a molar basis of the low and high dose group. The formation and excretion of MBuP was much lower. Only 6% was excreted as MBuP in the high dose group 24 hours after dosing. No excretion of MBuP was measured in the low dose group. On the second and sixth day after dosing no labelled phthalate monoester excretion was measured. This study indicates that the excretion of MBeP in the urine reflects exposure to BBP. Following exposure to ^{13}C-Dibutylphthalate (DBP) the excretion yield 24 hours post dosing was 64 and 73% MBuP on a molar basis of the low and high dose group, indicating that the excretion of MBuP in the urine mainly reflects exposure to DBP.

4.1.2.1.3 Summary toxico-kinetics, metabolism and distribution

In rats, the kinetics of BBP after oral administration was dose-dependent. Excretion of radiolabelled BBP in the urine was between 70% and 80% in the dose-range of 2 mg/kg p.o. and 200 mg/kg p.o. whereas 22.4% were excreted in the urine after administration of 2,000 mg/kg p.o. The excretion of radioactivity in the feces was 20% after intravenous administration which indicates that the absorption in the dose range between 2 mg/kg p.o. and 200 mg/kg p.o. is nearly complete. After dermal application, 30-40% of the applied amount

seems to be absorbed and reaches the systemic circulation. The extent of systemic availability of the substance administered by inhalation is not known as specific data are lacking.

BBP is metabolized to monobutyl phthalate or monobenzyl phthalate. This metabolism may take place in the gut wall and/or liver. In adult and immature rats, the ratio of monobutyl phthalate to monobenzyl phthalate found in the urine is 3:1. Both metabolites were found in the bile. Reabsorption from gut lumen may take place. There is no evidence of tissue accumulation. The percentages of excreted metabolites (MBuP and MBeP) in the urine in adult rats were shown to be higher compared to immature rats. The excretion of BBP metabolites in urine has also been studied in humans. Contrary to the metabolism of BBP in rats, BBP is mainly metabolised to MBeP in humans, which indicates that the excretion of MBeP in the urine reflects exposure to BBP. However, limited data on the metabolism of BBP in humans is available.

No half-life of BBP in the body has been calculated. However, the available data indicate a half-life of less than 24 hours.

In the risk characterization, 100% absorption is assumed for both inhalation and oral exposure, whereas the absorption for dermal exposure is set at 5%.

4.1.2.2 Acute toxicity

BBP has been assessed for acute toxicity in rats, mice and one study in rabbits. The acute toxicity information results from oral, dermal or intraperitoneal administration of BBP. There is no acute toxicity data following inhalation exposure. A summary of the acute toxicity data, and a table of the LD_{50} values are included in the end of this section.

Oral

The oral LD_{50} of BBP was determined to be 20,400 mg/kg by administration of (12,600, 15,300, 20,000 and 25,100 mg/kg) undiluted BBP by gavage to young Sprague-Dawley rats (220-240 g bw) (2-3/sex/group). The signs of intoxication were reduced appetite and activity, increasing weakness, collapse and death. The time to mortality was 1-2 days. Histological examination showed haemoragic areas of the lungs, liver discoloration and acute gastrointestinal inflammation. The survivors appeared normal. (Monsanto, 1976a; Hammond et al., 1987; Sibko and Blumenthal, 1973).

LD_{50} of BBP was found to be 2.35 ml/kg bw in rats. Exposure route and range, number, strain and sex of the animals was unknown. (Gupta et al., year unknown).

In an acute toxicity study in white mice and rats BBP was introduced once into the stomach at doses of 6000 to 15,000 mg/kg bw. Symptoms of intoxication developed mainly after 8-12 hours after BBP introduction when given at doses of 6,000 to 9,000 mg/kg bw. Intoxication was manifested as excitation alternating with depression, the development of paresis of the extremities, muscle tension and loss of body weight. The LD_{50} in this study was 13,000 ± 1,770 mg/kg bw. No more information of the study was available (Statsek, 1974).

The LD_{50} values for BBP in corn oil vehicle, calculated 14 days after administration of a single dose by gavage, were 2,330 mg/kg for male and female Fisher 344 rats, 6,160 mg/kg for male $B6C3F_1$ mice and 4,170 mg/kg for female $B6C3F_1$ mice (NTP, 1982a).

Inhalation

No studies were identified.

Dermal

An acute dermal toxicity study was performed by applying undiluted BBP (3,980, 6310 and 10,000 mg/kg) to shaved skin of 1-2 male or female New Zealand rabbits (2.0-2.2 kg bw) per group for 24 hours. The dermal LD_{50} from this study was > 10,000 mg/kg. The animals showed reduced appetite and activity after 2-4 days. No mortality was reported. (Monsanto study, project No. Y-76-54, 1976; Hammond et al., 1987).

In an acute dermal toxicity study in rats, BBP was applied to the skin on the back. In this study the LD_{50} was 6,700 mg/kg, and the local reaction was hyperaemia, maceration of the skin, erosions and fine-lamellar desquamation. No more information of the study was available (Statsek, 1974).

Intraperitoneally

A series of phthalate esters were administered intraperitoneally in Swiss Webster white mice of uniform weight and age in 4 dosage levels ranging from 500 mg/kg to 16,000 mg/kg. The experiment was conducted according to the methods described by Thompson and Weil (1952). LD_{50} of BBP was found to be 3,160 mg/kg (Calley et al., 1966).

In an acute toxicity study 16-18 rats were used to determine a LD_{50} of BBP. BBP was lethal to rats after intraperitoneal administration of doses > 1,800 mg/kg bw or after oral intake of doses > 4,000 mg/kg bw. The animals died after 4 - 8 days showing weight loss, apathy and leucocytosis. Histological examination revealed toxic spleenitis and degenerative lesions of the central nervous system with congestive encephalopathy, myelin degeneration and glial proliferation (Mallette and Von Haam, 1952).

Intraperitoneal injection was used to determine which systemic dose levels of BBP were non-lethal to AKR/JL female mice (age: 6 weeks - 6 months). Fourteen groups of mice with 5 mice per group were given intraperitoneal injections with BBP administered in doses: 500, 1,000, 2,000, 4,000, 5,000, 8,000 and 16,000 mg/kg. The administered volume was related to the amount of BBP, approximately 8 - 300 µl. The death or survival of animals was recorded for nearly three weeks. Control groups were given comparable volumes of 0.15M NaCl. 500 to 4,000 mg/kg of BBP produced no deaths. 60% of the mice given 5,000 mg/kg died and all the mice given 8,000 and 16,000 mg/kg died. There were no deaths in the control groups. The LD_{50} was determined to be between 4,000 mg/kg and 5,000mg/kg (Monsanto, 1983).

4.1.2.2.1 Summary acute toxicity

The acute toxicity of BBP in animals is low. The LD_{50} values are presented in **Table 4.22**. Signs of toxicity include: reduced appetite, weight loss, reduced activity, apathy, leucocytosis, collapse and death. Histological examination revealed toxic spleenitis, haemoragic areas of the lungs, liver discoloration, acute gastrointestinal inflammation and degenerative lesions of the Central Nervous System. The oral LD_{50} values of BBP ranged from 2,330 - 20,400 mg/kg bw for rats, and were 4,170 and 6,160 mg/kg bw in female and male mice. Dermal exposure of rabbits gave a LD_{50} value > 10,000 mg/kg bw. The dermal LD_{50} is in the same range as the LD_{50} values resulting from oral or i.p. administration, thus, supporting the conclusions from the dermal absorption studies with BBP. No information of exposure by inhalation is identified. The wide

range of LD_{50} values in rats after oral administration of BBP (from 2,330 mg/kg to 20,400 mg/kg) may be due to the relatively low water solubility of BBP. The highest LD_{50} value was obtained when BBP was given undiluted by gavage, and the lowest LD_{50} value was obtained when BBP was administered by gavage in corn oil vehicle.

Table 4.22 Acute toxicity

Study design	LD_{50} values	References
Oral		
Rats, Sprague-Dawley; 2-3/sex/group; Administration by gavage; 12,600, 15,300, 20,000 and 25,100 mg/kg.	20,400 mg/kg.	Hammond et al. (1987); Monsanto, (1976a)
Rats; 16-18 by average.	Killed animals at doses > 4,000 mg/kg	Mallette and Von Haam (1952)
Rats, Fisher -344; Administration by gavage.	2,330 mg/kg	NTP (1982a)
Mice, $B6C3F_1$, male and female; Administration by gavage.	Male: 6,160 mg/kg female: 4,170 mg/kg	NTP (1982a)
Intraperitoneally		
Rats; 16-18 in average.	Killed animals at doses > 1,800 mg/kg	Mallette and Von Haam (1952)
Mice, AKR/JL; 5 mice/group.	4,000 mg/kg < LD_{50} > 5,000 mg/kg.	Monsanto (1983); Robinson and Johannsen (1985)
Mice, Swiss Webster; 500 – 16,000 mg/kg.	3,160 mg/kg.	Calley et al. (1966)
Dermal		
Rats. Study poorly reported.	6,700 mg/kg	Statsek (1974)
Rabbits, New Zealand; 1-2/sex/group; 3,980, 6,310, 10,000 mg/kg.	> 10,000 mg/kg.	Hammond et al. (1987); Monsanto study Y-76-54.

4.1.2.3 Irritation

The skin irritating potential of BBP has been assessed in rabbits, mice and humans, and the eye irritation potential in rabbits. There is no data on respiratory irritation. A summary and a table of the various studies are included in the end of this section.

4.1.2.3.1 Studies in animals

Skin

Two - four white rabbits (strain or sex no information) were used for preliminary testing of skin irritability and sensitisation. A patch test was used and the reaction classified as slight, moderate or severe. The concentration of BBP used in white rabbits was 100% BBP. The study reported that BBP had a moderate irritating effect on white rabbits. Further information from the study was not available (Mallette and Von Haam, 1952).

0.2 ml phthalate emulsions in a concentration of 100 mg/ml were injected intradermally into cleanly shaven backs of rabbits. The inflammatory response at the injection site was measured by

injection of 1 ml/kg of 1% trypan blue into the marginal ear vein after an interval of 15 minutes. The irritating response was indicated by degree of dye extravasation and time lapses before appearance of the dye. The response was registered at 10, 15 and 26 minutes after injection and evaluated in a scale: - no colour, negative reaction, 1+ mild, 2++ moderate, 3+++ marked. BBP was negative after 10 minutes. 1+ after 15 minutes and 2++ after 26 minutes. (Calley et al., 1966).

Acute skin irritation was evaluated according to established procedures (Draize et al., 1944) using 2 separate groups of 6 New Zealand rabbits. No irritation was observed after 0.5 ml undiluted BBP was held in continuous 24-hour contact with intact and abraded rabbit skin (Monsanto, 1976a; Hammond et al., 1987).

The irritant level of BBP (1.44 mM, 14.4 mM, 144 mM and 1,440 mM) on AKR mouse ears was studied. BBP was applied epicutaneously to the right ear in 30 µl aliquots. Acetone/corn oil (4:1, 30 µl) was applied to the left ear. No significant changes were observed in ear thickness, in any group after 24 or 48 hours (Monsanto, 1983).

Eye

Acute eye irritation was evaluated according to established procedures (Draize et al., 1944) using 2 separate groups of 6 New Zealand rabbits. Installation of 0.1 ml of undiluted BBP into the conjunctival sac of the rabbit eye produced a slight degree of irritation at 1 and 24 hour, which subsided within 48 hours (Monsanto, 1976a; Hammond et al., 1987).

4.1.2.3.2 Studies in humans

A repeated human insult patch test was performed with BBP to assess its potential as a primary skin irritant/fatiguing agent and/or sensitiser. Patches containing undiluted BBP were applied to the skin of 200 human volunteers for 24 hours, 3 times a week for 5 weeks. Following a 2-week rest period when no patches were applied, human test subjects were re-challenged by applying patches containing BBP for 24 hours to virgin sites of the skin. During the induction and challenge phases, application sites were examined for signs of irritation/sensitisation. Neither primary irritation nor sensitisation reactions were observed in the 200 human volunteers after 24 hours applications, 3 times a week over a 5-week period and a subsequent challenge with BBP (Monsanto, 1980; Hammond et al., 1987).

15 to 30 humans were used for preliminary testing of skin irritability and sensitisation. A patch test was used and reaction classified as slight, moderate or severe. A 10% concentration of BBP was used. BBP showed a slight irritating reaction in 12% of the humans tested (Mallette and Von Haam, 1952).

4.1.2.3.3 Summary irritation

In two old skin irritation studies BBP was reported to have a moderate irritating effect on animals. In the first study little information was available, and in the second study the exposure route was intradermal, an exposure route which is not considered relevant for humans. The studies are therefore not used in the risk characterisation or classification according to EU criteria. In a well conducted Draize study no irritating effect was reported in rabbit skin. In an ear swelling test no irritating potential of BBP was observed. In humans BBP was found to have no skin irritating effect. The eye irritating potential of BBP was studied in rabbits using the Draize

procedures, and a slight eye irritation was reported. No human experience indicating eye irritation due to BBP exposure was located. Based on the available data, and according to EU criteria, BBP does not need to be classified as an irritant to skin or eye.

Table 4.23 Irritation

Study design	Critical effects	References
Skin		
Rabbits; 2-4 animals; Patch test; 100% BBP conentration.	Moderatly irritating.	Mallette and Von Haam (1952)
Rabbits; Intradermal injection (0.2 ml); 100 mg/ml BBP.	10 min no 15 min mild 26 min moderate	Calley et al. (1966)
Rabbits, New Zealand; 6/ group, 2 groups; Draize skin test; 0.5 ml BBP.	Negative.	Hammond et al. (1987); Monsanto (1976a)
Mouse, AKR/JL, epicutaneously injection in ear, 1.44, 14.4, 144 and 1,440 mM.	Negative.	Monsanto (1983)
Humans; 200 human volunteers; patch test; 0.2 ml/patch.	Negative.	Monsanto (1980); Hammond et al. (1987)
Humans; 15-30 humans; 10% concentration. Of BBP; patch test.	Slightly irritating in 12% of the human tested.	Mallette and Von Haam (1952)
Eye		
Rabbits, New Zealand; 6/group, 2 groups; 0.1 ml of BBP.	Slight degree of irritation.	Hammond et al. (1987); Monsanto (1976a)

4.1.2.4 Corrosivity

Based on the results from the irritation studies in animals and humans BBP are considered as not corrosive (see Section 4.1.2.3).

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

In several studies the ability of BBP to induce hypersensitivity in mice or guinea pigs were tested, after intraperitoneal, epicutaneous or foot pad administration using the ear swelling test. The probability of cross-reaction with another chemically related substance phthalic anhydride (PA) was also tested. In these studies the positive control was DNFB (2,4-dinitrofluorobenzene) and negative control acetone/corn oil (4:1). The following studies performed by Monsanto are described below (Monsanto, 1983).

The sensitising effect of BBP in female AKR/JL mice (5/group) was studied after epicutaneous administration. The initiating dose was: 0.036 M or 0.36 M BBP. After 5, 10 or 15 days a challenge dose was applied. The challenge dose was: BBP 0.036 M and 0.36 M. BBP did not induce delayed hypersensitivity in mice. A positive response was elicited with DNFB, whereas the negative control was negative (Monsanto, 1983).

BBP's ability to induce homologous hypersensitivity in female BALB/c mice and to cross-react with PA (phthalic anhydride, a chemical related to BBP, known to be sensitising in humans) was studied after epicutaneous administration. BALB/c mice (5/group) were used to minimize the risk of encountering a genetically unresponsive strain. The initial dose was: BBP 0.00036 or 0.036 M. Challenging doses with BBP or PA (0.36 M) were applied on day 7. Negative controls demonstrated very little change in ear thickness. Positive controls developed definite increase in ear thickness. The experimental groups initiated with BBP and challenged with BBP or PA did not show a positive response (Monsanto, 1983).

The homologous hypersensitivity after intraperitoneal administration of BBP or cross-reactivity with PA was studied in AKR and BALB/c mice (5group). The initiating dose was: BBP, 0.1, 1.0, 10 or 100 μMoles; PA, 0.02, 0.2, 2.2 or 22 μMoles. The challenging dose given by epicutaneous administration was either 0.36 M BBP or PA. BBP did not induce hypersensitivity in AKR or BALB/c mice. No cross reactivity with PA was reported. A positive response was found with DNFB, whereas the negative control was negative in both mice strains (Monsanto, 1983).

The induction of homologous hypersensitivity or cross-reactivity with PA in AKR mice (5/group) or guinea pigs (4/group) were studied after foot pad injection. The initiating dose was: 0.022, 0.22, 2.15 or 21.5 μMoles of BBP in mice and 0.2 μMoles of BBP in guinea pigs in Freud's complete adjuvant (CFA). A challenging dose was applied epicutaneous to the ear either 7 or 15 days after initiation in mice and to abdominal skin 14 days after initiation in guinea pigs. Five weeks later the guinea pigs were re-challenged with BBP or PA. The challenging dose was either BBP or PA (0.36 M in mice or 0.36 M, 0.036 M, 0.0036 M in guinea pigs). Mice or guinea pigs initiated or challenged with BBP or PA did not induce homologous or heterologous hypersensitivity with BBP or PA. In the positive control group a transient response was found in mice, whereas in guinea pigs the response was clearly positive. In the negative control groups no responses were reported (Monsanto, 1983).

BBP (2 and 20 μMoles) or TNP (Tri-Nitro-Phenyl)-BSA (0.04 μMoles TNP and 100 μg BSA) was tested for the induction of antibody formation in AKR mice (5/group). Single injections (0.2 ml) of BBP or TNP-BSA were given intraperitoneal per mouse. The TNP-BSA concentration corresponded to concentrations of TNP known to induced antibody formation without significant anti-BSA responses. To enhance the preferential IgE respons, BBP or TNP-BSA was adsorbed to the adjuvant, alumina (100 mg/ml). Two weeks after injection serum was isolated and assayed for antibody formation by the PCA (passive cutanous anaphylaxis) method. This method makes use of a characteristic of immediate type hypersensitivity i.e. the increased permeability of the post-capillary venules in the skin following antigen-antibody reaction. To produce the PCA reaction, antibody must be fixed to the skin mast cells (sensitisation) before challenged with antigen and intravenous dye to visualize the increased permeability. In this assay rats (2 per serum sample) were used for estimating the presence of antibody in mouse sera. The dorsal skin of the rats were clipped free of hair. Each testserum was injected intradermally, 50 μl per square. Twentyfour hours later, 50 μl of a challenge dose of antigen was given intradermally at the same site as the test antibody. Evans Blue dye was injected intravenously. After 15 minutes the colour changes in each square were observed. Finally, the rats were sacrificed, and the back skin was removed to visualize the reaction on the inside of the skin. One rat was challenged with 200 μMoles BBP, two rats were tested with the AKR antiserum from mice injected with BBP (2 and 20 μMoles BBP). Negative controls included normal AKR mouse serum (NMS) from uninjected mice, BBP (200 μMoles) on non-initiated skin and rabbit anti-TNP antibody. The positive control was Histamine Phosphate injection. The areas of skin which were injected with antibody and challenged with a corresponding antigen developed increased permeability of the post-capillary venules in that

area. This caused the intravenous dye to leak into the tissues surrounding the injection site, forming a blue spot. TNP-BSA immunisation induced IgE anti-TNP and a strong and consistent PCA respons was observed. The results from the BBP exposed group were equivocal. Inconsistency between the results in the two rats with these sera may be due to that blueing occurred as a result of local trauma (needle puncture site of BBP challenge injection only 15-20 minutes before injection of Evans Blue) or due to a local vasodilatory effect of the very high concentration of BBP. The positive control group, challenged with histamine, was positive in both rats tested. The rats challenged with Rabbit anti-TNP which is known to contain only IgG anti-TNP molecules produced negative or only trace positive results because IgG affinity for rat mast cells is sufficiently low as to permit their diffusion away from the test site within a few hours. It was commented in the study that it is easy to get false positive reactions in the PCA assay due to diverse mechanical or chemical stimuli that may results in enhanced vascular permeability at the test site, therefore, the interpretation of the data was difficult. Since no protein-conjugate could be formed with BBP (this was tested in a separate study, and it was concluded from this study that since BBP did not produce hapten-protein complexes when assayed with serum albumine, there was strong evidence that BBP is incapable of inducing immune hypersensitivity), it was necessary to inject BBP directly into the skin as a potenital challenge "antigen" to combine locally with the previously injected "antiserum". Diluted BBP was preferred, but solvents to be used (aceton, corn-oil or DMSO) were known to give false positive PCA reactions. Therefore, the experiment with BBP was having two highly suboptimal features; two injections were required at each test site, and the required concentration of BBP was undiluted BBP. These factors could have been responsible for the unexpected but inconsistent blueing reactions seen at BBP test sites. Due to the difficulties with the interpretention of the results, no conclusions can be drawn from this study on BBPs ability to induce antibody in AKR mice (Monsanto, 1983).

Assessment of sensitisation was performed two weeks after a primary irritation test on 2-4 white rabbits (no information on strain or sex) with a 100% concentration of BBP. BBP had a slight sensitising effect in rabbits when applied 2 weeks after the primary irritation test. Reactions were classified as slight, moderate and severe (Mallette and Von Haam, 1952).

4.1.2.5.2 Studies in humans

Assessment of sensitisation was performed two weeks after a primary irritation test on 15-30 humans with 10% concentration of BBP. BBP had no sensitising effect in humans (Mallette and Von Haam, 1952).

A repeated human insult patch test was performed with BBP to assess its potential as a primary skin irritant/fatiguing agent and/or sensitiser. Patches containing undiluted BBP were applied to the skin of 200 human volunteers for 24 hours, 3 times a week for 5 weeks. Following a 2-week rest period when no patches were applied, human test subjects were re-challenged by applying patches containing BBP for 24 hours to virgin sites of the skin. During the induction and challenge phases, application sites were examined for signs of irritation/sensitisation. Neither primary irritation nor sensitisation reactions were observed in the 200 human volunteers after 24 hours applications, 3 times a week over a 5-week period and a subsequent challenge with BBP (Monsanto, 1980; Hammond et al., 1987).

In a study by Bornehag et al. (2004) the potential association between persistent allergic symptomes in children and the concentration of phthalates in dust collected from their homes (single family houses) was studied. The investigation was a case control study nested within a cohort of 10,852 children. From the cohort 198 cases with persisten allergic symptomes and

202 controls without allergic symptomes were selected. In this study a higher median concentration of BBP was found among cases than among controls (0.15 versus 0.12 mg/g dust). When the case group was breaked down by allergic symptomes BBP was associated with rhinitis (p=0.001) and eczema (p=0.001) while higher levels of diethylhexyl phthalate (DEHP) in hous dust was associated with asthma (p=0.022). The concentration of DEHP in house dust among controls were 0.723 mg/g dust versus 0.828 mg/g dust among cases. No assocoation between levels of diethyl phthalate, diisobutyl phthalate, dibutyl phthalate and diisononyl phthalate and allergic symptomes in childern was found. In this study demographic factors such as levels of education, family income and recidency were not considered as well as pet ownership. Koo et al. (2002) has in a recent study indicated that there may be significant demographic variations in exposure and/or metabolism of phthalates. The only information in the Bornehag et al. (2004) study was that the analysis was resticted to single family houses. Due to these limitations, no clear conclusion can be drawn from the study on the relationship between BBP in house dust and allergic symptoms in children.

4.1.2.5.3 Summary sensitisation

In an old study BBP had a slight skin sensitasing effect in rabbits. It appears that BBP was negative in the various studies performed to assess a skin sensitisation potential of BBP using the ear swelling test in mice and guinea pigs. However, the test has not been fully evaluated and no standard protocols are available. Equivocal results were obtained when BBP was tested for antibody formation in mice. Furthermore, BBP did not form hapten-protein complexes which indicate that BBP is incapable of inducing immune hypersensitivity. No skin sensitisation was reported in two human studies with BBP. In a case-control study an association was found beetween childeren exposed to BBP in house dust and cases of allergic symptoms in children. However, in this study very small differences were found in the concentrations of BBP in house dust from controls and cases of allergic symptoms. Furthermore, demographic factors and pet ownership was not considered in this study. Based on the available data and according to EU criteria BBP does not need to be classified as a sensitiser.

4.1.2.6 Repeated dose toxicity

BBP has been assessed for potential toxic effects following repeated exposure almost exclusively in rats. Only one study in mice and a study in dogs were reported. Most of the available toxicological information results from oral exposure. However, a few inhalation studies and one dermal study were located. A summery of the most important findings in the repeated dose toxicity studies, and a table (**Table 4.24**) of the critical effects are included in the end of this section.

Oral

In two 2 weeks range finding studies groups of six male rats were given BBP in corn oil by gavage at doses of 160, 480 and 1,600 mg/kg bw/day. In the first study (1) Sprague-Dawley rats were given 160, 480 or 1,600 mg/kg bw/day of BBP, and in the second study (2) Wistar rats were given 480 or 1,600 mg/kg bw/day of BBP. In both studies histopathologic examination was performed on liver and testes. No mortality was reported. Growth retardation was reported at 1,600 mg/kg bw/day of BBP. In the first study testicular atrophy was reported in the highest dosage group, whereas dose-related histopathologic changes were reported from 480 mg/kg bw/day of BBP, however, at the 480 mg/kg bw/day BBP dose the changes were

minimal (in 1/6 animals > 5% of the tubules affected). Significant liver enlargement (120% of control) and reduced testis weight was reported at 1,600 mg/kg bw/day. Liver sections from animals receiving the highest dosage of BBP revealed ultrastructural changes with an increase in the number of peroxisomes. In the second study testicular atrophy was only reported in the highest dosage group. Microscopic changes in the liver were not determined (Hammond et al., 1987; Lake et al., 1978).

In a 4-week range finding study, groups of 5-10 Sprague-Dawley rats of each sex, approximately 4-7 weeks of age, were used to study the toxicity of BBP after oral administration in feed at doses of 500, 1,000, 1,500, 2,000, 3,000 and 4,000 mg/kg bw/day. Mortality was observed in male rats in the higher dosage group i.e. 2/5 at 1,500 mg/kg bw/day, 8/10 at 2,000 mg/kg bw/day, 7/10 at 3,000 mg/kg bw/day and 9/10 at 4,000 mg/kg bw/day. Dose-related growth retardation was observed at dose levels generally greater than 1,000 mg/kg and were usually more pronounced in male rats than in female rats. Adverse reactions were observed in males and occasionally in females at 2,000 – 4,000 mg/kg bw/day of BBP in the diet. At these doses, some animals exhibited stiffness of the hindlimbs while walking; bleeding around the nares was also observed in high dose animals. Dehydration and blue discoloration and/or inflammation of the extremities were observed in high-doses animals that died during the study. These animals also exhibited gross and microscopic evidence of widespread haemorrhaging in body tissues. Treatment-related gross pathological changes were restricted to high-dose animals and included testicular atrophy (in animals that died). Histopathologic changes were observed in a dose-related manner in the testes from 1,500 mg/kg bw/day. The few high-dose animals that survived were allowed to recover for 4 weeks. No microscopic evidence of haemorrhaging was apparent in these animals sacrificed after the 4-week recovery period although testicular atrophy was still evident in a few animals. The NOAEL from this study was 1,000 mg/kg bw/day of BBP (Hammond et al., 1987).

In a 28-day repeated dose toxicity study in young Cpb-WU male rats (28 days old), rats were treated daily with BBP by gavage (Piersma et al., 2000). The study was a part of a developmental toxicity study reported under section “Developmental studies, BBP”. The BBP doses used were 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg bw/day, with three animals per group. Necropsy was performed one day after the last dosage. The liver, kidney, thymus, thyroid, spleen and testis were weighted and processed for histology. The results were as follows: No changes in food consumption were reported after exposure to BBP. Body weight gain was decreased from 1,250 mg/kg bw/day. However, the decrease was not statistically significant. Relative liver weight was statistically significantly increased from 750 mg/kg bw/day (at 750 mg/kg bw/day 6.41, at 970 mg/kg bw/day 6.45, at 1,250 mg/kg bw/day 6.09, at 1,600 mg/kg bw/day 6.66, and at 2,100 mg/kg bw/day 6.64 compared to control value at 5.16). Liver PCoA as an index of peroxisome proliferation showed a similar response. A dose-depended increased trend in relative kidney weight was reported from 750 mg/kg bw/day. However, the increase was not statistically significant. No statistically significant decrease in thymus and thyroid weight was reported, however, a trend towards a decrease was observed. No effects in spleen were reported. In testis a dose-related decrease was reported in relative testis weight from 750 mg/kg bw/day, however, the decrease was statistically significant first at 1,250 mg/kg bw/day (at 1,250 mg/kg bw/day 0.41, at 1,600 mg/kg bw/day 0.28, and at 2,100 mg/kg bw/day 0.24, compared to control value at 1.15, data are given as percentage of body weight). Histopathologic analysis of the testis revealed severe atrophy from 970 mg/kg bw/day. FSH was significantly increased from 1,250 mg/kg bw/day (401 μg/l at 1,250, 532 μg/l at 1,600 mg/kg bw/day and 449 μg/l at 2,100 mg/kg bw/day, compared to control rats with 194 μg/l) and a statistically significant decrease in testosterone level was reported from 450 mg/kg bw/day (at 450 mg/kg bw/day 1.7 μg/l, at 580 mg/kg bw/day 2.0 μg/l, at

750 mg/kg bw/day 3.8 μg/l, at 970 mg/kg bw/day 2.7 μg/l, at 1,250 mg/kg bw/day 1.8 μg/l, at 1,600 mg/kg bw/day 2.7 μg/l, and at 2,100 mg/kg bw/day 1.1 μg/l compared to control level at 9.1 μg/l). The overall NOAEL for systemic toxicity, based on increased liver weight was 580 mg/kg bw/day.

In a sub-chronic rat feeding study groups of 10 Sprague-Dawley rats of each sex were fed diets containing 2,500 to 20,000 ppm of BBP (approximately 188, 375, 750, 1,125 and 1,500 mg/kg bw/day) for three months. A few deaths occurred randomly; however, since the mortality was not dose related, it was not likely to be attributed to BBP treatment. A reduction in body weight at 1,500 mg/kg bw/day of BBP in both male and female rats was reported, however, this was not compound related, since food consumption was not routinely measured, so that it was not possible to determine whether the reduction in body weight gain was related to changes in food consumption. A pair feeding study was also performed and the results from this study indicated that at the highest exposure levels the reduced body weight gain was caused by a reduction in food consumption, presumably related to unpalatability of the diet. No compound-related clinical changes were reported. A significant increase in relative liver weight was evident in females at 750 mg/kg bw/day and higher (16% increase at 750 mg/kg bw/day, 25% increase at 1,125 mg/kg bw/day, and 31% increase at 1,500 mg/kg bw/day), and in males at 1,125 mg/kg bw/day and higher (19% increase at 1,125 mg/kg bw/day, and 29% increase at 1,500 mg/kg bw/day). A significant increase in relative kidney weight was only evident in females at 750 mg/kg bw/day and higher (16% increase at 750 mg/kg bw/day, 25% increase at 1,125 mg/kg bw/day, and 31% increase at 1,500 mg/kg bw/day). No compound related histopathological changes in the liver, testes and pancreas were reported. The NOAEL based on altered liver weight in males was 750 mg/kg bw/day and in females 375 mg/kg bw/day of BBP in this study (Hammond et al., 1987).

In another 3 months sub-chronic study, groups of 27-45 Wistar rats of each sex were fed diets containing 2,500 to 12,000 ppm BBP (approximately 151, 381 and 960 mg/k bw/day). A few deaths occurred randomly. Since the mortality not was dose related, it was not considered to be related to BBP treatment. A reduction in body weight gain was reported in low-, mid- and high-dose groups, however, only at the highest dose level the reduction was shown to be compound related, since food consumption was reduced in the low- and mid dose group, however, at the highest dose group no reduction in food consumption was apparent. A slight anaemia at high-dose and decreased urinary pH at mid- and high-dose in male rats were reported. A significant increase in relative liver weight at 960 mg/kg bw/day (28% increase relative to control rats) and an increase in the relative kidney weight at 381 mg/kg bw/day (8% increase relative to control rats) and at 960 mg/kg bw/day (13% increase relative to control rats) was reported in males. In females a significant increase in relative liver and cecum weight was reported at all dose levels, (liver: 4% increase at 151 mg/kg bw/day, 5% increase at 381 mg/kg bw/day, and 21% at 960 mg/kg bw/day; cecum: 12% increase at 151 mg/kg bw/day, 19% increase at 381 mg/kg bw/day, and 27% increase at 960 mg/kg bw/day) whereas in the kidneys only at 381 mg/kg bw/day and higher (8% increase at 381 mg/kg bw/day, and 19% increase at 960 mg/kg bw/day). Gross pathological changes were limited to an increased incidence of red spots in the liver at 381 mg/kg bw/day and higher in male rats. Histopathologic changes were reported in the pancreas in males from 381 mg/kg bw/day and in the liver at 960 mg/kg bw/day. The changes in the liver were evident as small areas of cellular necrosis. In the endocrine pancreas cell vacuolization, peri-islet congestion and in some instances peri-islet inflammatory cell infiltration together with slight fibrosis was reported. Changes in the exocrine pancreas were less frequent and included occasional pycnotic nuclei, acinar atrophy and periacinar inflammatory cell infiltration. No histopathologic changes were reported in females. The overall NOAEL for systemic toxicity, based on histopathological changes in pancreas, gross

pathological changes in the liver, and significant increase in relative kidney weight was 151 mg/kg/day (Hammond et al., 1987).

In a sub-chronic study, groups of 3 male and 3 female beagle dogs were fed diets containing 10,000 to 50,000 ppm BBP (males approximately 400, 1,000 and 1,852 mg/kg bw/day, females approximately 700, 1,270 and 1,973 mg/kg bw/day) for 3 months. Adult animals of unspecified age were used. Haematologic evaluation, urinalyses and liver and kidney function tests were performed on all animals at monthly intervals. All animals were subjected to a gross necropsy and histopathologic examination was performed on approximately 30 tissues from high dose and control animals. No adverse reactions were reported. A decrease in body weight gain was reported in the highest dose group in male dogs (260% reduction relative to control animals), and in the two highest dose groups in females (130% reduction at 1,270 mg/kg bw/day, and 266% reduction at 1,973 mg/kg bw/day relative to control animals). However, food palatability complicated the interpretation of the reduced body weight in male and female dogs. Due to the poor palatability, dosing of high dosage animals was switched to capsule dosing at study day 39. The amount of BBP was equivalent to what would have been ingested in the diet. Body weight increased after initiation of capsule dosing but remained depressed relative to controls of the remainder of the study. No compound related clinical or histopathologic changes were reported. Due to the poor food palatability of the test diets, and since no adverse reactions of BBP were reported the NOAEL is set to 1,852 mg/kg bw/day for males and 1,973 mg/kg bw/day for females (Hammond et al., 1987).

In a 90-day sub-chronic toxicity study in B6C3F1 male and female mice, the animals were exposed to approximately 240, 464, 946, 1,875 and 3,750 mg/kg bw/day of BBP in the diet. No adverse toxicological effects were observed at any BBP doses studied. However, a decrease in body weight gain was observed at all doses tested in male mice (14% reduction at 240 mg/kg bw/day, 22% reduction at 464 mg/kg bw/day, 23% reduction at 946 mg/kg bw/day, 25% reduction at 1,875 mg/kg bw/day, and 35% reduction at 3,750 mg/kg bw/day compared to control mice) and at 1,875 mg/kg bw/day and higher in female mice (22% at 1,875 mg/kg bw/day, and 19% at 3,750 mg/kg bw/day compared to control mice). The LOEL for male mice was 240 mg/kg bw/day of BBP, and the NOEL for female mice was 946 mg/kg bw/day of BBP in this study based on decreased body weight gain (NTP, 1982b; Hammond et al., 1987).

In a 26-week dietary toxicity study male Fisher F344/N rats (15/group) were given 0, 300, 900, 2,800, 8,300 and 25,000 ppm BBP corresponding to 30, 60, 180 and 550 or 1,660 mg/kg bw/day. At the end of the study sperm morphology was analysed in the control, 2,800, 8,300 and 25,000 ppm group. On days 30, 60, 90, 120, 150 and at the end of the study, blood was collected from all rats. A necropsy was performed on all rats. The brain, heart, right kidney, liver, lung, prostate gland, seminal vesicle, right testis and thymus were weighed. A complete histopathologic examination was performed on all control and 25,000 ppm exposed rats. In addition, the epididymis, prostate gland, seminal vesicle, and testis were examined in all other exposure groups. The results from the study were: (1)- No deaths attributable to BBP exposure were reported, (2)- the final mean body weight was significantly lower in the 25,000 ppm group compared to control animals (365 g in control animals and 254 g in the 25,000 ppm exposed group), (3)- no clinical findings in any exposed groups related to BBP exposure were reported, and (4)- absolute and relative liver weight was significantly increased at 550 mg/kg bw/day, (absolute14.26, relative 37.92 compared to control rats 12.18 and 33.24), and at 1,660 mg/kg bw/day (relative 47.23 compared to control rats 33.24), (5) alterations in haematology values including; an exposure related anaemia in the 25,000 ppm group at all time points, statistically significant sporadically minimal erytrocyte count decreases in the 2,800 ppm

group (on day 150, 7.74 · 10^6/μL vs controls at 7.79 · 10^6/μL) and in the 8,300 ppm group (on day 60, 7.23 · 10^6/μL vs controls at 7.50 · 10^6/μL and on day 120, 7.81 · 10^6/μL vs controls at 8.05 · 10^6/μL), and statistically significant increases in cell haemoglobin concentrations in the 8,300 and 25,000 ppm group. Effects on the reproductive system associated with feeding BBP at 2.5% to male rats included; decreased testicular size and abnormal morphology; histopathologic evidence of virtual aspermia, and correlating total suppression of male reproductive capacity, accompanied with histopathologic changes in the epididymis and prostate. These effects were not reported in control animals (see also Section 4.1.2.9). The NOAEL for systemic toxicity was 2,800 ppm corresponding to 180 mg/kg bw/day based on an increase in cell haemoglobin concentration, that may be associated with the exposure related anemia observed at the highest dose tested, and increased relative liver weight. (As regards the NOAEL value for effects on the reproductive organs, see Section 4.1.2.9) (NTP, 1997). This study was performed in compliance with GLP.

In a 10-week modified mating study groups of 15 F344/N male rats were fed diets containing 0, 300, 2,800 and 25,000 ppm BBP corresponding to 0, 20, 200 or 2,200 mg/kg bw/day of BBP for 10 weeks.After 10 weeks the rats were allowed to recover for 2 days followed by a 7 days mating period and then necropsied. The effects on the reproductive organs are described in Section 4.1.2.9. A haematologic evaluation was performed on all exposed rats. The heart, brain, right kidney, liver, lung, prostate gland, seminal vesicle, right testis, and thymus were weighed. Histopathologic examinations were performed on 0 and 25,000 ppm male rats. In addition, the epididymis, prostate gland, seminal vesicle, and testis were examined in all other exposure groups. All rats survived to the end of the study. The final mean body weight gain in the 25,000 ppm group was significantly lower than those of controls (226 g compared to control rats 320 g). No clinical findings related to BBP exposure were noted. Changes in absolute and relative organ weight changes were only reported in rats exposed to 25,000 ppm BBP. Haematological changes occurred at 25,000 ppm. These included a statistically significant decrease in erythrocytes (7.25 · 10^6/μL versus 8.2 · 10^6/μL in control animals), a significant increase in mean cell haemoglobin (21.5 pg versus 19.7 pg in control animals), and a significant increase in platelets (963.5 · 10^3/μL versus 755.1 · 10^3/μL in control animals) There was some evidence of a minimal anemia. The NOAEL for systemic toxicity based on effects on haematologic parameters, and organ weight changes was 200 mg/kg bw/day. (As regards the NOAEL value for effects on the reproductive organs, see Section 4.1.2.9) (NTP, 1997). This study was performed in compliance with GLP.

In a two-year carcinogenicity study, F344 rats were fed 6,000 or 12,000 ppm BBP (corresponding to 360 and 720 mg/kg bw) 7 days/week, for 28 or 103 weeks. Groups of 50 male and female rats were used per dose level. Mean body weights of dosed female rats were lower than those of controls during most of the study. After week 14, an increasing number of dosed males died as a result of unexplained internal bleeding. Consequently, all living male rats were killed at 29 to 30 weeks. Thus, male rats were not adequately tested for carcinogenicity. No NOAEL for systemic effects in female rats could be established from this study. The LOAEL value was 360 mg/kg bw based on decreased body weight gain (NTP, 1982b). For further study description see Section 4.1.2.7.

In a recent NTP (1995) carcinogenicity study, male F344/N rats were fed 3,000, 6,000, 12,000 ppm (120, 240, 500 mg/kg bw) and females 6,000, 12,000, 24,000 ppm (300, 600, 1,200 mg/kg bw) of BBP for 24 months 7 days/week (NTP, 1997). Sixty animals were exposed per dose group. Survival of all exposed groups of male and female rats was similar to that of controls. There were no clinical findings in exposed groups of rats related to BBP exposure. At 12,000 ppm a decrease in the body weight of males and at 24,000 ppm in females, was noted. Mean

body weights of 3,000 and 6,000 ppm males and of 6,000 and 12,000 ppm females were similar to those of the controls throughout the study. In general, haematological changes were sporadic and minor. At 6 months, a minimal decrease in erythrocyte count and increase in mean cell haemoglobin occurred in male rats at 12,000 ppm. In females, a decreased haematocrit value occurred at 15 months in the 24,000 ppm exposure group. There was also a mild decrease in triiodothyronine concentrations in the 24,000 ppm females at 6 and 15 months and at the end of the study. The absolute right kidney weight of 12,000 ppm females and the relative right kidney weights of all exposed groups of males were increased (3.83 g at 120 mg/kg bw., 3.88 g at 240 mg/kg bw and 4.10 g at 500 mg/kg bw, compared to 3.52 g for controls) and the kidney weight of the 24,000 ppm females wase significantly greater than those of the controls at the 15-month interim evaluation. The severities of renal tubule pigmentation in 12,000 ppm males and in 24,000 ppm females were greater than those in the controls at 15 months and 2 years. At 2 years, the incidence of kidney mineralisation (0 ppm, 43/50; 6,000 ppm 34/50; 12,000 ppm 37/50; 24,000 ppm 35/50) in 6,000 ppm and 24,000 ppm females was significantly less than that in the controls. The incidences of nephropathy (34/50, 47/50, 43/50, 45/50) in exposed groups of females was significantly higher from 300 mg/kg bw after 2 year. An increased incidence of transitional epithelial hyperplasia after 2 years (0/50, 3/50, 7/50, 4/50) in the kidney was only significant at 600 mg/kg bw. The NOAEL value in male rats was 240 mg/kg bw based on a significant increase (more than 10%) in relative kidney weight at 500 mg/kg bw. The LOAEL value in female rats was 300 mg/kg bw based on nephropathy. For further study description see Section 4.1.2.7.

Neurotoxicity studies

In a 6-week range finding study groups of 10 Charles River CD rats of each sex, were used to study the neurotoxicity of BBP after oral administration in feed at doses of 500, 1,500 and 3,000 mg/kg bw/day. Histopathologic examination was performed on selected tissues from the central and peripheral nervous system. No mortality was reported. A transient stiffness when walking was observed at 3,000 mg/kg bw/day of BBP. No histopathologic changes were reported in the central nervous system (Robinson, 1991).

A 42-day study was conducted on laying hens. Three groups of 10 hens were treated orally with 5,000 mg/kg bw/day of BBP or 500 mg/kg bw/day tri-o-tolyl phosphate (positive control) for three consecutive days at the initiation of study. This schedule was repeated 21 days later. All birds dosed with tri-o-tolyl phosphate showed symptoms of neurotoxicity within 11-18 days following dosing. There were no gross symptoms detected in association with BBP exposure. No other toxic symptoms were evident (Monsanto, 1992).

Peroxisomal Proliferation

In a 21-day feeding study with Fisher rats (5/sex/group) the effects of BBP (0, 0.6, 1.2 or 2.5%) on the liver and liver lipids were studied A fifth group of five rats/sex, fed 1.2% DEHP, served as a positive study control. The absolute weight and relative weight of the liver were increased in all treated animals, while treatment with 2.5% BBP caused significantly lower testes weight with severe testicular atrophy. There was a reduction in cytoplasmic basophilia in the livers of rats given 2.5% BBP, and lower neutral lipid levels in female rats at all levels, and in males given 0.6% BBP. Serum triglyceride levels were lower in the treated males and higher in the treated females, while cholesterol levels were reduced in both sexes. Cyanide-insensitive palmitoyl-CoA oxidation levels increased in a dose-dependent manner, as well as lauric acid 11- and 12-hydroxylation. Electron microscopic examination of liver sections from two rats of each sex from the 2.5% BBP group (the only concentration tested) showed a moderate increase in

peroxisome numbers and size. The effects of DEHP at 1.2% were similar in type and magnitude to those previously reported (BIBRA, 1985). The LOAEL from this study was 0.6% BBP corresponding to a mean intake of 639 mg/kg bw/day of BBP in the diet (Barber et al., 1987). This study was performed in compliance with GLP.

In a 28-day oral study BBP was fed to groups of 5 male Fisher 344 rats at dietary levels of 0 (control), 0.01, 0.05, 0.1, 0.5 and 1.0% BBP. A further group of 5 male rats was fed 1.0% DEHP in the diet as a positive control. There were no statistically significant reductions in body weight after 28 days of treatment in rats fed 0.01 - 0.1 and 1.0% BBP or 1.0% DEHP in the diet. Liver weight, both absolute and relative to body weight, was statistically significantly increased at levels of 1.0% BBP and 1.0% DEHP. Testes weight was not affected by treatment of BBP or DEHP. At 1.0% BBP a small, but significant increase in whole homogenate protein content was reported. Hepatic palmitoyl-CoA oxidation activity was statistically significantly increased only at levels of 1.0% BBP and 1.0% DEHP in the diet. An increase in hepatocyte eosinophila was observed only in animals receiving 1.0% DEHP. No histopathologic changes were reported after exposure to BBP. The NOAEL observed in this study for induction of palmitoyl-CoA oxidation (as an index of hepatic peroxisome proliferation) was 0.5%, corresponding to a mean intake of 540 mg BBP/kg bw/day (BIBRA, 1992). This study was performed in compliance with GLP.

Female Fisher 344 rats were fed diets containing 0 (control), 0.6, 1.2 and 2.4% BBP for 12 months. Evidence of peroxisome proliferation in the liver, as measured as activities of carnitine acetyltransferase (CAT) and palmitoyl-CoA oxidation (PCoA), was present at 0.6% and above when measured as CAT, and 1.2% and above when measured as PCoA, after both 1 and 12 months of treatment. There was no evidence that treatment with BBP produced cell proliferation in the liver at any of the times examined (i.e. after 1 week, 3 months and 12 months of treatment). The immunotoxicity studies indicated that no significant immune suppression or enhancement was observed in rats treated with BBP for 1 and 12 months. The LOAEL for evidence of peroxisome proliferation was 0.6% BBP in the diet in this study (Monsanto, 1994).

Inhalation

In a 4-week inhalation study BBP was given as an aerosol-vapour to four groups of 20 male and female Spargue-Dawley rats per group (6-8 week old). The animals were exposed in a 1,750 L inhalation chamber 6 hours/day, 5 days/week for 4 weeks. Analytically determined exposure levels were 0, 360, 1,000 and 2,100 mg/m^3 of BBP. Approximately determined particle size was as following; 15% from 9 to 4.7 micron, 70% from 3.3 to 1.1 micron, and 15% from 0.7 to less than 0.4 micron. Toxicological effects, such as a statistically significant decrease in body weight gain (33% for males and 13% for females), death (3/20 males and 4/20 females), and in males atrophy of the spleen, and reproductive organs (see also Section 4.1.2.9), were seen only in the high exposure group. No organ weights were determined. The NOAEC was 1,000 mg/m^3 of BBP in this study (Monsanto, 1981). This study was performed in compliance with GLP.

In another 4-week inhalation study, BBP was given as an aerosol/vapour to four groups of 5 male or female Sprague-Dawley rats per group (6-8 week old). The animals were exposed in a 500 L inhalation chamber 6 hours/day, 5 days/week for 4 weeks. Analytical determined chamber concentrations were 0, 49, 144 and 526 mg/m^3 of BBP. Body weight gain was reduced (17-19%) relative to control animals at 526 mg/m^3 of BBP of both sexes. Clinical parameters were not affected by BBP treatment and no changes in organ weights or microscopic abnormalities were observed. The NOAEC from this study was 144 mg/m^3 of BBP (Hammond et al., 1987).

In a 13-week inhalation toxicity study, Santicizer 160 (BBP) was given as an aerosol-vapour to four groups of 25 male and female Sprague-Dawley rats per group (6 to 8 week old). The

animals were exposed in a 10,000 L inhalation chamber 6 hours/day, 5 days/ week for 13 weeks. Exposure levels were analytically determined as 0, 51, 218 and 789 mg/m^3 of BBP. Approximately determined particle size was as following; 12% from 9 to 4.7 micron, 80% from 4.7 to 1.1 micron and 8% from 1.1 to less than 0.4 micron. No changes in body weights relative to control animals were observed. Clinical observations of urine-stained fur, piloerection and alopecia were reported at 218 and 789 mg/m^3 in both sexes, however, these findings were sporadic and inconsistent, and were not considered as reliable indicators of toxicity of the test compound. Significant increase in absolute and/or relative organ weights were observed for the liver and kidney at 789 mg/m^3 in both sexes (male 21 and 15% increase and female 12 and 15% increase,), whereas a significant increase in kidney weigh at 218 mg/m^3 was only reported in males at interim sacrifice. Since no changes in body weight was reported, the increased weight of liver and kidney was considered to be treatment related. A marked decrease in serum glucose was observed in males only at 789 mg/m^3. No compound-related macroscopic or microscopic lesions were detected in any tissues, including kidneys and liver, from all animals. The NOAEC for both sexes of rats exposed by inhalation to BBP 6 hours/ day, 5 days/week for 13 weeks was 218 mg/m^3 in this study (Monsanto, 1982). This study was performed in compliance with GLP.Dermal

A dermal toxicity study was carried out with repeated skin applications of BBP at doses of 1, 5, 10 and 100 mg/kg for 5 months (no information regarding the species used). BBP was reported to have a local-irritating action. No mortality was observed during the study. The threshold dose of BBP was 1 mg/kg when applied repeatedly. The study was poorly reported (Statsek, 1974).

4.1.2.6.1 Studies in humans

Workers were exposed to phthalic acid esters (PAE) by inhalation, mainly DEHP, DIDP or BBP in the polyvinyl chloride processing industry (Nielsen et al., 1985). A group of 54 workers were studied. The average age was 38 years (range 21-64) and the subjects had been employed for an average of eight years (range 1-21). Two hours samples were obtained on glass fiber filters by personal sampling and analysed by high pressure liquid chromatography (HPLC). The detection limit was 0.01 mg/m^3. The exposure ranged from 0.02 to 2 mg/m^3 PAE in different job categories. The workers excreted slightly but sinificantly higher levels of PAE metabolites in urine (U-PAEM) than controls, 25, 23 or 18 μmol/L U-PAEM in exposed workers, compared to a control level at 17 μmol/L. In 54 workers studied clinically, there were no indications of peripheral nerve or respiratory system effects, however, some biochemical tests were abnormal but within the normal reference range, the only exception was S-IgA in subjects heavily exposed in the last year. However, since the workers were exposed to a mixture of phthalates these data are of limited value in the risk characterisation of BBP.

The health status was studied in workers exposed to phthalate plasticizers, predominantly DBP and higher alkyl phthalates (DAP-789) or periodically dioctyl phthalate (DOP), diiso octyl phthalate (DIOP) and BBP (Milkov et al., 1973). A group of 147 workers (87 women and 60 men) were studied. The majority (75%) was not older than 40 years. The duration of occupational contact with the plasticizer was 0.5 to 5 years for 54 workers, 6-10 years for 28 workers, and > 10 years (up to a maximum of 19 years) for 65 workers. The ambient levels of vapours or aerosols of the plasticizers (mixed esters) at the working zone ranged from 1.7 to 66 mg/m^3. In the workers a moderately pronounced toxic polyneuritis was found, the frequency and degree increased with increasing working time. A study of the sensory functions revealed an early lowering of the excitability of the vestibular and olfactory receptors and cutaneous sensitivity. However, this study is of limited value in the risk characterisation of BBP since the

exposure to BBP only was periodically and the main exposure was to DBP or higher alkyl phthalates.

4.1.2.6.2 Summary Repeated Dose Toxicity, oral and inhalation

Repeated exposure of rats to BBP resulted in decreased body weight gain, alterations in haematological parameters, and damage to the testes, epididymis, prostate, liver, kidney, spleen and pancreas. In mice and dogs the only reported effects are a reduction in body weight gain. The derived NOEL/NOAEL/NOAEC/LOAEL of the various studies is given in **Table 4.24**.

Table 4.24 Repeated dose toxicity

Study design	Effect level	Critical effect	References
Oral			
Sprague-Dawley rats; 6 male/group; 14 days; gastric intubation; 160, 480, 1,600 mg/kg bw/day		At 480 mg/kg bw/day histopathologic changes in testis in 1/6 rats. At 1,600 mg/kg bw/day body weight decrease, liver enlargement and ultrastructural changes with an increase in peroxisome numbers in the liver. Testicular atrophy and decreased testis weight.	Lake et al. (1978)
Charles River CD rats; 10/sex/group; 6 weeks; Administration in diet; 500, 1,500, 3,000 mg/kg bw/day.		No evidence of neurologic impairment.	Robinson (1991)
Sprague-Dawley rats; 5-10/sex/group; 4 weeks; Administration in diet; 500, 1,000, 1,500, 2,000, 3,000 and 4,000 mg/kg bw/day	NOAEL: 1,000 mg/kg bw/day	At doses ≥ 1,500 mg/kg bw/day body weight decrease. From 1,500 mg/kg bw/day testicular atrophy. From 2,000 mg/kg bw/day stiffness while walking and bleeding around nares.	Hammond et al. (1987)
Wistar rats; 10/sex/group; 2 weeks; gastric intubation; 160, 480 and 1,600 mg/kg bw/day		At 1,600 mg/kg bw/day body weight decrease and testes atrophy.	Hammond et al. (1987)
Cpb-WU male rats; 4 weeks of age; Administration of BBP by gavage 28 days; 3/group; 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600, 2,100 mg/kg bw/day.	NOAEL for systemic toxicity (increased liver weight): 580 mg/kg bw/day	Liver weight increase from 750 mg/kg bw/day. A dose related decrease in testis weight from 750 mg/kg bw/day, however, statistically significant from 1,250 mg/kg bw/day. Severe testicular atrophy from 970 mg/kg bw/day. Decreased testosterone levels from 450 mg/kg bw/day.	Piersma et al. (2000)
Sprague-Dawley rats; 10/sex/group; 3 month; Administration in diet; 2,500 - 20,000 ppm (corresp. to approx. 188, 375, 750, 1,125, 1,500 mg/kg bw/day)	NOAEL female: 375 mg/kg bw/day NOAEL male: 750 mg/kg bw/day	At doses ≥ 750 mg/kg bw/day kidney and liver weight increase in females, at doses ≥ 1,125 mg/kg bw/day liver weight increase in males.	Hammond et al. (1987)

Table 4.24 continued overleaf

Table 4.24 continued Repeated dose toxicity

Study design	Effect level	Critical effect	References
Oral			
F344 rats; 50/sex/group; 2 years: Administration in diet; 600, 12,000 ppm corresponding to 360 and 720 mg/kg bw.	LOAEL female: 360 mg/kg bw	Decreased body weight in female from 360 mg/kg bw. Male rats died due to internal bleeding.	NTP (1982b)
Fisher 344 rats; 15/male/group; 10 weeks; Administration in diet; 300, 2,800 and 25,000 ppm (20, 200 and 2,200 mg/kg bw/day)	NOAEL: 2,800 ppm (200 mg/kg bw/day) for systemic toxicity	At 2,200 mg/kg bw/day changes in absolute and relative organ weights, changes in haematological parameters, and some evidence of minimal anemia.	NTP (1997)
F344/N rats; 60/sex/group; 2 years: Administration in diet; male 3,000, 6,000 and 12,000 ppm, corresponding to 120, 240 and 500 mg/kg bw; female: 6,000, 12,000 and 24,000 ppm, corresponding to 300, 600 and 1,200 mg/kg bw.	NOAEL male: 240 mg/kg bw based on increased kidney weight. LOAEL female: 300 mg/kg bw based on increased incidence of nephropathy	Male: From 120 mg/kg bw increased relative kidney weight, at 15 month interim evaluation (more than 10% only at 500 mg/kg bw). From 500 mg/kg bw decreased body weight and haematological changes. Female: Increased incidence of nephropathy from 300 mg/kg bw. Increased relative kidney weight 600 mg/kg bw. At 1,200 mg/kg bw decreased body weight, haematological changes.	NTP (1997)
Fisher –344 male rats; 15 male/group; 26 weeks; Administration in diet; 300, 900, 2,800, 8,300 and 25,000 ppm (30, 60, 180, 550 and 1,660 mg/kg bw/day).	NOAEL: 2,800 ppm (180 mg/kg bw/day)	At 180 and 550 mg/kg bw/day sporadically decreased erytrocyte count. At 550 mg/kg bw/day increased cell haemoglobin concentrations, and increased relative liver weight. At 1,660 mg/kg bw/day anaemia, decreased body, testes and epididymis weight, and decreased sperm concentrations. Histopathologic changes in testes, epididymis, prostate and kidney.	NTP (1997)
B6C3F1 mice; 90 days; Administration in diet; (240, 464, 946, 1,850 and 3,750 mg/kg bw/day)	LOEL male: 224 mg/kg bw/day NOEL female: 946 mg/kg bw/day	Reduced body weight in male at all doses, reduced body weight in females from 1,750 mg/kg bw/day.	NTP (1982b); Hammond et al. (1987)
Beagle dogs; 3/sex/group; 3 month; Administration in diet; 10,000 - 50,000 ppm (400, 1,000 and 1,850 mg/kg bw/day for males, and 700, 1,270 or 1,973 mg/kg bw/day for females)	NOAEL: 1,852 mg/kg bw/day for males and 1,973 mg/kg bw/day for females	At 1,852 mg/kg bw/day (males) or 1,973 mg/kg bw/day (females) decreased body weight.	Hammond et al. (1987)
Wistar rats; 10/sex/group; 3 month; Administration in diet; 2,500 - 12,000 ppm (corresp. to approx. 151, 381, 960 mg/kg bw/day)	NOAEL male: 151 mg/kg bw/day	At doses ≥ 381 mg/kg bw/day in males kidney weight increase, urinary pH decrease, and histopathological changes in pancreas, and gross pathological changes in the liver. At 960 mg/kg bw/day body weight decrease, liver weight increase, and slight anaemia. At 151 mg/kg bw/day in females a marginal increase in relative liver and cecum weight. Increases in relative kidney weight from 381 mg/kg bw/day.	Hammond et al. (1987)

Table 4.24 continued overleaf

Table 4.24 continued Repeated dose toxicity

Study design	Effect level	Critical effect	References
Inhalation			
Sprague-Dawley rats; 20/sex/group; 4 weeks; Inhalation; 360, 1,000 and 2,100 mg/m^3	NOAEC: 1,000 mg/m^3	At 2,100 mg/m^3 decreased body weight, atrophy in spleen and testes.	Monsanto (1981)
Sprague-Dawley rats; 5/sex/group; 4 weeks; Inhalation; 49, 144 and 526 mg/m^3	NOAEC: 144 mg/m^3	At 526 mg/m^3 decreased body weight.	Hammond et al. (1987)
Sprague-Dawley rats; 25/sex/group; 13 weeks; Inhalation ; 51, 218 and 789 mg/m^3	NOAEC: 218 mg/m^3	From 218 mg/m^3 urine-stained fur, and piloerection, however, not considered treatment related. At 789 mg/m^3 increased kidney and liver weight, decreased serum glucose.	Monsanto (1982)

The most relevant oral studies in rats are two 90-day sub-chronic studies in male and female Sprague-Dawley rats and Wistar rats (Hammond et al., 1987), and a 26-week oral toxicity study in male Fisher rats (NTP, 1997). In Wistar rats a slight anaemia, a decrease in urinary pH, and slight changes in relative organ weights were reported. Histopathologic changes were limited to the liver, pancreas and testes. The NOAEL in male Wistar rats was 151 mg/kg bw/day of BBP and the LOAEL 381 mg/kg bw/day based on histopathological changes in the pancreas, gross pathological changes in the liver, increased kidney weight and decreased urinary pH. In Sprague-Dawley rats an increase in relative liver and kidney weight were observed at 750 mg/kg bw/day of BBP, whereas no compound related lesions were observed at necropsy or upon histopathologic examination. The NOAEL in Sprague-Dawley rats was 375 mg/kg bw/day of BBP. In the 26 week study in Fisher rats, anaemia, decreased testes weight, hypospermia and atrophy of the seminiferous tubules were reported at 1,600 mg/kg bw/day, and at 550 mg/kg bw decreased liver weight and increased cell haemoglobin concentrations were reported. At 180 and 550 mg/kg bw/day minimal erytrocyte count occurred sporadically. The NOAEL in Fisher rats was 180 mg/kg bw/day, and the LOAEL 550 mg/kg bw/day. In the 3 month oral study in Beagle dogs the only effect reported was a decrease in body weight at 1,852 mg/kg bw/day. The effect reported in dogs at relative high concentrations of BBP compared to effects of BBP reported in mice and rats could be due to pharmacokinetic differences. In beagle dogs approximately 90% of unchanged BBP was measured in faeces over a 4 hours period (Erickson, 1965, see Section 4.1.2.1).

The most relevant inhalation study is a 90-day sub-chronic study with male and female Sprague-Dawley rats performed according to current Guidelines and GLP. Relative organ weight changes were observed in the liver and kidney in both sexes at 789 mg/m^3 of BBP, and a decrease in serum glucose. At 218 mg/m^3 an increase in kidney weight was reported in male rats at interim sacrifice. However, no compound related macroscopic or microscopic lesions were observed. The NOAEC from the study was 218 mg/m^3 of BBP, and the LOAEC 789 mg/m^3 based on increased kidney and liver weight in male and female rats.

BBP has been shown to cause peroxisom proliferation in both male and female rats. There was no obvious sex difference in the induction of peroxisome proliferation based on the 21-day feeding study by Barber et al. (1987). Compared with DEHP, BBP appears to be somewhat less effective in causing peroxisome proliferation, at least in male rats. Peroxisome proliferation was in all studies measured as an increase in PCoA and/or CAT activity as an index for peroxisome

proliferation or lauric acid 11- and 12-hydroxylation. The most relevant study is the 21-day oral study in male and female rats performed according to GLP. Dose-dependent increases in liver weight, hepatic PCoA as well as lauric acid 11- and 12-hydroxylation were reported after exposure to 0.6, 1.2 or 2.5% of BBP in the diet. In the 2.5% group a moderate increase in peroxisome numbers measured by electron microscopy (EM) were evident. The LOAEL from this study was 0.6% of BBP in the diet corresponding to 639 mg/kg/day of BBP, and this LOAEL value correlate well with the NOAEL and LOAEL values reported from a 28-day oral study and a 12-month oral study in rats.

In the risk characterisation for BBP for repeated dose toxicity a NOAEL at 151 mg/kg bw/day from a 90-day oral study in Wistar rats is used (Hammond et al., 1987). This NOAEL value is based on histopathological changes in the pancreas, and gross pathological changes in the liver fom 381 mg/kg bw/day. In the other repeated dose toxicity studies with a duration of three month or longer the NOAEL values are based on organ weight changes (Hammond et al., 1987, Sprague-Dawley rats), decreased body weight (NTP, 1982), or slight, but statistically significant changes in haematological parameters (NTP, 1997). For inhalation exposure to BBP a NOAEC at 218 mg/m^3 (Monsanto, 1982) from a 13-week inhalation study in Sprague Dawley rats is considered used. This NOAEC value is based on a significant increase in absolute and/or relative kidney or liver weight at 789 mg/m^3. No NOAEL value is derived for dermal exposure to BBP due to only one poorly reported study available.

The results from the repeated dose toxicity studies indicate that no classification for repeated dose toxicity (R48) is warranted.

4.1.2.7 Mutagenicity

The mutagenic activity of BBP has been assessed in several *in vitro* and *in vivo* studies. The information available from *in vitro* studies includes gene mutation, cytogenicity, as well as cell transformation. The *in vivo* studies include the induction of sex-linked recessive lethals in *Drosophila melanogaster* and in mice the induction of dominant lethal mutations and SCE and CA in bone marrow. A summary of the overall results and a table are included in the end of this section.

4.1.2.7.1 Studies *in vitro*

Studies in prokaryotes and eukaryotes

Mutations

The mutagenic activity of BBP (Santicizer 160) was studied in *Salmonella typhimurium* strain TA98, TA100, TA1535, TA1537, and TA1538, and *Saccharomyces cerevisiae,* strain D4. BBP (0.1, 1.0, 5.0, and 10.0 µl/plate) was tested directly and in the presence of liver microsomal enzyme preparations from Arclor-induced rats. BBP did not demonstrate mutagenic activity in any of the assays conducted (Monsanto, 1976b).

The mutagenic activity of BBP (Santicizer 160) was studied in *Salmonella typhimurium* strain TA98, TA100, TA1535, TA1537, and TA1538. BBP was tested at 0.001, 0.01, 0.1, 1.0, 5.0 and 10.0 µl/plate with and without mammalian metabolic activation. BBP gave no indication of toxic effects at the concentration range tested. No mutagenic activity was reported in any of the strains tested (Monsanto, 1976c).

The mutagenic activity of BBP was studied in *Salmonella typhimurium* Strain TA98, TA100, TA1535 and TA1537 at BBP concentrations of 0, 100, 333, 1,000, 3,333 and 10,000 µg/plate with or without a metabolic activation system (S9). BBP did not demonstrate mutagenic activity in any of the strains tested, and was considered non mutagenic in this study (NTP, 1997).

In a review article on phthalates, Omori (1976) reported on the mutagenicity of BBP in bacterial systems (original ref. Kurata, 1975). No mutagenic activity was detected neither in *E. coli* nor in the repair tests with *B. subtilis* or *E. coli* at a dose of 30 mg/plate.

Studies in mammalian cells

Gene mutation

BBP (Santicizer 160) (0.06, 0.16, 0.32, 0.65, 1.25, 2.5 or 5.0 µl/ml) was evaluated for its potential to induce specific locus forward mutations in L5178Y trifluorothymidine resistant (TK) mouse lymphoma cells with and without the addition of an exogenous metabolism system (S9 from mice). No statistical increase in mutant frequency was detected either in the presence or absence of S9 with doses up to 1,25 µl/ml. BBP was tested up to it solubility limit (At 1.25, 2.5 and 5.0 BBP was incompletely soluble) (Monsanto, 1976d).

In a mouse lymphoma L5178Y assay BBP (5, 10, 20, 30, 40 or 60 nl/ml) was tested for induction of trifluorothymidine resistant (TK) cells with and without the addition of an exogenous metabolism system (S9) (precipitate was formed at a dose level of 60 nl/ml). No induction of TK resistance was obtained with concentrations of BBP that formed stable solutions. Increases in mutant colonies were reported in the absence of S9 in cultures treated with concentrations of BBP that produced precipitation. Such responses were not considered valid by the experimental quality control parameters. Therefore the test was concluded to be negative (NTP, 1997).

Cytogenicity

Sister Chromatide Excanges (SCE)

A well conducted *in vitro* cytogenetic assay was available using Chinese hamster ovaries (CHO) cells for induction of SCE, with and without metabolic activation (S9). BBP was tested in doses up to 1,250 µg/ml. In the first SCE trial without S9, the response was considered to be equivocal due to a significant positive trend (P=0.004) in the absence of a significant increase in SCE at any dose level. The results of the second trial were clearly negative, as was the single test conducted with S9. It was concluded that no induction of SCE were observed in CHO cells treated with BBP (Galloway et al., 1987)

Chromosome Aberrations (CA)

A well conducted *in vitro* cytogenetic assay was described usign CHO cells for the induction of CA, with and without metabolic activation (S9). BBP was tested in doses up to 1,250 µg/ml. No induction in CA were observed in CHO cells treated with BBP (Galloway et al., 1987).

Cell transformation

BBP (98% purity) has been tested for induction of cell transformation in the pH 6.7 Syrian hamster embryo cell transformation assay. Concentrations of BBP in the 24 hours study were 25, 50, 150 and 250 µg/ml and in the normal 7 days study 1, 2, 5, 10 and 20 µg/ml. No information

regarding the use of an exogenous metabolism system was found. Concentrations ≥ 25 μg/ml resulted in a globular precipitate indicating insolubility of BBP at these concentrations. No morphological cell transformation was found in the 24 hours study, whereas, a positive result was obtained with 2, 5 and 10 μg/ml of BBP when using a 7 days exposure regime. The fact that BBP was positive only in the 7 days study suggests that transformation occurs via a non-mutagenic mechanism (e.g. changes in gene expression) (Le Boeuf et al., 1996)This study was performed in compliance with GLP.

BBP (Santicizer 160) has also been assessed for transformation potential in BALB/3T3 cells. The low solubility of BBP in the cell culture medium was a problem when performing the test. A series of dilutions from 0.49 nl/ml to 8,000 nl/ml, (based on a cytotoxicity test) of BBP were used No morphological cell transformation was detected with BBP (Monsanto, 1985).

4.1.2.7.2 Studies *in vivo*

Drosophila melanogaster

BBP was tested for the induction of sex-linked recessive lethals in *Drosophila melanogaster*. No induction of sex-linked recessive lethal mutations was observed in germ cells from male *Drosophila melanogaster* administrated 500 ppm by injection or up to 50,000 ppm in dosed feed (Valencia et al., 1985).

Mice, Sister Chromatide Exchanges (SCE)

As part of a NTP carcinogenicity study in rats (NTP, 1997) BBP was assessed for SCE in mouse bone marrow. In the SCE study, BBP was given as a single *i.p.* injection to groups of 5 male B6C3F1 mice at doses of 0, 1,250, 2,500 and 5,000 mg/kg bw. The standard harvest time of 23 hours and a delayed harvest time of 42 hours were used. In the SCE test, a single trial with sample time 23 hours yielded a positive trend when the highest dose was excluded from the analysis because of a reduction in response at the 5,000 mg/kg level. The SCE test conducted with a 42 hours sample time also gave a weak positive response in trend analysis. Neither trial was repeated (NTP, 1997).

Mice, Chromosomal Abberations (CA)

As part of NTP carcinogenicity study in rats (NTP, 1997) BBP was assessed for CA in mouse bone marrow cells. Groups of 10 male mice (B6C3F1 mice) were injected *i.p.* with BBP doses of 0, 1,250, 2,500 and 5,000 mg/kg bw/day. Four hundred cells were examined in the control group, and at each dose level. Responses were evaluated as the percentage of abarrant metaphase cells, excluding gaps. Positive trend analyses were obtained in each of the two trials in the CA test that used the standard harvest time of 17 hours post injection. In trial 1 and trial 2 the percentage of cells with CA was 0.75 and 1.00 in the control group, 1.50 and 2.25 at 1,250 mg/kg, 0.75 and 2.0 in the 2,500 mg/kg dose group and 3.25 and 4.15 in the 5,000 mg/kg dose group (statistically significant at the highest dose tested). However, CA/cells or number of CA were not statistically different from the control group and the exposed groups in trial 1 and 2. In the single trial that used a delayed harvest time of 36 hours the percentage of cells with CA was 0.25 in the control group, 1.50 at 1,250 mg/kg, 0.25 at 2,500 mg/kg and 0.50 in the 5,000 mg/kg dose groups, (not statistically significant at any dose levles). Furthermore, no statistically significant difference in the number of CA or CA/cells was reported in the control group or exposed groups (NTP, 1997).

Mice, dominant lethal mutation

BBP was tested for its ability to induce dominant lethal mutations in mice. On each of three days (days 1, 5, and 10 of the study), 24 CD-1 and 36 B6C3F1 male mice were given s.c. injections of BBP equivalent to doses of 400-600, 1,280-1,840 and 3,200-4,560 mg/kg bw/day. Each male mouse was mated for 4 day intervals to three untreated virgin females of the same strain beginning on days 2, 6, 11, 15, 22, 29, 42 and 49. Females were sacrified 17 days after the beginning of the mating period. No increase in foetal deaths and no decrease in various fertility parameters were found. BBP was concluded to be negative in the mouse dominant lethal assay (Bishop et al., 1987).

Rats, micronucleus

As part of a developmental toxicity study in rats (see Section 4.1.2.9) BBP was tested for the induction of micronucleus in 19 female Alpk:AP_fSD rats (aged between 10 and 12 weeks). The rats were exposed to BBP via drinking water during gestation and lactation. The exposure to BBP was estimated to be 182.6 μg/kg/day. Bone marrow smears were prepared at termination of the dams, and stained with acridine orange. Slides were assessed for the presence of micronucleated polychromatic erythrocytes (MPE) among 2,000 PE and the erythropoietic ratio was determined among 1,000 PE and normochromatic erythrocytes. No induction of micronucleus was reported in this study, however, very low doses of BBP were used in this study (182.6 μg/kg/day) (Ashby et al., 1997).

4.1.2.7.3 Summary mutagenicity

BBP showed no evidence of mutagenicity in *Salmonell typhimurium* or mouse lymphoma cells. BBP did not induce sister chromatid exchanges (SCE) or chromosomal aberrations (CA) in CHO hamster cells. BBP induced morphological transformation in Syrian hamster embryo cells, but not in the BALB/3T3 cell transformation system. BBP did not induce sex-linked recessive lethals *in Drosophila melanogaster* or dominant lethal mutations in mice. Positive results were obtained in a mouse bone marrow test for SCE, however the responses were week, and the SCE test was not repeated. For the induction of CA conflicting results were reported when different observations times were compared. No inductions of micronucleus were reported in female rats after exposure to low doses of BBP (182.6 μg/kg bw/day during gestation and lactation). An overview of the studies is presented in **Table 4.25**. Based on the available data, and according to EU criteria, BBP should not be considered a mutagen.

Table 4.25 Mutagenicity

Study design	Results	Reference
In vitro, prokaryotes and lower eukaryotes		
Salmonella typhimurium Strain TA98, TA100, TA1535, TA1537, TA1538; S9 +/-; 0.1, 1.0, 5.0, 10.0 μl BBP/plate	Negative	Monsanto (1976b)
Salmonella typhimurium Strain TA98, TA100, TA1535, TA1537, TA1538; S9 +/-; 0.001, 0.01, 0.1, 1.0, 5.0, 10.0 μl BBP/plate	Negative	Monsanto (1976c)
Salmonella typhimurium Strain TA98, TA100, TA1535 and TA1537; S9 +/-; 100, 333, 1,000, 3,333 and 10,000 μgBBP/plate.	Negative	NTP (1997)

Table 4.25 continued overleaf

Table 4.25 continued Mutagenicity

Study design	Results	Reference
In vitro, Mammalian cells		
E. Coli; 30 mg/plate; mutation test.	Negative	Omori (1976), orgianl data Kurata (1975)
B. Subtilis; 30 mg/plate; repair test.	Negative	Omori (1976), orginal data Kurata (1975)
Saccharomyces cerevisiae Strain D4, S9 +/-; 0.1, 1.0, 5.0, 10.0 μl BBP/plate; mutation test.	Negative	Monsanto(1976b)
Mouse lymphoma cells L5178Y TK; S9 +/-; 0.08, 0.16, 0.32, 0.65, 1.25, 2.5 or 5.0 μl BBP/ml, insoluble at 1.25, 2.5 and 5.0 μl/ml. Mutation test	Negative	Monsanto (1977d)
Mouse lymphoma cells L5178Y TK; S9 +/-; 5, 10, 20, 30, 40, 60 nl BBP/ml. Mutation test.	Negative	NTP (1997)
Chinese hamster ovary (CHO) cell; S9 +/-; Up to 1,250 μg BBP/ml; CA and SCE assay.	Negative or ambiguous	Galloway (1987)
Syrian hamster embryo cells; 25, 50, 100, 150, 250 μg BBP/ml in the 7 days study, 1,2 5,10 and 20 μg BBP/ml in the 24 hours study, precipitation at conc. ≥ 25 μg BBP/ml. Cell transformation test.	Negative in the 24 hours study; positive at 2.5 and 10 μg/ml in the 7 days study.	Le Boeuf (1996)
BALB/3T3 cells; 10, 20, 40, 80, 160 nl BBP/ml. Transformation test.	Negative	Monsanto (1985)
In vivo		
Drosophila melanogaster; 250, 10,000 and 50,000 ppm BBP in feed; sex-linked recessive lethal mutation.	Negative	Valencia (1985)
Mouse bone marrow; 1,250, 2,500 and 5,000 mg BBP/kg bw i.p; SCE and CA assay.	SCE weak positive after 23 and 42 hours, CA positive at 17 hours at highest dose, negative all doses at 36 hours.	NTP (1997)
Alpk:AP$_f$SD (AP) rats. 182.6 μg/kg/day of BBP during gestation and lactation; 19 rats; micronucleus	No induction of micronucleus	Ashby et al. (1997)
B6C3F1 mice, CD-1 mice; 400-600, 1,280-1,840, 3,200-4,560 mg BBP/kg bw, s.c.; dominant lethal mutations.	No increase in foetal deaths.	Bishop (1987)

4.1.2.8 Carcinogenicity

BBP has been tested for carcinogenicity by the oral route in rats and mice. Studies using other routes of exposure (i.e. dermal or inhalation) or other animal species have not been reported.

BBP was tested for carcinogenicity in F344 rats fed 6,000 or 12,000 ppm BBP (corresponding to 360 and 720 mg/kg bw) 7 days/week, for 28 or 103 weeks. Groups of 50 male and female rats were used per dose level. Mean body weights of dosed female rats of each sex were lower than those of controls during most of the study. After week 14, an increasing number of dosed males died as a result of unexplained internal bleeding. Consequently, all living male rats were killed at 29 to 30 weeks. Thus, male rats were not adequately tested for carcinogenicity. Mononuclear cell leukemias occurred at a statistically significant (P=0.011) increased incidence in the high-dose group of female rats when compared to controls and with a significantly (P=0.006) increased incidence: Controls 7/49 (14%); low-dose 7/49 (14%); high-dose 18/50 (36%). Tumour rates

were decreased in female rats for fibroadenomas of mammary glands: controls 13/50; low-dose 11/49; high-dose 4/50. The conclusion of the study by NTP Peer Review Panel was that BBP was probably carcinogenic for F344/N rats, causing an increased incidence of mononuclear cell leukemias.

In an identical study to the NTP study cited above, 50 mice (B6C3F1) per sex were exposed to 6,000 or 12,000 ppm BBP (corresponding to 840 and 1,680 mg BBP/kg bw) in feed for 24 months (NTP, 1982b). The exposure of mice to BBP was not associated with increased incidences of any type of tumour among male and female mice.

In a more recent NTP carcinogenicity study, male F344/N rats were fed 3,000, 6,000, 12,000 ppm (120, 240, 500 mg/kg bw) and females 6,000, 12,000, 24,000 ppm (300, 600, 1,200 mg/kg bw) of BBP for 24 months 7 days/week (NTP, 1997). Sixty animals were exposed per dose group. Survival of all exposed groups of male and female rats was similar to that of controls. There were no clinical findings in exposed groups of rats related to BBP exposure. At 12,000 ppm a decrease in the body weight of males and at 24,000 ppm in females, were noted. Mean body weights of 3,000 and 6,000 ppm males and of 6,000 and 12,000 ppm females were similar to those of the controls throughout the study. In general, haematological changes were sporadic and minor. At 6 months, a minimal decrease in erythrocyte count and increase in mean cell haemoglobin occurred in male rats at 12,000 ppm. In females, a decreased haematocrit value occurred at 15 months in the 24,000 ppm exposure group. There was also a mild decrease in triiodothyronine concentrations in the 24,000 ppm females at 6 and 15 months and at the end of the study.

At 2 years, the incidences of pancreatic acinar cell adenoma (0 ppm 3/50; 3,000 ppm 2/49; 6,000 ppm 3/50; 12,000 ppm 10/50) and adenoma and carcinoma (combined) (3/50, 2/49, 3/50, 11/50) in 12,000 ppm males were significantly greater than in controls and exceeded the historical controls (19/1, 191 [1.6% ± 2.4%]; range 0% - 10%) in other 2-year NTP feed studies. One carcinoma was observed in one 12,000 ppm male, and two adenomas were observed in the 24,000 ppm females. At 2 years, the incidence of focal hyperplasia of the pancreatic acinar cell in 12,000 ppm males was significantly greater than in the controls (4/50, 0/49, 9/50, 12/50). Transitional epithelial papillomas in the urinary bladder were observed in one control female and in two 24,000 ppm females (1/50, 0/50, 0/50, 2/50). The incidence of this neoplasm exceeded the range of historical controls from NTP 2-years studies (4/1, 182 [0.3% ± 0.8%]; range 0% - 2%). The incidence of transitional epithelial hyperplasia in 24,000 ppm females was significantly greater than that in controls (4/50, 0/50, 1/50, 10/50).

The absolute right kidney weight of 12,000 ppm females and the relative right kidney weights of all exposed groups of males and of 24,000 ppm females were significantly greater than those of the controls at the 15 month interim evaluation. The severities of renal tubule pigmentation in 12,000 ppm males and in 24,000 ppm females were greater than those in the controls at 15 months and 2 years. At 2 years, the incidence of kidney mineralisation (0 ppm, 43/50; 6,000 ppm 34/50; 12,000 ppm 37/50; 24,000 ppm 35/50) in 6,000 ppm and 24,000 ppm females was significantly less than that in the controls. The incidences of nephropathy (34/50, 47/50, 43/50, 45/50) in exposed groups of females and of transitional epithelial hyperplasia (0/50, 3/50, 7/50, 4/50) of the kidney in 12,000 ppm females were significantly greater than those of the controls.

Under these conditions the conclusions (by the NTP) of the 2-year NTP carcinogenicity study were that there was some evidence of carcinogenic activity of BBP in male F344/N rats based on the increased incidences of pancreatic acinar cell adenoma and of acinar cell adenoma and carcinoma (combined). There was equivocal evidence of carcinogenic activity of BBP in female F344/N rats based on the marginally increased incidences of pancreatic acinar adenoma and

transitional epithelial papilloma of the urinary bladder. Exposure of rats to BBP in feed for 2 years resulted in focal hyperplasia in the pancreas in male rats and in transitional hyperplasia in the urinary bladder of female rats.

BBP has also been studied for carcinogenicity using protocols employing dietary restriction (NTP, 1995). This study was conducted along with the 1995 NTP rat study (NTP, 1997) to understand the influence of dietary restriction on the sensitivity of the bioassay and the effects of weight-matched controls on the sensitivity of the bioassay. Groups of 50 to 60 F344/N rats were fed NIH-07 diet containing BBP, either *ad libitum* or in amounts that restricted mean body weights to approximately 85% of the mean *ad libitum* control body weights. BBP caused an increased incidence of pancreatic acinar cell neoplasms in *ad libitum*-fed male rats relative to *ad libitum*-fed or weight-matched controls. This change did not occur in rats in the restricted feed protocols after 2 years, although acinar cell adenomas were observed in three exposed, feed-restricted males at 30 months. BBP also caused an increased incidence of urinary bladder neoplasms in female rats in the 32-month restricted feed protocol, but not in any of the 2-years protocols.

Groups of 27 female Sprague-Dawley rats, 43 days of age were given BBP (purity unspecified) at doses of 250 or 500 mg/kg/day intragastrically in corn oil for seven days prior to intragastric administration of 31 mg/kg/day dimethylbenz(a)anthracene (DMBA) in corn oil. After 15 weeks, rats were killed and mammary tumour incidences were determined. Administration of BBP did not affect body weight gain. The incidence of palpable mammary tumours was significantly inhibited by pre-treatment with BBP (58 and 71% for 250 and 500 mg/kg/day of BBP) ($p < 0.05$). The number of adenocarcinomas per rat was also significantly reduced (4.0 for DMBA alone, 1.6 for 250 mg/kg/day of BBP and 1.2 for 500 mg/kg/day of BBP) ($p < 0.05$) (Singletary et al., 1997).

According to the International Agency for Research on Cancer, no evaluation could be made to determine the carcinogenic risk of BBP to humans.

Table 4.26 Summary of the incidence of neoplasms in rats

	NTP 1982			NTP 1997					NTP 1999 Dietary restriction 24 months		NTP 1999 Dietary restriction 32 months	
	0 ppm	6,000 ppm	12,000 ppm	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	24,000 ppm	0 ppm	24,000 ppm*	0 ppm	24,000 ppm*
Male												
Pancreas: adenoma	Terminated. after 30 weeks			3/50 (6%)	2/49 (4%)	3/50 (6%)	10/50 (20%)		0/50 (0%)	0/51 (0%)	0/50 (0%)	3/41 (6%)
Female												
Pancreas adenoma	0/47 (0%)	3/46 (7%)	0/46 (0%)	0/50 (0%)		0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Mononuclear cell leukaemia	7/49 (14%)	7/49 (14%)	18/50 (36%)	21/50 (42%)		20/50 (40%)**	21/50 (42%)	19/50 (38%)	16/50 (32%)	18/50 (36%)	29/50 (58%)	39/50 (78%)
Bladder, papilloma	No data			1/50 (2%)		0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	2/49 (4%)	1/50 (2%)	2/50 (4%)
Carcinoma	No data			0/50 (0%)		0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)

Bold indicates significant increase compared to control.

* 12,000 ppm for males.

** The historical controls for mononuclear cell leukaemia varies today from 16% to 42% with an average of 29.3% (NTP, 2000)

4.1.2.8.1 Summary carcinogenicity BBP

Butyl benzyl phthalate was tested for carcinogenicity by oral administration in one experiment in mice and in three experiments with rats, including a dietary restriction study. No increases in the incidence of tumours were observed in mice. The results from the rat studies are summarised in **Table 4.26**. An increased incidence of mononuclear cell leukemias was reported in female rats at 12,000 ppm BBP. The increase was within the historical controls and frequency was actually similar to the frequencies found in the two control groups in the second experiment. No significant increase in the incidence of mononuclear cell leukemias was, however, found in two later studies with the same rat strain although a higher concentration was tested. An increased incidence of benign pancreatic tumours was seen at the highest dose in one conventional study in male rats, but not at a two times higher dose after dietary restriction. A marginally increased incidence of pancreatic adenomas occurred in female rats in a convential study (p = 0.49), but not after dietary restriction. Pappilomas of the urinary bladder was marginally increased in female rats both in the conventionally study (p = 0.49) and after dietary restriction. Moreover, after dietary restriction and 32 months a non-significant (p = 0.12) increase in bladder carcinomas was found. The latter results are difficult to interpret as no historic controls are available. In one study in rats, butyl benzyl phthalate given prior to 7,12-dimethylbenz(a)antracene inhibited mammary carcinogenesis. BBP may be a borderline case between no classification for carcinogenicity and Carc. Cat. 3. However, due to the lack of genotoxic effects no classification is proposed.

4.1.2.9 Fertility, development and endocrine activity.

BBP has been assessed for potential toxic effects on fertility, reproductive organs, development, and endocrine activity following exposure almost exclusively in rats. Only one developmental study in mouse and one *in vivo* study for estrogenic activity in mice was reported. Most of the available reproduction toxicity information results from oral exposure, however, the endocrine activity in rats and mice have also been evaluated after subcutaneous injection of BBP. A summary of the most important observations and a table of the critical effects of BBP with respect to fertility including effects on the reproductive organs, development and endocrine activity are included in the end of each section (**Table 4.27** and **Table 4.29**).

4.1.2.9.1 Fertility studies BBP, animals

In a new 2-generation study male and female CD (Sprague-Dawley) rats (40-45 days old), 30 animals/sex/dose (F0 generation) were administered Butyl Benzyl Phthalate (BBP) in the feed at doses of 0, 750, 3,750, and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day for 10 weeks (Tyl et al., 2004). Animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with exposure continuing. F0 and F1 males were necropsied after the delivery period, and a histopathologic evaluation was performed on 10 animals from the high dose group and the control group. The following organs were evaluated; pituitary, liver, thyroid gland, seminal vesicles with coagulating glands and fluids, epididymis with contents and fluids, prostate, testes and pancreas. An andrological assessment was also performed which included; reproductive organ weights, epididymal sperm number, motility and morphology, testicular homogenisation-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production. On the day of birth, post natal day (pnd) 0 anogenital distance (AGD) was measured and body weights

recorded for all live F1 pups in all litters. F1 litters were standardised to 10 pups (5/sex) on pnd 4. On pnd 11-13 all F1 male pups were examined for retained nipples/aerolae on the ventrum. At weaning on pnd 21, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as F1 parents of the F2 generation. Any remaining F1 male pups not selected as parents or for necropsy, which exhibited retained nipples, were also necropsied. On pnd 21 F0 or F1 females were necropsied, and histopathology was performed on 10 animals from the high dose group and from the control group. The following organs were evaluated: ovaries, vagina, uterus with oviducts and cervix, pancreas, pituitary, thyroid gland and liver, and other tissues with gross lesions identified as being treatment related. Selected F1 weanlings 30/sex/dose were administered BBP in the diet for a 10 weeks prebreed exposure period. Acquisition of vaginal patency in females and preputial separation in males were assessed. Vaginal cytology for estrus cyclicity in F1 selected females was evaluated during the last three weeks of the prebreed exposure period, and they were mated for a two-week period as described above. F1 males were necropsied after the F2 litters, parental F1 females were necropsied with histopathology, as described above, and F2 weanlings, up to three/sex/litter were necropsied. For all surviving F0 and F1 parental animals the following organs were weighed at scheduled sacrifice: ovaries, uterus with oviducts and cervix, pituitary, adrenal glands, liver, thyroid gland, seminal vesicles, epididymis with contents and fluid, spleen, prostate, testes, brain, kidneys, and pancreas. Results F0 parental systemic toxicity; Males and females: at 750 mg/kg bw/day significantly increased absolute and relative liver weight and relative kidney weight. Histopathological lesions in the liver mostly graded as minimal, and more abundant in female rats. At 250 mg/kg bw/day significantly increased absolute (male) and absolute and relative (female) kidney weight was reported. In females, at 750 mg/kg bw/day a significantly decreased body weight from study day 0 to 70 and during gestation and lactation was reported. Results F0 parental reproductive toxicity; In males no reproductive effects were reported, since the exposure to BBP started after they had achieved puberty. In females at 750 mg/kg bw/day significantly reduced absolute and relative paired ovaries weight and uterus weight were reported. Results F1 offspring toxicity; At 750 mg/kg bw/day a significant decrease in pup body weight per litter on pnd 0, 4, 7, 14 and 21 and in the 250 mg/kg bw/day group at pnd 7. In male offspring AGD was significantly ($p < 0.001$) decreased in a dose-related pattern from 250 mg/kg bw/day (1.89 mm compared to controls at 2.06 mm) and at 750 mg/kg bw/day (1.7 mm compared to controls at 2.06 mm). When the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values at 250 and 750 mg/kg bw/day were still significantly reduced. At 750 mg/kg bw/day significant increase ($p < 0.01$) in male pups with one or more nipples (19.23% compared to 0% in the control group), and in the number of nipples per male (0.72 compared to 0 in the controls). In the 750 mg/kg bw/day group a significanlty increase ($p < 0.001$) in the percentage of male pups with one or more areolae (32.31% compared to 2.63% in the controls), and in the number of areolae per male (1.29 compared to controls at 0.07, $p < 0.01$). At weanling necropsy in males at 750 mg/kg bw/day a significant decrease in terminal body weight, in absolute thymus weight, in absoulte and relative spleen and testis weight, and in absolute epididymis weight was reported. In postwean males F1 a significant delay in the acquisition of puberty in F1 males was seen, evident as delayed age at preputial separation (45.2 compared to 40.9 in controls), and in the adjusted age at preputial separation (45.4 compared to 41.0 in controls). At weanling necropsy in females a significant decrease in terminal body weight, in absolute thymus weight, in absolute and relative spleen weight, and in absolute ovaries and uterus weight was reported. In postwean females F1 a significant delay in the acquisition of puberty was seen, evident as a delay in vaginal patency at 750 mg/kg bw/day (34.1 compared to 31.4 in controls) and in adjusted age at vaginal patency (34.4 compared to 31.5 in controls). Results F1 systemic toxicity; males: at 750 mg/kg bw/day significantly decreased body weight during preebreed exposure and at

necropsy, significantly increased relative liver weight, relative adrenal gland weight, absolute and relative pancreas weight and relative pituitary weight. At 250 mg/kg bw/day significantly increased absolute and relative liver, kidney and pancreas weight. Females: at 750 mg/kg bw/day significantly decreased body weight at necropsy. Histopathological lesions graded as minimal were reported, and were more abundant in females. Results F1 reproductive toxicity; At 750 mg/kg bw/day significantly reduced mating (70.0 compared to 96.7 in controls) and fertility (81.0 compared to 100.0 in controls) indices in F1 parents to make F2 offspring. Males: at 750 mg/kg bw/day significantly reduced absolute paired testis weight (2.8585 g compared to controls 3.5980 g), paired epididymis weight (1.2076 g compared to controls 1.3507 g), prostate weight (0.5626 g compared to controls 0.7556 g) and seminal vesicle with coagulating gland weight (1.7515 compared to controls 2.1455). The number of rats with histopathological changes in testis and epididymis in the 750 mg/kg bw/day group was 23 and 15 compared to 3 and 2 in controls. Furthermore, the epididymal sperm concentration (649.51 mil/g compared to 825.59 mil/g in controls), the percentage of motile sperms (52.1 compared to 68.6 in controls), and the percentage progressively motile sperm (42.1 compared to 57.3 in controls) were significantly decreased at 750 mg/kg bw/day compared to controls. In the 750 mg/kg bw/day group a significant increase in the number of males with one or more reproductive tract malformations was reported (16 compared to 1 in controls), as well as in the percentage of males with one or more reproductive tract malformations (53.3 compared to 3.33 in controls). These included in the testis: abnormal, missing, reduced in size, and/or undescended, and in the epididymis missing (right, left or bilateral) or reduced in size (right, left or bilateral). Microscopic findings in the 750 mg/kg bw/day dose group included in the epididymis; aspermia (8/24) and chronic inflammation 4/24, in the prostate gland; chronic inflammation (13/30), and in the testis; atrophy seminiferous tubule (15/29) and dilatation duct rete testis (7/29). Furthermore, at 750 mg/kg bw/day the number of implants sites per litter (12.35 compared to 15.86 in controls), number of total pups per litter and the average number of live pups per litter on pnd 0 (11.4 compared to 14.2 in controls) and on pnd 4 (10.9 compared to 14.0 in controls) was significantly reduced compared to control animals. In females the absolute and relative uterus weight was increased compared to control animals. Results F2 offspring toxicity; During lactation at 750 mg/kg bw/day significantly reduced number of total pups per litter and live pups per litter on pnd 0 compared to control animals was reported. Furthermore, the average pup body weight per litter on pnd 7 (14.52 g compared to 16.91 g in controls), pnd 14 (29.53 g compared to 33.87 g in controls) and pnd 21 (44.63 compared to 50.01 g in controls) was significantly reduced compared to control animals. A significantly ($p < 0.05$) reduced AGD was reported in males at 250 mg/kg bw/day (1.99 mm compared to 2.05 mm in controls) and at 750 mg/kg bw/day (1.77 mm compared to 2.05 mm in controls, $p < 0.001$). When the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values at 250 and 750 mg/kg bw/day were still significantly reduced (1.99 mm at 250 mg/kg bw/day and 1.79 mm at 750 mg/kg bw/day compared to 2.04 in controls). No effect on AGD was reported in females. In males a significant increase in the percentage of male pups with one or more nipples (16.46 compared to 0 in the control group) and in the number of nipples per male (0.51 compared to 0 in controls), and in the number of areolae per male (3.14 compared to 0.05 in controls) was reported at 750 mg/kg bw/day. At weanling necropsy in males a significantly reduced terminal body weight (45.89 g compared to 51.78 in conrols), absolute thymus (0.2048 g compared to 0.2360 g in controls), absolute (0.1549 g compared to 0.2106 g in controls) and relative (0.3335 g compared to 0.4056 g in controls) spleen weight, and paired testis weight (0.1949 g compared to 0.2432 g in controls) was reported at 750 mg/kg bw/day. In the 750 mg/kg bw/day group a significant increase in gross lesions were reported. These included missing epididymis in twenty male weanlings (20/54) (full or caput or corpus), missing seminal vesicle or reduced size in 5 male weanlings (5/54), and one male in the

250 mg/kg bw/day group had a missing testis. In females weanling at necropsy a significant reduced terminal body weight, reduced absolute thymus and ovaries weight, and reduced absolute and relative spleen weight was reported at 750 mg/kg bw/day. At 250 mg/kg bw/day a significant increase in uterus weight was reported. F2 offspring was not evaluated as postweanlings. In this study the NOAEL for parental systemic toxicity is 250 mg/kg bw/day based on organ weight changes and histopathological lesions in the liver. The NOAEL for effects on the reproductive system in offspring is 50 mg/kg bw/day based on a dose-realted reduction in AGD in both F1 and F2 offspring from 250 mg/kg bw/day. This effect was still statistically significant at 250 and 750 mg/kg bw/day when the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate. The study was performed in compliance with Good Laboratory Practice, and the US EPA OPPTS Testing Guideline. The NOAEL value for fertility was 250 mg/kg bw/day based on statistically singificantly reduced mating and fertility indices in F1 parents to make F2 offspring.

A recent 2-generation study is available (Nagao et al., 2000). In this study Sprague-Dawley rats (8 week old) (25 male or female/group) were administered oral doses of 0, 20, 100 or 500 mg/kg bw/day BBP by gavage. F0 male rats were treated for 12 weeks prior to 2-week cohabitation, and until necropsy (confirmation of fertility by pairing). F0 female rats were treated for 2 weeks prior to cohabitation until necropsy (including gestation, delivery, and lactation through postpartum day 21). F1 animals were treated by oral gavage after weaning (postnatal day 22) until necropsy (confirmation of fertility by pairing). At 13 weeks of age mating was permitted. The F0 animals were observed for clinical signs daily during the study. In female F0 rats estrous cycling was evaluated. Furthermore, brain, hearth, lung, liver, spleen, kidneys, adrenal glands, thymus, ovaries, uterus, thyroid gland, and pituitary gland were weighed. The levels of prolactin, luteinizing hormone (LH), FSH, thyroidstimulating hormone (TSH), triiodothyronine (T_3), thyroxine (T_4) and estradiol (E_2) were measured in serum. Histopathologic examination of ovaries, uterus, vagina, liver, kidneys, mammary glands, thyroid gland, parathyroid gland, pituitary gland, and adrenal glands was performed in 10 dams from the 500 mg/kg bw/day dose group and the control group. F0 male rats were necropsied after confirmation of fertility by pairing with females. The brain hearth, lung, liver, spleen, kidneys, adrenal glands, thymus, testes, epididymis, ventral prostate, seminal vesicles, thyroid gland, and pituitary gland were weighed. The number of sperm in the right cauda epididymis and the percentage of motile sperm was determined. The levels of testosterone, LH, FSH, TSH, T_3, and T_4 were measured in serum. Histopathologic examination of testes, epididymis, prostate, seminal vesicle with coagulating gland, parathyroid gland, liver, kidneys, mammary glands, thyroid gland, pituitary gland, and adrenal glands was performed in 10 males each from the 500 mg/kg bw/day dose group and the control group. Observations in F1 offspring included; Numbers of live and dead pups for each litter were recorded after post natal day (pnd) 0 to 21, and the viability from pnd 0 to 4 (preculling) and from pnd 4 (postculling) to pnd 21 in each litter. Anogenital distance (AGD) was determined for each pup on pnd 4, 7, 14, and 21. On pnd 4 litters were culled randomly to eight (4 pups/sex/litter). Two pups/sex/litter in each group were examined for the development of neural reflexes, and for physical development. Sexual maturation measured as vaginal opening for female offspring (beginning on pnd 28) and preputial separation for male offspring (beginning on pnd 35) was assessed (2/sex/litter). On pnd 22 offspring (2/sex/litter) were necropsied. The testes, epididymis, and seminal vesicle with prostate in males, and ovaries and uterus in females were weighed. The testes in 10 male weanlings and ovaries in 10 female weanlings from all groups, and the epididymis, ventral prostate, and seminal vesicle with coagulating gland in 10 male weanlings and the uterus in 10 female weanlings from the 500 mg/kg group and the control group were examined histologically. Levels of testosterone, LH, TSH, FSH, T_3 and T_4 in male weanlings and of prolactin, LH, FSH, TSH, T_3, T_4, and E_2 in female weanlings were determined. One male and

one female offspring from each litter of each group was subjected to behvioral and functional tests. At 13 weeks of age mating was permitted by pairing on a 1:1 basis within the same treatment group. The same measurements described above for pregnancy, delivery, lactation, and the evaluation of histology of internal organs including reproductive tissues, sperm motions and counts, and serum hormone levels were performed in male and female offspring. F2 pups were necropsied on pnd 21.

The results from the two-generation study are as following; *In parent animals* (F0) a significant decrease in body weight gain was reported in males at 500 mg/kg/day compared to control males, although no decrease in food consumption was evident. In females no significant difference among groups in body weight and food consumption prior to mating or during pregnancy or lactation were reported. No dose-related changes were reported in estrous cyclicity, fertility and lactation. A dose-dependent increase in kidneys weight in rats of both sexes (significant at 100 and 500 mg/kg bw/day in females, and at 500 mg/kg bw/day in males), and an increase in liver weight in males (significant at 500 mg/kg bw/day), and a decrease in the weight of the ovaries in females (significant at 500 mg/kg bw/day) were reported compared to control animals. No macroscopic or microscopic changes were observed in the reproductive system of males or females. A decrease in serum testosterone, T_3 and T_4 levels (significant at 500 mg/kg bw/day), and an increase in FSH (significant from 100 mg/kg bw/day) were reported in males compared to control males. In females a significant increase in serum concentrations of prolactin, and a significant decrease in T_4 were reported at 500 mg/kg bw/day compared to control females. *Preweanling (F1);* The viability in percentage during pnd 0-4 was significantly decreased at 500 mg/kg bw/day (96.7% versus 100% in controls). Body weight of male and female offspring at birth in the 100 and 500 mg/kg bw/day dose group was significantly decreased compared to control animals (male offspring: 6.4 g at 100 mg/kg bw/day and 6.3 g at 500 mg/kg bw/day compared to 6.8 g in control offspring, female offspring: 6.0 g at 100 and 500 mg/kg bw/day compared to 6.4 g in control offspring), and the body weight at 500 mg/kg bw/day was lower throughout the study, however, the viability was not affected. In the 500-mg/kg bw/day group a significant decrease in AGD at birth was reported in male offspring, and an increase in AGD was reported in female offspring compared to control animals. A significant decrease in testis and epididymis weight in males, and a significant decrease in ovary weight and increase in uterus weight in females was reported in the 500 mg/kg bw/day group compared to control animals. Furthermore, a significant decrease in FSH concentration in males at 500 mg/kg bw/day, and in TSH concentrations in males at 100 and 500 mg/kg bw/day were observed compared to control animals. In females the level of T_3 was significantly decreased in the 100 and 500 mg/kg bw/day dose group compared to control females. Histopathologic examination revealed a significant decrease in the numbers of spermatocytes in the seminiferous tubules in the 500 mg/kg bw/day group compared to control males. Cryptorchidism or hypospadia was not observed in any dose groups. In females no histopathologic abnormalities were considered to be related to BBP exposure. *Postweanling (F1);* Preputial separation for male offspring in the 500 mg/kg bw/day group was significantly delayed compared to control males at pnd 40, while vaginal opening for female offspring in this group was not affected. BBP did not affect the reproductive ability, including delivery and lactation at any dose levels, whereas a significant reduction in the absolute weights of the testis, epididymis, prostate, seminal vesicle and spleen were reported at 10 or 18 weeks of age, and a significant increase in relative weight of the thyroid gland, adrenal glands, and liver weights was reported in males at 500 mg/kg bw/day compared to control males. A significant increase in the relative weight of kidneys in the 100 and 500 mg/kg bw/day dose group was reported compared to control males. However, no significant organ weight changes were reported in females. A significant decrease in serum concentrations of testosterone, LH, and T_4 were reported in male offspring at 500 mg/kg bw/day compared to control males. Furthermore, in the

500 mg/kg bw/day dose group histopathologic examination revealed significant increases in the incidence of atrophy of the seminiferous tubules with a decreased number of germ cells, a significant increase in the incidence of interstitial edema, and a significant increase in the incidence of decreased number of sperm in the epididymis compared to control males. In females no adverse changes in the ovaries or uterus in the 500 mg/kg bw/day dose group were reported. As regards the behavioral function tests, the only effect observed related to BBP exposure was a significant increase in the spontaneous motor activity in females in the 500 mg/kg bw/day dose group compared to control females, however, no effect was reported in males. *Preweanling F2;* In this group no significant adverse effects related to BBP exposure were reported including pup weight, viability, and development. From this study no NOAEL value for effects on fertility could be derived. The NOAEL value for effects on the reproductive organs in males was 100 mg/kg bw/day. This NOAEL value is based on atrophy of the testis, epididymis, and seminal vesicle at 10 or 18 weeks of age, and reduced reproductive organ weights in the F1 generation at the next higher dose. The NOAEL value for effects on development was 20 mg/kg bw/day based on reduced body weight in male and female offspring at birth at 100 and 500 mg/kg bw/day

As part of a NTP BBP feeding carcinogenicity study in F344/N rats (NTP, 1997) a modified mating study were conducted. Groups of 15 male F344/N rats were given 0, 300, 2,800 or 25,000 ppm BBP in feed (corresponding to approximately 20, 200 or 2,200 mg/kg bw/day of BBP) for 10 weeks. After the ten weeks exposure period the animals recovered for 2 days. Then a 7-days mating period was started. After mating the male rats were necropsied. All rats survived to the end of the study. The final mean body weight of the 25,000 ppm group (226 g) was significantly lower than those of the controls (320 g). No clinical findings related to BBP exposure were noted. A few minor haematological changes occurred at 25,000 ppm. There was some evidence of a minimal anemia, and the platelet count was increased. The absolute and relative prostate gland (0.276 and 1.23) and testis weight (0.442 and 1.97) of the 25,000 ppm males were significantly less than those of the controls (prostate gland; absolute 0.609, relative 1.93, testis; absolute 1.497, relative 4.73). Degeneration of the seminiferous tubule epithelium was observed in all males at 25,000 ppm. A dose-dependent decrease in epididymal spermatozoa concentration was reported. At 300 ppm the epididymal spermatozoa concentration per gram epididymal tissue was $324.14 \cdot 10^6$, at 2,800 ppm $261.47 \cdot 10^6$ and at 25,000 ppm $0.57 \cdot 10^{6.}$ The control value was $373.94 \cdot 10^6$. The decrease was statistically significant according to the published report at 2,800 ppm ($P \leq 0.05$). However, in this study the days of allowed recovery [days between mating (detection of sperm plug) and counting of epididymal spermatozoa concentration (necropsy)] varied within animals and BBP dose. In the 2,800 ppm group a higher number of rats were shown to have a shorter recovery period compared to control animals. The epididymal spermatozoa concentration after mating increased almost back to normal in the control group after two days, whereas in the 300 and 2,800 ppm group it was almost back to normal after 4 or 5 days, however, this information is based on a limited number of observations. When days of recovery were taken into account in a covariate analysis of variance on the epididymal spermatozoa concentration from the control, 300 and 2,800 ppm group, the decrease in epididymal spermatozoa concentration was not statistically significant at 2,800 ppm at the 5% level ($p = 0.07$), however, a dose-dependent decrease was still evident (control; $382.5 \cdot 10^6$, 300 ppm; $340.7 \cdot 10^6$ and 2,800 ppm $282.2 \cdot 10^6$). Ten females mated to 25,000 ppm males were initially found to be sperm positive, none of these females were pregnant at necropsy. There were no significant differences in litter data between the controls and the 300 and 2,800 ppm groups. The NOAEL from this study based on reduced epididymal spermatozoa concentration was 300 ppm corresponding to 20 mg/kg bw/day of BBP. The NOAEL for fertility was 2,800 ppm corresponding to 200 mg/kg bw/day of BBP. This study was performed in compliance with GLP.

A reproductive toxicity screening study according to OECD 421 Test Guideline was performed in rats. This study was performed to validate the OECD 421 Test Guideline protocol. BBP was chosen for this validation because BBP was known to have effects both on fertility parameters and on development on the conceptus (Agarwal et al., 1985; Hammond et al., 1987; NTP, 1989). The conclusion from the authors was that the OECD 421 Test Guideline scores BBP correctly as a reproductive toxicant. In the study RIVM-bred WU rats (10 males and 10 females/group) were exposed by gastric intubation to 250, 500 or 1,000 mg/kg/day of BBP. After dosing of both sexes for 14 days, males and females were paired (1:1), and allowed to mate for a maximum of 14 days, whilst dosing was continued. Males were dosed further daily, and killed and necropsied after a total dosage period of 29 days. Female rats were dosed until postpartum day 6, and then killed and necropsied. At 1,000 mg/kg effects were found on the body weight gain and food consumption in both male rats (63 g compared to control values at 80 g) and pregnant female rats (69 g compared to control values at 118), the pregnancy rate was reduced, testis and epididymis weights were significantly reduced (4.2 g compared to control values at 4.9 g), testicular degeneration accompanied by interstitial (Leydig) cell hyperplasia and appearance of cellular debris were increased as were time to conception and postimplantation loss. Corpora lutea, implants per dam, and pre-implantation loss were not different between controls and exposed groups. The number of live pups at day 1 and 6 after birth were lower in the 1,000 mg/kg/day group, 1.5 and 0.8 compared to control rats, 9.4 and 9.2. A reduced mean pup weight at day 1 was reported at 500 mg/kg/day of BBP (7.0 g in control animals and 6.5 g at 500 mg/kg/day), however, the pups were of similar weight compared to controls on post natal day 6. Furthermore, statistical significance was observed on a per pup basis as opposed to a litter basis which is more appropriate for these studies. No abnormalities were found in the offspring except for one pup in the low-dose group with a displaced digit of one paw. The NOAEL for effects on reproductive organs were 500 mg/kg bw/day. The NOEL for effects in offspring was 250 mg/kg bw/day of BBP based on reduced pup weight on postantal day 1 from 500 mg/kg bw/day (Piersma et al., 1995).

A one-generation reproduction study was performed in Wistar rats (Crl:WI (WU) BR) (male, 225 - 272 g 12/group, female, 185 - 266 g 24/group). BBP was administered at dietary concentrations of 0 (control), 0.2, 0.4 and 0.8% over one generation producing 2 litters. Clinical signs, body weight, food consumption, fertility and reproductive performance were evaluated, combined with microscopic examination of male and female reproductive organs (ovaries, uterus, vagina, testes, epididymis, seminal vesicle, prostate, coagulating gland, pituitary) and the liver. No mortality or clinical signs reported, were considered to be caused by the treatment; one male and one female parent rat in the control group were killed during the study. Live birth index was 97, 98, 100 and 99% in the control, 0.2, 0.4 and 0.8% BBP group and viability index day 4-21 was 100, 97, 100 and 97% in the control, 0.2, 0.4 and 0.8% BBP group. Gross necropsy findings in parent rats or pups that died during lactation did not indicate the presence of any significant treatment related effects. A reduction in mean body weight was observed in the females in the 0.8% BBP group (263.3 ± 3.6) when compared to control animals (266.8 ± 3.5) during the gestational and lactational periods of the two litters. Reduced food consumption was reported at 0.8% BBP during both gestational and lactational periods, and was considered related to BBP exposure. A slight increase in absolute liver weights was found in the females of the 0.8% BBP group (8.71 and control 8.24) and in the males of the 0.4% (14.81) and 0.8% (14.98) BBP groups compared to control levels at (14.11). An increase in relative liver weight was only statistically significant for the females in the 0.8% BBP group (33.04 and control 30.88). Microscopic examination of the organs of the reproductive tract did not reveal any treatment related effects. On the basis of the results described above, the NOAEL for parental toxicity was 0.4% BBP in the diet based on effects on the liver (206 mg/kg bw/day for males, 217 mg/kg bw/day for females), and the NOAEL for reproductive performance and development

of the offspring was 0.8% BBP in the diet, the highest dose tested (418 mg/kg bw/day for males, 446 mg/kg bw/day for females). The study was performed in compliance with GLP. The study was performed according to EEC Annex 5 Directive 79/831/EEC and OECD Guidelines No. 415 (Monsanto, 1993).

4.1.2.9.2 Effects on the reproductive organs, aimals

A 14-day dietary fertility study was conducted in adult, male Fisher 344 rats given levels of 0, 0.625, 1.25, 2.5 and 5% of BBP in the diet corresponding to approximately 0, 312, 625, 1,250 or 2,500 mg/kg bw/day of BBP. When expressed relative to body weight significant increases in liver and kidney weights were reported in all BBP groups (liver; 4.52, 5.19, 4.97, 4.46, compared to control value at 3.88, kidney; 0.809, 0.857, 0.840, 0.882, compared to control value at 0.763). A statistically significant reduction in total body, and absolute thymus, testis, epididymis, prostate and seminal vesicle weights were reported in the 2.5% and 5% BBP dose groups, whereas a statistically significant decrease in the relative organ weight was only reported in the thymus, testes, epididymis (only in the 5% group) and seminal vesicle at 2.5 and 5%. Histology revealed dose-dependent and statstistically significant atrophy of the testis, prostate and seminal vesicles at 2.5 and 5%, atrophy of the thymus and epididymis at 5%, and the presence of immature sperm cells in the tubular lumens and necrosis of the tubular epithelium in the caput epididymis at 2.5% (5/8 and 8/10 animals) and 5% BBP (3/9 and 10/10 animals). In the liver a mild multifocal chronic hepatitis was reported in the 5% BBP dose group, and in the kidney scattered cases of renal proximal tubular regeneration in a small number of animals in all dose groups were evident. Plasma testosterone concentration was significantly decreased in the 5% BBP group while follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were increased at 2.5% and 5.0% BBP. Since exposure to 5% BBP produced a generalised toxicosis this general toxicity may have caused or contributed to the organ-specific lesions observed at this dose. The NOAEL for effects on male reproductive organs was 1.25% corresponding to 625 mg/kg bw/day of BBP. The LOAEL for increases in relative liver and kidney weight was 0.625% of BBP in the diet corresponding to 312 mg/kg/day (Agarwal et al., 1985).

In a 28 days repeated dose toxicity study in young Cpb-WU male rats (28 days of age), rats were treated daily with BBP by gavage (Piersma et al., 2000). The study was a part of a developmental toxicity study reported under the section “Developmental studies, BBP”. The BBP doses used were 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg bw/day, with three animals per group. Necropsy was performed one day after the last dosage. The liver, kidney, thymus, thyroid, spleen and testis were weighted and processed for histology. The results were as following: No changes in food consumption were reported after exposure to BBP. Body weight gain was decreased from 1,250 mg/kg bw/day, however, the decrese was not statistically significant. Relative liver weight was statistically significantly increased from 750 mg/kg bw/day (6.14, 6.45, 6.09, 6.66 and 6.64 compared to control value at 5.16). Liver PCoA as an index of peroxisome proliferation showed a similar response. A dose-dependent increased trend in relative kidney weight was reported from 750 mg/kg bw/day. However, the increase was not statistically significant. No statistically significant decrease in thymus and thyroid weight was reported, however, a trend towards a decrease was observed. No effects in spleen were reported. In testis a dose-related decrease was reported in relative testis weight from 750 mg/kg bw/day, however, the decrease was statistically significant first at 1,250 mg/kg bw/day (1.03 at 750 mg/kg/day, 0.74 at 970 mg/kg/day, 0.41 at 1,250 mg/kg/day, 0.28 at 1,600 mg/kg/day and 0.24 at 2,100 mg/kg/day compared to control value at 1.15). Histopathologic analysis of the testis revealed severe atrophy from 970 mg/kg bw/day. LH and FSH was increased at

1,250 mg/kg bw/day and a statistically significant decrease in testosterone level was reported from 450 mg/kg bw/day (1.7 at 450 mg/kg/day, 2.0 at 580 mg/kg/day, 3.8 at 750 mg/kg/day, 2.7 at 970 mg/kg/day, 1.8 at 1,250 mg/kg/day, 2.7 at 1,600 mg/kg/day, and 1.1 at 2,100 mg/kg/day compared to control value at 9.1). The study concluded a LOAEL of 970 mg/kg bw/day based on testis atrophy as the most critical reproductive effect in male rats in this study. The testicular atrophy was reported in the presence of a 20% increase in relative liver weight. In summary, the NOAEL for effects on the reproductive organs was 750 mg/kg bw/day. The NOAEL for systemic toxicity based on increased liver weight was 580 mg/kg bw/day.

In a 26 week dietary toxicity study (NTP Rapport No. 458, 1997), male Fisher F344/N rats (15/group) were given 0, 300, 2,800, 8,300 and 25,000 ppm BBP corresponding to 30, 180 and 550 or 1,660 mg/kg bw/day. The non-reproductive effects from the 26 week study are described in Section 4.1.2.6. The main effects on male sex organs and fertility are described in this section. Effects on fertility were studied at doses of 0, 2,800, 8,300 and 25,000 ppm BBP, and were only observed in the 25,000 ppm group. The pregnancy rate for females mated with 25,000 ppm dosed BBP males was 0/30. Epididymal spermatozoa concentrations at 0, 2,800, 8,300 and 25,000 ppm BBP were $284 \cdot 10^6$, $285 \cdot 10^6$, $376 \cdot 10^6$ and $2.06 \cdot 10^6$. In this study the control animals were shown to have an epididymal spermatozoa concentration lower than expected ($284 \cdot 10^6$ per gram cauda tissue). This phenomenon was explained in a study remark by the study director to may be due to an inadequate mincing of the cauda epididymis in these animals. Other control values for epididymal spermatozoa concentrations/gram epididymis from various studies are $365 \pm 107 \cdot 10^6$ in Fisher 344 rats (Balzak et al., 1985), $522 \cdot 10^6$ (Aajfes et al., 1980), $574.9 \pm 37.6 \cdot 10^6$ in Sprague-Dawley rats (NTP, 1991), $443 \pm 40 \cdot 10^6$ in Fisher 344 rats (van Birgelen et al., 1999), and $298.7 \pm 14.3 \cdot 10^6$ in Wistar rat (TNO, 1998). No information is given about mincing of the BBP treated tissue. The absolute right cauda, epididymis, and testis weight at 25,000 ppm was significant less than in control animals. The incidence of hypospermia and atrophy of the seminiferous tubule in the testis, and hypospermia in the epidydimis was significantly greater than in control animals. The degenerative changes of the testis and epididymis in the 25,000 ppm males were qualitatively and quantitatively similar to those observed in males in the 10-week study. The NOAEL for fertility and reduced epididymal spermatozoa concentration in this 26 weeks study was 8,300 ppm BBP corresponding to 550 mg/kg bw/day (NTP, 1997). This study was performed in compliance with GLP.

Several studies with BBP were conducted by Lake et al. (1978) and are reported in the following paragraphs. It should be noted that these studies are less comprehensive and detailed than the NTP studies.

Sprague-Dawley rats (6 animals/group) were treated daily by gastric intubation at dose levels of 0, 160, 480 or 1,600 mg/kg/day for 14 days. Control animals received corresponding quantities of corn oil vehicle. At 1,600 mg/kg/day a statistically significant increase in relative liver weight was reported (6.3 g compared to control value at 5.2 g). The administration of 1,600 mg/kg/day of BBP was found to cause a marked depression of both the absolute (0.82 compared to control value at 1.89) and relative testis weight (0.44 compared to control value at 0.99). At 1,600 mg/kg/day histopathologic examination revealed severe testicular atrophy in > 50% of the seminiferous tubules in all animals examined. Similar, but less severe lesions were observed by histopathologic examination in one of three animals examined from the 480 mg/kg/day group. However, the effect at 480 mg/kg/day was not statistically significant. The NOEL from this study was 160 mg/kg bw/day based on testicular atrophy reported in one of three animals at 480 mg/kg/day (Lake et al., 1978).

A similar test was performed with 480 and 1,600 mg/kg/day of BBP for 14 days in both Sprague-Dawley and Wistar rats. A significant depression in absolute and relative testis weight

was only observed in rats receiving 1,600 mg/kg/day in Sprague-Dawley rats (0.68 and 0.34 compared to control levels at 2.03 and 0.95) and in Wistar rats (1.03 and 0.60 compared to control levels at 1.93 and 1.02). Histopathologic examination was performed on testis from all animals. 480 mg/kg/day did not induce any histopathologic changes in Wistar rats, however, in one Sprague-Dawley rat testicular atrophy < 25% was reported in one of six animals examined. In the 1,600 mg/kg/day group all animals from both strains were affected and the extent of the lesions being more severe in the Sprague-Dawley rats. For Wistar rats the NOAEL from this study was 480 mg/kg bw/day, and for Sprague-Dawley rats the LOAEL was 480 mg/kg bw/day (Lake et al., 1978).

In a 4-day study the testicular effects of BBP and the monoester derivatives of BBP were examined after oral administration. Administration of 1,600 but not 800 mg/kg/day of BBP was sufficient to reduce both the absolute (0.69 compared to control 0.87) and the relative (0.64 compared to control 0.76) testis weight of Sprague-Dawley rats within 4 days. Histopathologic examination revealed atrophic changes in three of six animals in the 800 mg/kg/day group and in five of six animals in the 1,600 mg/kg/day group. The severity of the atrophic lesions was enhanced at the highest dose level of BBP. Mono-n-butyl phthalate (MBuP) administered in doses equimolar to 1,200 mg/kg/day of the diester, caused a depression of both the absolute (0.96 compared to control 1.28) and relative (0.72 compared to control 0.95) testis weight, whereas monobenzyl phthalate (MBeP) only depressed the absolute testis weight (1.06 compared to control 1.28). Histopathologic examination of section of the testis from all animals treated with either MBuP or MBeP revealed atrophic changes. The effects of MBuP were more severe than those with MBeP (Lake et al., 1978).

In two 3 months subchronic studies in Sprague-Dawley and Wistar rats the animals were fed diets containing 2,500 to 20,000 ppm or 2,500 to 12,000 ppm BBP (corresponding to 188, 375, 750, 1,125, 1,500 or 151, 381, 960 mg/kg bw/day). In these studies no effects on testis were reported either as weight changes or histopathologically. However, in Sprague-Dawley rats an increase in the relative liver weight was reported at 1,125 mg/kg bw/day and higher, and in Wistar rats at 960 mg/kg bw/day. An increase in relative kidney weight was reported from 381 mg/kg bw/day in Wistar rats and histopathological changes in the pancreas from 381 mg/kg bw/day and in the liver at 960 mg/kg bw/day. For more detailed description of these studies see Section 4.1.2.6 (Hammond et al., 1987). In Wistar rats the NOAEL was 151 mg/kg bw/day based on increased kidney weight at doses ≥ 381 mg/kg bw/day. In female Sprague-Dawley rats the NOAEL was 375 mg/kg bw/day based on liver and kidney weight increases at doses ≥ 750 mg/kg/day, and in males the NOAEL was 750 mg/kg/day based on liver weight increase at doses ≥ 1,125 mg/kg/day.

In a 4 week oral subacute toxicity study, Sprague-Dawley rats were administered BBP in food at doses from 500 to 4,000 mg/kg bw/day. Adverse reactions were reported in males from 2,000 to 4,000 mg/kg bw/day, and included testicular atrophy. Histopathological changes in the testis were reported in a dose-dependent manner from 1,500 mg/kg bw/day, whereas no changes were reported in the liver. The few high-dose animals that survived were allowed to recover for 4 weeks, and in these animals testicular atrophy was still evident in some animals. For more detailed description of the study see Section 4.1.2.6. The NOAEL for testicular atrophy was 1,000 mg/kg bw/day (Hammond et al., 1987).

4.1.2.9.3 Effects on the reproducitve organs, humans

Duty et al. (2003) studied whether the general population levels of phthalate monoesters in urine were associated with altered semen quality. The levels of mono-esters measured in urine reflected recent exposure to phthalates, since phthalates have short half-lives, and from all routes of exposure, oral, dermal, inhalation and ingestion. In this study male partners (168 men between 20 and 54 years of age) of sub-fertile couples were recruited. Semen parameters were dichotomized based on WHO (1999) reference values for sperm concentration (less than 20 million/ml) and motility (less than 50% motile) and Tygerberg Strict criteria for morphology (less than 4% normal). The comparison group was men with all three semen parameters above the reference values. Eight urinary phthalate monoesters were measured in a single spot urine sample collected on the same day as the semen sample [monoethyl phthalate (MEP), monomethyl phthalate (MMP), monoethylhexyl phthalate (MEHP), monobutyl phthalate (MBuP) monobenzyl phthalate (MBeP), monooctyl phthalate (MOP), monoisononyl phthalate (MINP), and monocyclohexyl phthalate (MCHP)]. The phthalate metabolites were measured with high performance liquid chromatography and tandem mass spectrometry. Specific gravity adjusted phthalate levels were dichotomized using median into high and low categories. The unadjusted median levels of urinary phthalate monoester concentrations in μg/L urine were; 156 for MEP, 10.3 for MBeP, 15.9 for MBuP, 5.7 for MEHP, and 7.5 MMP, reflecting exposure to diethyl phthalate, butyl benzyl phthalate, dibutyl phthalate, diethyl hexyl phthalate and dimethyl phthalate. These levels can be compared to phthalate monoester levels measured in Blount et al. (2000); Hoppin et al. (2002); NHANES, see Section 4.1.2.1. However, it has to be taken into account that in this study the levels of phthalate monoesters were only measured in males. The results from the Duty et al. (2003) study indicated that median monobutyl phthalate (MBuP) levels were associated with sperm motility and sperm concentration below the reference values with odds ratio (95% confidence interval) of 2.37 (1.13 to 5.00) and 2.41 (0.80 to 7.23) which means that they were 2.37 and 2.41 times more likley to have sperm motility or sperm concentrations below the reference value. The median mono benzyl phthalate (MBeP) levels were also associated with sperm motility, morphology, and sperm concentration below the reference values with odds ratio of 1.8 (0.9 to 3.9), 2.1 (0.9 to 5.1) and 2.7 (0.8 to 8.5). The authors concluded from the study that there were dose-response relations for MBuP and MBeP for one or more of the semen parameters studied, and suggestive evidence for MMP for sperm morphology. For the other monoesters, no clear correlations were found.

4.1.2.9.4 Summary fertility and effects on the reproductive organs, BBP

Reproductive effects of BBP and its major metabolites MBuP and MBeP in rats following oral administration both by gavage or in the diet have been investigated in studies of different duration (from 4 days to 26 weeks, and in 2-generation studies). The main effects reported include a decrease in the relative weight of testis, damage to the testis, epididymis, prostate, seminal vesicle and to reduced epididymal sperm concentrations, and at high BBP concentrations reduced fertility, in addition to increases in relative liver and kidney weights. The determined NOEL/NOAEL/LOAEL values form the various studies are given in **Table 4.27**.

Table 4.27 Fertility and effects on the reproductive organs

Study Design	Effect Level	Critical Effect	Reference
CD Sprague-Dawley rats; 2-generation study; 30/sex/group; Administration in feed; 0, 750, 3750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day of BBP.	NOAEL for fertility: 250 mg/kg bw/day of BBP based on reduced mating and fertility indices in F1 parents to make F2 offspring at 750 mg/kg bw/day. NOAEL for developmental effects: 50 mg/kg bw/day of BBP based on reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring	Fertility: reduced mating and fertility indices in F1 parents to make F2 offspring at 750 mg/kg bw/day. Development: reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day	Tyl et al. (2004)
Sprague-Dawley rats; two-generation study; 25/sex/group; Administration by gavage; 0, 20, 100 and 500 mg/kg bw/day BBP	NOAEL: 20 mg/kg bw/day BBP for developmental effects based on decreased body weight in offspring from 100 mg/kg bw/day. No NOAEL value could be derived for effects on fertility. NOAEL for effects on the reproductive organs: 100 mg/kg bw/day	F_0; decrease i body weight gain in males at 500 mg/kg/day. A dose-dependent increase in kidney weight of both sexes, (significant from 100 mg/kg/day in females and at 500 mg/kg/day in males), a significant increase in liver weight in males at 500 mg/kg/day, and a significant decrease in ovarie weight in females at 500 mg/kg/day. A decrease in testosteron (significant at 500 mg/kg/day), and increase in FSH (significant from 100 mg/kg/day) in males. F1: significantly decreased body weight at birth from 100 mg/kg/day, and at 500 mg/kg/day throughout the study. AGD was decreased and preputial separation delayed in males at 500 mg/kg/day. Macroscopic and microscopic changes of testis, and decreased testosterone levels at 500 mg/kg/day after puberty. Significantly decreased testis, epididymis, and seminal vesicle weight at 500 mg/kg/day in F1 postweaning. Decreased number of germ cells in the seminiferous tubules, and sperm in the epididymis at 500 mg/kg/day as well. BBP did not affect reproductive ability, including delivery and lactation. F2: no significant effects related to BBP exposure up to pnd 21.	Nagao et al. (2000)

Table 4.27 continued overleaf

Table 4.27 continued Fertility and effects on the reproductive organs

Study Design	Effect Level	Critical Effect	Reference
Fisher 344 rats male; 14 days; Administration in diet; 0.625, 1.25, 2.5 and 5% (312, 625, 1,250 and 2,500 mg/kg bw/day BBP)	NOAEL: 625 mg/kg bw/day BBP for effects on reproductive organs LOAEL: 312 mg/kg/day BBP for effects on liver and kidney	At doses ≥1,250 mg/kg bw/day body, thymus, testes, epididymis and prostate weight decrease, histopathologic changes in testes, prostate and seminal vesicle with the presence of immature sperm and necrosis in tubular epithelium, increased levels of LH and FSH. At 2,500 mg/kg bw/day decreased progesterone levels, general toxicosis.	Agrawal et al. (1985)
Cpb-WU male rats, 4 weeks of age; Administration of BBP by gavage 28 days; 3/group; 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600, 2,100 mg/kg bw/day BBP	NOAEL for effects on the reproductive organs (reduced testis weight): 750 mg bw/kg/day BBP NOAEL for systemic toxicity (increased liver weight): 580 mg/kg bw/day	Liver weight increase from 750 mg/kg bw/day. A dose related decrease in testis weight from 750 mg/kg bw/day, however, statistically significant from 1,250 mg/kg bw/day. Decreased testosterone levels from 450 mg/kg bw/day. Severe testicular atrophy from 970 mg/kg bw/day.	Piersma et al. (2000)
Fisher 344 rats; 15/male/group; 10 weeks; Administration in diet; 300, 2,800 and 25,000 ppm (20, 200 and 2,200 mg/kg bw/day BBP)	NOAEL: 300 ppm (20 mg/kg bw/day BBP) for sperm effects. NOAEL: 2,800 ppm (200 mg/kg bw/day BBP) for fertility	At doses ≥ 200 mg/kg bw/day decreased epididymal spermatozoa concentration. At 2,200 mg/kg bw/day alterations in haematological values, decreased body, prostate and testes weight, degeneration in seminiferous tubules, no pregnancy after mating.	NTP (1997)
Fisher-344 male rats; 15 male/group; 26 weeks; Administration in diet; 0, 2,800, 8,300, and 25,000 ppm (0, 180, 550, 1,660 mg/kg bw/day BBP.)	NOAEL: 8,300 ppm (550 mg/kg bw/day BBP) for fertility and sperm effects.	Fertility; At 25,000 ppm decreased fertility, testis, epididymis weight, and epididymal spermatozoa conc. Degenerative changes in testis and epididymis. Other toxic effects see Table 4.24.	NTP (1997)
RIVM-bred WU-rats; 10/sex/group; 14 days prior to and throughout mating; gastric intubation; 250, 500 and 1,000 mg/kg bw/day BBP.	NOEL: 250 mg/kg bw/day BBP based on reduced pup weight at 500 mg/kg bw/day; NOAEL 500 mg/kg bw/day for effects on reproductive organs	At 1,000 mg/kg bw/day decreased body weight, pregnancy rate, live pups, pup weight, and epididymis weight, testicular degeneration. At 500 mg/kg bw/day slightly reduced pup weight.	Piersma et al. (1995)
Wistar rats; Administration in diet over one generation producing two litters; 0.2, 0.4 and 0.8% BBP	NOAEL parental: 0.4% (206 mg/kg bw/day BBP male and 217 mg/kg/day BBP female) based on increased liver and kidney weight NOAEL reproductive performence and developmental effects: 0.8% (418 mg/kg bw/day BBP male and 446 mg/kg bw/day BBP female). Based on reduced reproductive performance.	At 0.8% reduced body weight gain and food intake in dams. Slight increase in absolute and relative liver weight in female.	Monsanto (1993)

Table 4.27 continued overleaf

Table 4.27 continued Fertility and effects on the reproductive organs

Study Design	Effect Level	Critical Effect	Reference
Sprague-Dawley rats; 6 male/group; 14 days; gastric intubation; 160, 480 and 1,600 mg/kg bw/day BBP.	NOEL: 160 mg/kg bw/day BBP	At 480 mg/kg bw/day histopathologic changes in testis in one of three rats examined, at 1,600 mg/kg bw/day decreased testes weight with testicular atrophy.	Lake et al. (1978)
Wistar rats and Sprague-Dawley rats; 6/male/group; 14 days; gastric intubation; 480 and 1,600 mg/kg bw/day BBP.	NOAEL Wistar rat: 480 mg/kg bw/day BBP LOAEL Sprague-Dawley rats: 480 mg/kg bw/day BBP	At 480 mg/kg bw/day testicular atrophy in one Sprague-Dawley rat. At 1,600 mg/kg bw/day decreased testes weight with testicular atrophy in all rats. Sprague-Dawley rats were more severely affected than Wistar rats.	Lake et al. (1978)
Sprague-Dawley rats; 6/male/group; 4 days; gastric intubation; 800 and 1,600 mg/kg bw/day of BBP, 855 mg/kg bw/day of MBuP and 985 mg/kg bw/day of MBeP		At doses ≥ 800 (BBP), 855 (MBuP) and 985 MBeP) mg/kg bw/day reduced testes weight and testicular atrophy.	Lake et al. (1978)
Sprague-Dawley rats; 5-10/sex/group; 4 weeks; Administration in diet; 500, 1,000, 1,500 2,000, 3,000 and 4,000 mg/kg bw/day BBP.	NOAEL: 1,000 mg/kg bw/day BBP	At doses ≥ 1,500 mg/kg bw/day body weight decrease. From 1,500 mg/kg bw/day testicular atrophy. From 2,000 mg/kg/day stiffness while walking and bleeding around nares.	Hammond et al. (1987)
Sprague-Dawley rats; 10/sex/group; 3 month; Administration in diet; 2,500 – 20,000 ppm (corresp. to approx. 188, 375, 750, 1,125, 1,500 mg/kg bw/day BBP)	NOAEL female: 375 mg/kg bw/day BBP NOAEL male: 750 mg/kg bw/day BBP	At doses ≥ 750 mg/kg bw/day kidney and liver weight increase in females, at doses ≥ 1,125 mg/kg bw/day liver weight increase in males.	Hammond et al. (1987)
Wistar rats; 10/sex/group; 3 month; Administration in diet; 2,500 – 12,000 ppm (corresp. to approx. 151, 381, 960 mg/kg bw/day BBP)	NOAEL male and female: 151 mg/kg bw/day BBP	At doses ≥ 381 mg/kg bw/day kidney weight increase, urinary pH decrease. At 960 mg/kg bw/day body weight decrease, liver weight increase, slight anaemia, and histopathologic changes in liver and pancreas.	Hammond et al. (1987)

As regards the effects on fertility or reproductive organs following administration of BBP to rats in the diet (Tyl et al., 2004; Agarwal et al., 1975; NTP, 1997; Hammond et al., 1987) or via gavage (Piersma et al., 1995; Piersma et al., 2000; Lake et al., 1978; Nagao et al., 2000), reduced mating and fertility indices, decreases in testis weight, histopathological changes in testis, and hormonal changes have been reported. These effects have in the majority of the studies been reported at BBP doses equal to (Hammond et al., 1987, 4-week diet study) or higher than those which have induced other effects, such as variations in absolute and relative weights of the liver and kidney and histopathological changes such as atrophy in the liver and pycnotic nuclei, acinar atrophy and slight fibrosis in the pancreas. Exceptions includes, when BBP is administered by gavage, a 14 days and a 4 days study in Sprague-Dawley rats (Lake et al., 1978), and a 28 days study in Cpb-WU rats (Piersma et al., 2000). In the Lake 14 days study, minimal testicular atrophy was reported in one of three animals examined at 480 mg/kg bw/day (Lake et al., 1978). In the 4 days study atrophic changes in the testis in 3 of 6 animals at 800 mg/kg bw/day of BBP were reported. In the 28 days study by Piersma et al. (2000) a decrease in testosterone level was reported from 450 mg/kg/day. Exceptions include, when BBP is administered in the diet, a

10 week fertility study (NTP, 1997). In this study a dose-related decrease in epididymal spermatozoa concentration compared to control animals was reported from 200 mg/kg bw/day (2,800 ppm) ($p \leq 0.05$) of BBP and the NOAEL from this study was 20 mg/kg bw/day of BBP. When taking into account days of recovery in males in the 10 week NTP study (days from positive sperm plug to necropsy) in a covariate analysis of variance, on the epididymal spermatozoa concentration, the decrease in epididymal spermatozoa concentration at 2,800 ppm was not statistically significant at the 5% level compared to control animals ($p = 0.07$). However, the dose-dependent decrease in epididymal spermatozoa concentration was still evident. In the parallel 26-week oral toxicity study in rats where BBP was administered in the diet (NTP, 1997) the control value for epididymal spermatozoa concentration may not be valid due to a reported possible inadequate mincing of the cauda epididymis tissue from control animals. The NOAEL for reduced epididymal spermatozoa concentration and fertility in the 26 week study was 550 mg/kg bw/day. In a new 2-generation study (Tyl et al., 2004) significantly reduced mating and fertility indices were reported in F1 parents to make F2 offspring at 750 mg/kg bw/day. In the same study a significantly reduced relative and absolute paired ovaries and uterus weight was reported in F0 females. In adult F1 males a significant increase in reproductive tract malformations was reported (53.33% compared to 0% in controls). No increases in reproductive tract malformations were reported in females. Systemic toxicity reported at 750 mg/kg bw/day was limited to organ weight changes (liver, kidney) in males and females and histopathological lesions graded as minimal in females. The NOAEL for fertility was 250 mg/kg bw/day from this study. In another recent two-generation study (Nagao et al., 2000) increased serum FSH in F0 males was reported from 100 mg/kg bw/day, and at 500 mg/kg bw/day a decreased testosteron level. In F1 males (18 weeks old) a decrease in testis, epididymis and ventral prostate weight, a decrease in testosterone and LH levels, and athrophy of the seminiferous tubules with decreased number of germ cells, and a decreased number of sperm in the epididymis were reported, accompanied with reduced body weight and an increased relative liver and kidney weight. No effect on fertility was reported in this study at any dose levels (20, 100 or 500 mg/kg bw/day). From the Nagao (2000) study no NOAEL value could be derived for effects on fertility. The NOAEL value for effects on the reproductive organs in males was 100 mg/kg bw/day, based on weight changes and atrophy of the reproductive organs in the F1 generation at 10 or 18 weeks of age at 500 mg/kg bw/day. The NOAEL value for developmental effects was 20 mg/kg bw/day based on reduced body weight in male and female F1 offspring from 100 mg/kg bw/day.

Only one human study is available where the relation between exposure to phthalates and semen quality was evaluated. In this study an association was found between high levels of mono butyl phthalate and/or mono benzyl phthalate in the urine and altered semen quality including semen concentration, semen motality and semen morphology (Duty et al., 2003). Due to the mixed exposure to various phthalates it is difficult to conclude that the effect observed on semen quality is related only to BBP exposure. Furthermore, the phthalates were only measured in a single spot urine sample in a relative small group of men (168) derived from subfertile couples.

The National Toxicology Program (NTP) Center for the evaluation of risk to human reproduction has used a phthalate expert panel to evaluated the reproductive and developmental toxicity of BBP and other phthalates. This expert panel has concluded that the database on reproductive toxicity is suffucient to judge that oral exposure to BBP can cause reproductive toxicity in rats (Kavlock et al., 2002).When taking the available data base into account a NOAEL value at 100 mg/kg bw/day for effect on the reproducitve organs is considered to be used in the risk characterisation from the study of Nagao et al. (2000). This NOAEL value is based on atrophy of the testis, epididymis, and seminal vesicle, and reduced reproductive organ weights at 10 or 18 weeks of age in the F1 generation at 500 mg/kg bw/day.

In the above summarised studies, effects on male reproductive organs and/or fertility are reported after administration of BBP in doses equal to or higher than those which induce minimal systemic toxicity such as relative organ weight changes, and in some studies histopathological changes in the liver and pancreas. Furthermore, since signs of testicular toxicity, evident as a dose-dependent decrease in epididymal spermatozoa concentration and atrophy of the testis, and decreased testosterone and FSH levels, are reported in the absence of effects in other organs, BBP may affect fertility. Based on the available data BBP is proposed classified with Xn R62 (Repro. Cat. 3) "Possible risk of impaired fertility", according to EU criteria.

4.1.2.9.5 Developmental studies, BBP, animals

Developmental toxicity of BBP was studied in Swiss DC-1 mice. Timed-pregnant mice received BBP (~ 96% purity) in feed 0 (control), 0.1, 0.5, 1.25 and 2.0% corresponding to 0, 182, 910, 2,330 and 4,121 mg/kg bw/day from gestation day (gd) 6 to 15 (major organogenesis). Dams (27-30/group) were sacrificed on gd 17. No maternal or embryo/foetal effects were reported at 0.1% BBP (182 mg/kg bw/day). At 0.5% BBP (910 mg/kg bw/day), maternal effects were limited to a reduction (15%) in dam weight gain (gd 6-15), however no reduction was reported in adjusted weight gain; prenatal mortality per litter (15% versus 8% for controls) and malformed foetuses per litter (14% versus 4% for controls) were significantly increased. At 1.25% BBP (2,330 mg/kg bw/day) dam weight gain was reduced by 66% (gd 6-15) and 25% (gd 0-17 corrected for uterine weight). Absolute liver weight was decreased and relative liver and kidney weights were increased in the absence of treatment-related microscopic lesions; foetal weight per litter was 83% of controls; prenatal mortality per litter (93% versus 8% for controls) and malformed foetuses per litter (89% versus 4% for controls) were increased. The 2.0% BBP group (4,121 mg/kg bw/day) was eliminated after evaluation of 14 dams since all conceptuses were resorbed. The NOAEL for maternal and developmental toxicity, according to the author was 0.1% BBP (182 mg/kg bw/day). 0.5% BBP (910 mg/kg bw/day) is by the authors described to produce minimal evidence of maternal effects (15% reduction in maternal body weight gain during treatment), and significant developmental toxicity, evident as foetal death per litter (15% versus 8% for controls) and percent malformed foetuses per litter (14% versus 4% for controls) (NTP, 1990).

Developmental toxicity of BBP was studied in Sprague-Dawley rats. Time-pregnant rats received BBP (~96% purity) in feed 0 (control), 0.1, 0.5, 1.25 and 2.0% corresponding to 0, 419, 1,102 and 1,641 mg/kg bw/day from gestation day (gd) 6-15 (major organogenesis). Dams (27-30/group) were sacrificed on gd 20. At 1.25% BBP (1102 mg/kg bw/day), dam weight gain (gd 6-15) was reduced by 37% and relative liver weight was increased. The percent foetuses with variations/litter were increased (41.03% versus 19.04% for controls). The percent malformed foetuses per litter (5.9% versus 2% for controls) were increased. At 2% BBP (1,641 mg/kg bw/day), dam weight gains (gd 6-15 and gd 0-20 corrected for uterine weight) were reduced by 93% and 17%; foetal weight was 80% of controls; resorptions per litter (40% versus 4% for controls) and malformations per litter (53% versus 2% for controls) were increased. Maternal food/water intake was unchanged or increased, except for reduced food intake at 2% BBP (gd 6-9) in rats. The NOAEL for maternal and developmental toxicity was 0.5% BBP (419 mg/kg bw/day) (NTP, 1989).

Developmental toxicity of BBP was investigated in a recent study in pregnant and non-pregnant Cpb-WU rats (8 weeks of age) (Piersma et al., 2000). Pregnant rats received BBP from gd 6-15 (short time exposure) or gd 6-20 (long time exposure) by gavage. The study was performed to

investigate developmental toxicity following both the classic exposure duration from gd 6-15 or from gd 6-20, the last which is considered to be a more sensitive exposure duration used for the study of developmental effects in animals. Ten dose groups of BBP were studied for both exposure durations, 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg bw/day. Ten pregnant rats per group for 270, 350, 580, 970, 1,600 or 2,100 mg/kg bw/day and 25 animals per group for 450, 750 or 1,250 mg/kg bw/day. To each dose group 3 nonmated females were added for comparison with pregnant female rats exposed to BBP. The nonpregnant rats were exposed to BBP for 10 or 15 days. Body weight and food consumption was determined on gd 0, 6, 11, 16 and 21. Necropsy was performed on gd 21. Before termination blood was taken for haematological and biochemical analysis which included serum ALAT (alanin aminotransferase), ASAT (aspergine aminotransferase), testosterone, progesterone, LH, FSH, PRL (prolactin) and estradiol. Liver protein and PCoA activity (palmitoyl CoA oxidase) was also assessed as an index for peroxisome proliferation. After termination the weight of liver kidneys, thymus, thyroid and spleen were determined and a liver lobe was isolated for biochemical analysis. A histological examination was performed on the respective organs. Corpus Lutea, implantation sites, early and late resorptions and foetuses with early and late death were counted, and external anomalies of living foetuses were registered. Odd foetuses were processed for skeletal staining and examination, even foetuses were fixed for morphological analysis according to Barrow and Taylor (1969). The results in pregnant rats and non-pregnant rats were as following:

Maternal effects

At 1,600 and 2,100 mg/kg bw/day several animals died during the first five days of exposure due to toxicity of BBP. Two animals in the 1,600 mg/kg bw/day group died at dosing on day 10. Decreased food consumption was observed from 1,250 mg/kg bw/day during the first 5 days of dosing. Afterwards there was no difference in food consumption between the dose groups. Pregnant rats showed a statistically significant dose-related reduction in body weight gain from 750 mg/kg bw/day. The reduction was more pronounced in the long exposure group than in the short. In non-pregnant rats no dose-related differences in body weight gain were reported. A statistically significant dose-related increase in relative liver weight was reported from 750 mg/kg bw/day in the short time exposure group, and from 580 mg/kg bw/day in the long time exposure group. Similar observations were reported in the non-pregnant rats. Haematological biochemical analysis of ALAT, ASAT, PCoA showed increases from 750, 970 and 270 mg/kg bw/day after long time exposure, whereas, only minor increases were reported after short time exposure. Peroxisome proliferation was reported only at the three highest doses of BBP. Effects on kidneys of pregnant rats included a statistically significant increase in kidney weight from 750 mg/kg bw/day in both exposure groups, and from 970 mg/kg bw/day in the non-pregnant long time exposure group. Effects on thymus, thyroid and spleen were reported at doses from 970, 1,250 and 750 mg/kg bw/day in pregnant rats. In non-pregnant rats only an effect on spleen weight was reported at 2,100 mg/kg bw/day. Haeamatologic evaluation revealed an increase in haematocrit from 750 mg/kg bw/day in pregnant rats, whereas no effects were reported in non-pregnant rats. Endocrinology showed a statistically significant reduction in progesterone levels in pregnant rats from 270 mg/kg bw/day in the long time exposure group, and from 1,250 mg/kg bw/day in the short time exposure group, indicating that the effects at lower doses may be reversible, as short time exposure ended 6 days before necropsy. It was concluded from the study that the LOAEL for maternal toxicity was 580 mg/kg bw/day based on a dose-related liver weight increase, and the corresponding NOAEL 450 mg/kg bw/day.

Developmental effects

No effects on Corpora Lutea were reported. Early resorption was increased from 1,600 mg/kg bw/day, whereas late resorption was increased from 750 mg/kg bw/day in both exposure groups. Foetal weights were statistically significantly decreased from 450 mg/kg bw/day after short time exposure and from 350 mg/kg bw/day after long time exposure. From 750 mg/kg bw/day skeletal anomalies were reported to increase. A low incidence of foetal ovary malformations was found from 580 mg/kg bw/day, mainly after long time exposure. Rather than the spheric shape in unaffected animals, these ovaries had an elongated shape reminiscent of an earlier developmental stage, suggesting a retardation of morphologic development. The incidence of retarded foetal testicular caudal migration showed a dose-related increase from 580 mg/kg bw/day, the incidence being higher after long time exposure as compared to short time exposure to BBP. A decrease in relative foetal testis weight was only reported after long time exposure in a dose-related way from 270 mg/kg bw/day. At 270 mg/kg bw/day a statistically significant reduction was reported (0.282 compared to control value at 0.337). At the two next higher doses 350 and 450 mg/kg bw/day, the foetal testis weight was 0.315 and 0.305. This reduction was not statistically significant, however, from 580 mg/kg bw/day the reduction in foetal testis weight was statistically significant. It was concluded from the study that the most sensitive foetotoxic effect of BBP was a relative foetal testicular weight reduction with a LOAEL at 270 mg/kg bw/day. The morphological effects on testis and ovary at 580 mg/kg bw/day and higher were testicular dislocation and ovary malformation. In summary, the maternal NOAEL was 450 mg/kg bw/day based on statistically significant incresed liver weight at 580 mg/kg/bw/day. The LOAEL based on a dose-related reduction (statistically significant at 270, 580, 750 mg/kg bw/day and further, but not at 350 and 450 mg/kg bw/day) in relative foetal testis weight was 270 mg/kg bw/day. The NOAEL for reduced foetal weight was 270 mg/kg bw/day.

The embryotoxic effects of butyl benzyl phthalate (BBP) and its two main metabolites mono-*n*-butyl (MBuP) and mono-*n*-benzyl phthalate (MBeP) were studied in 0F1 mice and Sprague-Dawley rats, *in vivo* and in whole embryo cultures (Saillenfait et al., 2003). *In vivo*, pregnant mice (15-23/group) and pregnant rats (7-13/group) received a single oral dose of BBP, MBuP or MBeP on gestaion day (gd) 8 and 10. The concentrations of BBP, MBuP and MBeP tested were 0, 0.9, 1.8, 3.6 and /or 5.4 mmol/kg, corresponding to 0, 280, 560, 1,120 and 1,690 mg/kg BBP; 0, 200, 400, 800, 1,200 mg/kg MBuP and 230, 460, 920 and 1,380 mg/kg MBeP. The foetuses were examined externally on gd 18 (mice) and 21 (rats). Results mice *in vivo*: Maternal deaths occurred within 24 hours after administration of 1,120 (1) and 1,690 (3) mg/kg BBP, 800 mg/kg (1) MBuP, and 920 (2) and 1,380 (5) mg/kg MBeP. BBP caused a statistically significant decrease in body weight gain on gd 9-18 in the 1,120 and 1,690 mg/kg dose group (13.2 ± 1.5 and 10.5 ± 1.3 compared to 22.9 ± 1.3 in controls). MBuP caused a statistically significant decrease in body weight gain on gd 9-18 in the 400, 800 and 1,200 mg/kg dose group (13.4 ± 1.4, 7.6 ± 1.4 and 7.8 ± 1.0 compared to 22.7 ± 1.4 in controls). MBeP caused no statistically significant reduction in body weight gain on gd 9-18. No changes were reported in the corrected body weight gain (body weight gain on gd 0-18 minus gravid uterus weight) following exposure to BBP, MBuP and MBeP. In the two highest BBP dose groups a statistically significant reduction in live foetus/litter was reported and in the three highest dose groups of MBuP a statistically significant reduction in live foetus/litter was reported. No statistically significant effect on live foetus/litter was reported following exposure to MBeP. A statistically significant increase in the percentage of resorptions/litter was reported in the three highest dose groups of BBP and MBuP, and in the highest dose group of MBeP. A dose-depended increase in malformed foetuses per litter was reported from 560 mg/kg BBP, 200 mg/kg MBuP and 920 mg/kg MBeP. The mean foetal weight/litter was statistically significantly decreased only at the highest doses of BBP and MBuP. Results rats *in vivo*:

Maternal lethality occurred after administration of 1,690 (1) mg/kg BBP, and 920 (1) and 1,380 (5) mg/kg MBeP. No statistically significant decrease in body weight gain, or corrected body weight gain was reported following exposure to BBP, MBuP and MBeP. No effects on live foetus/litter and percentage of post-implantation loss/litter were reported following exposure to BBP, MBuP and MBeP. A slight increase in malformed foetuses per litter was reported from 1,120 mg/kg BBP, and only at 460 mg/kg MBeP. Results *in vitro* mice: gd 8 mouse embryos were cultured for 46 hours in the presence of MBuP and MBeP at the following concentrations 0.5, 1, 2 and 5 mM. Exposure to MBuP resulted in concentration-related effects on growth, development and morphology. All parameters assessed (yolk sac diameter, crown-rump length, head length, number of somites and morphological score) were significantly different from the controls at 5mM MBuP. MBeP induced significantly reductions in the crown-rump length, head length and number of somites at concentrations ≥ 1 mM, and in the yolk sac diameter and developmental score at concentrations ≥ 2 mM. Results *in vitro* rats: gd 10 rat embryos were cultured for 46 hours in the presence of MBeP at the following concentrations 0.5, 1, 2 and 3 mM. MBeP induced concentration related effects on growth and development. There was a slight but statistically significant reduction in head length and morphological score from 1 mM. All parameters assessed (see above) were significantly lower than the control from 2 mM (by 16-31%). These data indicate that the cultured mouse embryos did not appear intrinsically more sensitive to MBuP and MBeP, than the rat embryos. The authors suggested that the species sensitivity observed *in vivo* after oral administration of BBP, MBuP or MBeP during early organogenesis, might be due to maternal factors i.e. toxicity and/or kinetics.

In a developmental toxicity study performed by Gray and coworkers (Gray et al., 2000) pregnant Sprague-Dawley rats (5/group, block 1) were gavaged daily with 750 mg/kg bw/day of BBP or corn oil from gestation day 14 through postnatal day (pnd) 3. A second block with the same dosing regime was conducted to repeat the positive effects reported in the first block. Eight Sprague-Dawley rats/group were used in block 2. In block 1 the animals were necropsied at 3-4 month of age, and in block 2 at 4-7 monthes of age. Reproductive organ weights were taken from almost every male from each litter (45 male pups/11 litters versus 77 male pups/19 litters in controls in block 1 and 30 male pups/10 litters versus 45 male pups/17 litters in block 2). No reduction in maternal weight gain was reported during gestation and up to pnd 3. The mean pup weight on a per litter basis was statistically significant reduced at birth (5.78 g compared to 6.84 g in the control group). In male offspring treated with BBP a reduced anogenital distance (about 30%), and reduced paired testis weight (about 35%) at day 2 of age was reported on a per litter basis, furthermore, significant decreases in seminal vesicle (1,154 versus 1,857 in controls), ventral prostate (398 versus 685 in controls), paired epididymis (966 versus 1,293 in controls), cauda epididymis (182 versus 312 in controls) were reported on a per litter basis. As infants, 70% of males displayed femalelike areolas/nipples at day 13 of age compared to 0% in the control group on a per litter basis. At necropsy, malformations in the androgen-dependent organs and testis were reported in 84% of male offspring treated with BBP on a per litter bais. Hypospadias were reported in 29% of the male offspring on a per litter basis. Di(2-ethylhexyl) phthalate (DEHP) was also tested in this study, and the results demonstrated that exposure to 750 mg/kg bw/day BBP or DEHP from gestation day 14 through pnd 3 severely altered sexual differentiation in the male rat with about equal potency, and that BBP altered male rat sexual differentiation in an antiandrogenic fashion. The developmental effects of BBP were also studied by Parks et al. (1999). In this study pregnant Sprague-Dawley rats were dosed by gavage with 750 mg/kg bw/day BBP from gestational day 14 to pnd 3. On pnd 2 anogenital distance (AGD), testes weight and *in vitro* testosterone production were measured. Testes weight and AGD was decreased for BBP exposed male pups, and the incidence of areolas on pnd 13 was increased. Testosterone production was not reduced by BBP treament. The authors describe

that these antiandrogenic-like effects may results from reduced androgen production in the fetal Leydig cells and suggests that the testis is the target organ directly affected by perinatal BBP exposure. However, it remains to be determined whether these effects are mediated via direct action of BBP on fetal Leydig cells or through alterations of Sertoli cell paracrine secretions.

In a new 2-generation study male and female CD (Sprague-Dawley) rats (40-45 days old), 30 animals/sex/dose (F0 generation) were administered Butyl Benzyl Phthalate (BBP) in the feed at doses of 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day for 10 weeks (Tyl et al., 2004). Animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with exposure continuing. F0 and F1 males were necropsied after the delivery period, and a histopathologic evaluation was performed on 10 animals from the high dose group and the control group. The following organs were evaluated; pituitary, liver, thyroid gland, seminal vesicles with coagulating glands and fluids, epididymis with contents and fluids, prostate, testes and pancreas. An andrological assessment was also performed which included; reproductive organ weights, epididymal sperm number, motility and morphology, testicular homogenisation-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production. On the day of birth, post natal day (pnd) 0 anogenital distance (AGD) was measured and body weights recorded for all live F1 pups in all litters. F1 litters were standardised to 10 pups (5/sex) on pnd 4. On pnd 11-13 all F1 male pups were examined for retained nipples/aerolae on the ventrum. At weaning on pnd 21, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as F1 parents of the F2 generation. Any remaining F1 male pups not selected as parents or for necropsy, which exhibited retained nipples, were also necropsied. On pnd 21 F0 or F1 females were necropsied, and histopathology was performed on 10 animals from the high dose group and from the control group. The following organs were evaluated ovaries, vagina, uterus with oviducts and cervix, pancreas, pituitary, thyroid gland and liver, and other tissues with gross lesions identified as being treatment related. Selected F1 weanlings 30/sex/dose were administered BBP in the diet for a 10 weeks prebreed exposure period. Acquisition of vaginal patency in females and preputial separation in males were assessed. Vaginal cytology for estrus cyclicity in F1 selected females was evaluated during the last three weeks of the prebreed exposure period, and they were mated for a two-week period as described above. F1 males were necropsied after the F2 litters, parental F1 females were necropsied with histopathology, as described above, and F2 weanlings, up to three/sex/litter were necropsied. For all surviving F0 and F1 parental animals the following organs were weighed at scheduled sacrifice: ovaries, uterus with oviducts and cervix, pituitary, adrenal glands, liver, thyroid gland, seminal vesicles, epididymis with contents and fluid, spleen, prostate, testes, brain, kidneys, and pancreas. Results F0 parental systemic toxicity; Males and females: at 750 mg/kg bw/day significantly increased absolute and relative liver weight, and relative kidney weight. Histopathological lesions in the liver mostly graded as minimal, and more abundant in female rats. At 250 mg/kg bw/day a significantly increased absolute (male) and absolute and relative (female) kidney weight was reported. In females, at 750 mg/kg bw/day significantly decreased body weight from study day 0 to 70, and during gestation and lactation was reported. Results F0 parental reproductive toxicity; In males no reproductive effects were reported, since the exposure to BBP started after they had achieved puberty. In females at 750 mg/kg bw/day significantly reduced absolute and relative paired ovaries weight and uterus weight were reported. Results F1 offspring toxicity; At 750 mg/kg bw/day a significant decrease in pup body weight per litter on pnd 0, 4, 7, 14 and 21, and in the 250 mg/kg bw/day group at pnd 7. In male offspring AGD was significantly ($p < 0.001$) decreased in a dose-related pattern from 250 mg/kg bw/day (1.89 mm compared to controls at 2.06 mm) and at 750 mg/kg bw/day (1.7 mm compared to controls at 2.06 mm). When the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values at 250 and 750 mg/kg bw/day were

still significantly reduced. At 750 mg/kg bw/day significant increase ($p < 0.01$) in male pups with one or more nipples (19.23% compared to 0% in the control group), and in the number of nipples per male (0.72 compared to 0 in the controls). In the 750 mg/kg bw/day group a significanlty increase ($p < 0.001$) in the percentage of male pups with one or more areolae (32.31% compared to 2.63% in the controls), and in the number of areolae per male (1.29 compared to controls at 0.07, $p < 0.01$). At weanling necropsy in males at 750 mg/kg bw/day a significant decrease in terminal body weight, in absolute thymus weight, in absoulte and relative spleen and testis weight, and in absolute epididymis weight was reported. In postwean males F1 a significant delay in the acquisition of puberty in F1 males was seen, evident as delayed age at preputial separation (45.2 compared to 40.9 in controls), an in the adjusted age at preputial separation (45.4 compared to 41.0 in controls). At weanling necropsy in females a significant decrease in terminal body weight, in absolute thymus weight, in absolute and relative spleen weight, and in absolute ovaries and uterus weight was reported. In postwean females F1 a significant delay in the acquisition of puberty was seen, evident as a delay in vaginal patency at 750 mg/kg bw/day (34.1 compared to 31.4 in controls) and in adjusted age at vaginal patency (34.4 compared to 31.5 in controls). Results F1 systemic toxicity; Males: at 750 mg/kg bw/day significantly deceased body weight during preebreed exposure and at necropsy, significantly increased relative liver weight, relative adrenal gland weight, absolute and relative pancreas weight and relative pituitary weight. At 250 mg/kg bw/day significantly increased absolute and relative liver, kidney and pancreas weight. Females: at 750 mg/kg bw/day significantly decreased body weight at necropsy. Histopathological lesions graded as minimal were reported, and were more abundant in females. Results F1 reproductive toxicity; At 750 mg/kg bw/day significantly reduced mating (70.0 compared to 96.7 in controls) and fertility (81.0 compared to 100.0 in controls) indices in F1 parents to make F2 offspring. Males: at 750 mg/kg bw/day significantly reduced absolute paired testis weight (2.8585 g compared to controls 3.5980 g), paired epididymis weight (1.2076 g compared to controls 1.3507 g), prostate weight (0.5626 g compared to controls 0.7556 g) and seminal vesicle with coagulating gland weight (1.7515 compared to controls 2.1455). The number of rats with histopathological changes in testis and epididymis in the 750 mg/kg bw/day group was 23 and 15 compared to 3 and 2 in controls. Furthermore, the epididymal sperm concentration (649.51 mil/g compared to 825.59 mil/g in controls), the percentage motile sperm (52.1 compared to 68.6 in controls), and the percentage progressively motile sperm (42.1 compared to 57.3 in controls) was significantly decreased at 750 mg/kg bw/day compared to controls. In the 750 mg/kg bw/day group a significant increase in the number of males with one or more reproductive tract malformations were reported (16 compared to 1 in controls), and in the percent males with one or more reproductive tract malformations (53.3 compared to 3.33 in controls). These included in the testis abnormal, missing, reduced in size, and/or undescended, and in the epididymis missing (right, left or bilateral) or reduced in size (right, left or bilateral). Microscopic findings in the 750 mg/kg bw/day dose group included in the epididymis; aspermia (8/24) and chronic inflammation 4/24, in the prostate gland; chronic inflammation (13/30), and in the testis; atrophy seminiferous tubule (15/29) and dilatation duct rete testis (7/29). Furthermore, at 750 mg/kg bw/day the number of implants sites per litter (12.35 compared to 15.86 in controls), number of total pups per litter and the average number of live pups per litter on pnd 0 (11.4 compared to 14.2 in controls) and on pnd 4 (10.9 compared to 14.0 in controls) was significantly reduced compared to control animals. In females the absolute and relative uterus weight was increased compared to control animals. Results F2 offspring toxicity; During lactation at 750 mg/kg bw/day significantly reduced number of total pups per litter and live pups per litter on pnd 0 compared to control animals was reported. Furthermore, the average pup body weight per litter on pnd 7 (14.52 g compared to 16.91 g in controls), pnd 14 (29.53 g compared to 33.87 g in controls) and pnd 21 (44.63 compared to 50.01 g in controls) was significantly reduced

compared to control animals. A significantly ($p < 0.05$) reduced AGD was reported in males at 250 mg/kg bw/day (1.99 mm compared to 2.05 mm in controls) and at 750 mg/kg bw/day (1.77 mm compared to 2.05 mm in controls, $p < 0.001$). When the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values at 250 and 750 mg/kg bw/day were still significantly reduced (1.99 mm at 250 mg/kg bw/day and 1.79 mm at 750 mg/kg bw/day compared to 2.04 in controls). No effect on AGD was reported in females. In males a significant increase in the percentage male pups with one or more nipples (16.46 compared to 0 in the control group) and in the number of nipples per male (0.51 compared to 0 in controls), and in the number of areolae per male (3.14 compared to 0.05 in controls) was reported at 750 mg/kg bw/day. At weanling necropsy in males a significantly reduced terminal body weight (45.89 g compared to 51.78 in conrols), absolute thymus (0.2048 g compared to 0.2360 g in controls), absolute (0.1549 g compared to 0.2106 g in controls) and relative (0.3335 g compared to 0.4056 g in controls) spleen weight, and paired testis weight (0.1949 g compared to 0.2432 g in controls) was reported at 750 mg/kg bw/day. In the 750 mg/kg bw/day group a significant increase in gross lesions were reported. These included missing epididymis in twenty male weanlings (20/54) (full or caput or corpus), missing seminal vesicle or reduced size in 5 male weanlings (5/54), and one male in the 250 mg/kg bw/day group had a missing testis. In females weanling at necropsy a significant reduced terminal body weight, reduced absolute thymus and ovaries weight, and reduced absolute and relative spleen weight was reported at 750 mg/kg bw/day. At 250 mg/kg bw/day a significant increase in uterus weight was reported. F2 offspring was not evaluated as postweanlings. In this study the NOAEL for parental systemic toxicity is 250 mg/kg bw/day based on organ weight changes and histopathological lesions in the liver. The NOAEL for effects on the reproductive system in offspring is 50 mg/kg bw/day based on a dose-realted reduction in AGD in both F1 and F2 offspring from 250 mg/kg bw/day. This effect was still statistically significant at 250 and 750 mg/kg bw/day when the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate. The study was performed in compliance with Good Laboratory Practice, and the US. EPA OPPTS Testing Guideline.

A recent 2-generation study is available (Nagao et al., 2000). In this study Sprague-Dawley rats (8 week old) (25 male or female/group) were administered oral doses of 0, 20, 100 or 500 mg/kg bw/day BBP by gavage. F0 male rats were treated for 12 weeks prior to 2-week cohabitation, and until necropsy (confirmation of fertility by pairing). F0 female rats were treated for 2 weeks prior to cohabitation until necropsy (including gestation, delivery, and lactation through postpartum day 21). F1 animals were treated by oral gavage after weaning (postnatal day 22) until necropsy (confirmation of fertility by pairing). At 13 weeks of age mating was permitted. The F0 animals were observed for clinical signs daily during the study. In female F0 rats estrous cycling was evaluated. Furthermore, brain, hearth, lung, liver, spleen, kidneys, adrenal glands, thymus, ovaries, uterus, thyroid gland, and pituitary gland were weighed. The levels of prolactin, luteinizing hormone (LH), FSH, thyroidstimulating hormone (TSH), triiodothyronine (T_3), thyroxine (T_4) and estradiol (E_2) were measured in serum. Histopathologic examination of ovaries, uterus, vagina, liver, kidneys, mammary glands, thyroid gland, parathyroid gland, pituitary gland, and adrenal glands was performed in 10 dams from the 500 mg/kg bw/day dose group and the control group. F0 male rats were necropsied after confirmation of fertility by pairing with females. The brain hearth, lung, liver, spleen, kidneys, adrenal glands, thymus, testes, epididymis, ventral prostate, seminal vesicles, thyroid gland, and pituitary gland were weighed. The number of sperm in the right cauda epididymis and the percentage of motile sperm were determined. The levels of testosterone, LH, FSH, TSH, T_3, and T_4 were measured in serum. Histopathologic examination of testes, epididymis, prostate, seminal vesicle with coagulating gland, parathyroid gland, liver, kidneys, mammary glands, thyroid gland, pituitary gland, and adrenal glands was performed in 10 males each from the

500 mg/kg bw/day dose group and the control group. Observations in F1 offspring included; Numbers of live and dead pups for each litter were recorded after post natal day (pnd) 0 to 21 and the viability from pnd 0 to 4 (preculling) and from pnd 4 (postculling) to pnd 21 in each litter. Anogenital distance (AGD) was determined for each pup on pnd 4, 7, 14 and 21. On pnd 4 litters were culled randomly to eight (4 pups/sex/litter). Two pups/sex/litter in each group were examined for the development of neural reflexes, and for physical development. Sexual maturation measured as vaginal opening for female offspring (beginning on pnd 28) and preputial separation for male offspring (beginning on pnd 35) was assessed (2/sex/litter). On pnd 22 offspring (2/sex/litter) were necropsied. The testes, epididymis, and seminal vesicle with prostate in males, and ovaries and uterus in females were weighed. The testes in 10 male weanlings and ovaries in 10 female weanlings from all groups, and the epididymis, ventral prostate, and seminal vesicle with coagulating gland in 10 male weanlings and the uterus in 10 female weanlings from the 500 mg/kg group and the control group were examined histologically. Levels of testosterone, LH, TSH, FSH, T_3 and T_4 in male weanlings and of prolactin, LH, FSH, TSH, T_3, T_4, and E_2 in female weanlings were determined. One male and one female offspring from each litter of each group was subjected to behvioral and functional tests. At 13 weeks of age mating was permitted by pairing on a 1:1 basis within the same treatment group. The same measurements described above for pregnancy, delivery, lactation, and the evaluation of histology of internal organs including reproductive tissues, sperm motions and counts, and serum hormone levels were performed in male and female offspring. F2 pups were necropsied on pnd 21.

The results from the two-generation study

In parent animals (F0) a significant decrease in body weight gain was reported in males at 500 mg/kg/day compared to control males, although no decrease in food consumption was evident. In females no significant difference among groups in body weight and food consumption prior to mating or during pregnancy or lactation was reported. No dose-related changes were reported in estrous cyclicity, fertility and lactation. A dose-dependent increase in kidneys weight in rats of both sexes (significant at 100 and 500 mg/kg bw/day in females, and at 500 mg/kg bw/day in males), and an increase in liver weight in males (significant at 500 mg/kg bw/day), and a decrease in the weight of the ovaries in females (significant at 500 mg/kg bw/day) were reported compared to control animals. No macroscopic or microscopic changes were observed in the reproductive system of males or females. A decrease in serum testosterone, T_3 and T_4 levels (significant at 500 mg/kg bw/day), and an increase in FSH (significant from 100 mg/kg bw/day) were reported in males compared to control males. In females a significant increase in serum concentrations of prolactin, and a significant decrease in T_4 were reported at 500 mg/kg bw/day compared to control females. *Preweanling (F1);* The viability in percentage during pnd 0-4 was significantly decreased at 500 mg/kg bw/day (96.7% versus 100% in controls). Body weight of male and female offspring at birth in the 100 and 500 mg/kg bw/day dose group was significantly decreased compared to control animals (male offspring: 6.4 g at 100 mg/kg bw/day and 6.3 g at 500 mg/kg bw/day compared to 6.8 g in control offspring, female offspring: 6.0 g at 100 and 500 mg/kg bw/day compared to 6.4 g in control offspring), and the body weight at 500 mg/kg bw/day was lower throughout the study, however, the viability was not affected. In the 500 mg/kg bw/day group a significant decrease in AGD at birth was reported in male offspring, and an increase in AGD was reported in female offspring compared to control animals. In this study the AGD was not adjusted for individual body weights. Only the absolute AGD was measured. A significant decrease in testis and epididymis weight in males, and a significant decrease in ovary weight and increase in uterus weight in females was reported in the 500 mg/kg bw/day group compared to control animals. Furthermore, a significant decrease in FSH concentration in males at 500 mg/kg bw/day, and in TSH concentrations in males at 100

and 500 mg/kg bw/day were observed compared to control animals. In females the level of T_3 was significantly decreased in the 100 and 500 mg/kg bw/day dose group compared to control females. Histopathologic examination revealed a significant decrease in the numbers of spermatocytes in the seminiferous tubules in the 500 mg/kg bw/day group compared to control males. Cryptorchidism or hypospadia was not observed in any dose groups. In females no histopathologic abnormalities were considered to be related to BBP exposure. *Postweanling (F1);* Preputial separation for male offspring in the 500 mg/kg bw/day group was significantly delayed compared to control males, while vaginal opening for female offspring in this group was not affected. BBP did not affect the reproductive ability, including delivery and lactation at any dose levels, whereas a significant reduction in the absolute weights of the testis, epididymis, prostate, seminal vesicle and spleen were reported, and a significant increase in relative weight of the thyroid gland, adrenal glands, and liver weights were reported in males at 500 mg/kg bw/day compared to control males. A significant increase in the relative weight of kidneys in the 100 and 500 mg/kg bw/day dose group was reported compared to control males. However, no significant organ weight changes were reported in females. A significant decrease in serum concentrations of testosterone, LH, and T_4 were reported in male offspring at 500 mg/kg bw/day compared to control males. Furthermore, in the 500 mg/kg bw/day dose group histopathologic examination revealed significant increases in the incidence of atrophy of the seminiferous tubules with a decreased number of germ cells, a significant increase in the incidence of interstitial edema, and a significant increase in the incidence of decreased number of sperm in the epididymis compared to control males. In females no adverse changes in the ovaries or uterus in the 500 mg/kg bw/day dose group were reported. As regards the behavioral function tests, the only effect observed related to BBP exposure was a significant increase in the spontaneous motor activity in females in the 500 mg/kg bw/day dose group compared to control females, however, no effect was reported in males. *Preweanling F2;* In this group no significant adverse effects related to BBP exposure were reported including pup weight, viability, and development. From this study no NOAEL value for effects on fertility could be derived. The NOAEL value for effcts on development was 20 mg/kg bw/day based on reduced body weight in male and female offspring at birth at 100 and 500 mg/kg bw/day.

Several studies of the developmental toxicity of BBP and the major BBP metabolites (MBeP and MBuP) have been performed by Ema and coworkers (1990, 1991, 1992a, 1992b, 1992c, 1993a, 1994a, 1995a, 1995b, 1996 and 1998a; 2002) in Wistar rats after oral exposure. The following studies are described below.

The developmental toxicity of BBP was evaluated in pregnant rats exposed to various concentrations of BBP from day 0 of gestation to day 20, and killed on day 20 of pregnancy. The rats were exposed to 0.25, 0.5, 1.0 or 2.0% BBP in the diet corresponding to approximately 185, 375, 654 or 974 mg/kg bw/day (Ema et al., 1990) or to 2% BBP corresponding to 974 mg/kg bw/day (Ema et al., 1991). The number of pregnant rats/group was 13 to 17. Reduced maternal weight gain during pregnancy was observed at 375, 654 and 974 mg/kg bw/day. However, a reduced adjusted weight gain was only observed at 654 and 974 mg/kg bw/day. Embryotoxic effects were observed in the 0.5% group with a significant reduction in the number of live fetuses per litter (11.3 ± 3.8 versus 13.9 ± 1.6 in controls). In the 1.0% group a significantly reduced body weight of male and female foetuses, and in the 2.0% group complete resorption of all the implanted embryos were reported. Morphological examination of the foetuses revealed no evidence of teratogenesis. The maternal NOAEL from the Ema et al. (1990) study was 375 mg/kg bw/day based on a reduced adjusted weight gain from 654 mg/kg bw/day, and the NOAEL for offspring was 185 mg/kg bw/day based on reduced number of live fetuses per litter at 375 mg/kg bw/day. A pair-feeding study was performed in which the pregnant pair-fed rats received the same amount of diet consumed by the 2% BBP-treated pregnant rats.

The pair-fed and 2% BBP-treated rats showed the same reductions in the adjusted weight gain. Higher incidence of post implantation losses were reported in pair-fed rats compared to control rats, however, the complete resorption of all the implanted embryos was not found in any of the pair-fed rats. It was concluded that the embryolethality in the 2% BBP exposed rats could be attributable to the effects of dietary BBP and not from reduced food consumption during pregnancy (Ema et al., 1991).

In another study the teratogenic potential of BBP was investigated in pregnant Wistar rats (10 pregnant rats/group). The rats were given BBP in corn oil once daily by gastric intubation (0, 500, 750 or 1,000 mg/kg bw/day) throughout the period of major organogenesis i.e. on days 7-15 of pregnancy. The rats were killed on day 20 of pregnancy. At 500 mg/kg bw/day reduced food consumption in the dams were reported, and in the 750 mg/kg bw/day group reduced body weight gain. However, a reduced adjusted body weight gain was only reported at 1,000 mg/kg bw/day. High maternal lethality was observed in the 1,000 mg/kg bw/day group. Complete resorption of all implanted embryos was observed in 3 of 10 dams at 750 mg/kg bw/day and in all of the 6 pregnant rats in the 1,000 mg/kg bw/day groups. At 750 mg/kg bw/day a significant decrease in foetal weight, and a decrease in the number of live fetuses per litter was reported, and a significant increase in the incidence of foetal malformations per litter. These malformations included external malformations, skeletal malformations (fusion of sternebrae), and internal malformations (dilatation of renal pelvis). The LOAEL maternal and NOAEL offspring from this study was 500 mg/kg bw/day (Ema et al., 1992a).

To determine if periods of exposure during pregnancy would modify the developmental toxicity of BBP, pregnant Wistar rats were given BBP by oral exposure at a concentration of 2% in the diet (974 mg/kg bw/day). The exposure period in the first (Ema et al., 1992b) study was on days 0-20, days 0-7 (the pre-implantation and pre-organogenesis period), days 7-16 (the organogenesis period) or days 16-20 (the foetal period) of pregnancy and from day 0-20, 0-11 and 11-20 in the second (Ema et al., 1992c) study. The number of pregnant rats/group was 11 in both studies. In both studies, a pair-fed group of rats was included which received the same amount of feed as the feed intake of rats fed a diet containing 2% BBP. The pregnant rats were killed on day 20 of pregnancy in both studies. Pronounced effects on maternal body weight gain, adjusted body weight gain and food consumption during pregnancy were found regardless of the days on which BBP was given. No effect on preimplantation loss per litter was reported on exposure day 0-20, 0-7 or 0-11. A statistically significant increase in postimplantation loss per litter was reported after exposure in the early phase of pregnancy (day 0-20, 0-7, 0-11, and 7-16) as compared to control animals and pair fed rats, whereas no increase was found after exposure in the late phase of pregnancy (day 11-20 or 16-20). After exposure on pregnancy day 11-20 or 16-20 the incidence of teratogenic effects (cleft palate and fusion of the sternebrae) in the foetuses were significantly and markedly higher than in the control and pair-fed groups. The authors concluded that the teratogenic effect of BBP after oral administration during the organogenic period is primarily the result of the effect of exposure to BBP.

The teratogenic phase specificity of BBP during gestation on developmental toxicity was examined by a shorter duration of treatment. Pregnant Wistar rats were dosed once daily by gastric intubation with BBP dissolved in olive oil at a dose of 600, 750 or 1,000 mg/kg bw on days 7-9, 10-12 or 13-15 of pregnancy. Control rats received olive oil only, on the corresponding days. The pregnant rats were killed on day 20 of pregnancy (10 litters/group were examined). No information was provided regarding the evaluation of maternal effects. After exposure on pregnancy day 7-9 post-implantation the loss/litter and the number of dead foetuses per litter was significantly increased and the number of live foetuses per litter and body weight of live foetuses was significantly decreased at 750 and 1,000 mg/kg bw/day of BBP. After exposure on

pregnancy day 10-12 postimplantation loss and the number of dead foetuses per litter was significantly increased at 750 and 1,000 mg/kg bw/day of BBP and number of litters totally resorbed was significantly increased at 1,000 mg/kg bw/day of BBP. Number of live foetuses per litter, and body weight of live foetuses were significantly decreased at 1,000 mg/kg bw/day of BBP. After exposure on pregnancy day 13-15, post-implantation loss and the number of dead foetuses per litter was significantly increased at 750 and 1,000 mg/kg bw/day of BBP, and number of live foetuses per litter was significantly decreased at 750 and 1,000 mg/kg bw/day of BBP. However, different patterns of malformations were induced during the different exposure periods. These included malformations [external malformations (cleft palate), skeletal malformations (fusion and/or absence of cervical vertebral arches or thoracic vertebral arches)] after exposure to 750 or 1,000 mg/kg bw/day of BBP on pregnancy day 7-9 or 13-15, whereas no malformations were reported after exposure on pregnancy day 10-12. The sex ratio of live foetuses was comparable across all groups, except for the group treated on days 10-12 with 1,000 mg/kg bw/day. No significant effects in any reproductive parameter were found in pregnant rats given a dose of 600 mg/kg bw/day. The authors concluded also from this study that the susceptibility to the teratogenic effect of BBP varies with the developmental stage at the time of BBP administration. The NOAEL for offspring from this study was 600 mg/kg bw/day (Ema et al., 1993a).

In the Ema et al. (1994a) study, the embryolethality of BBP during early pregnancy was investigated by studying the effect of BBP on the uterine and ovarian weight and plasma progesterone levels. The rats were exposed to BBP (2%) on day 0-7, 0-9 and 0-11, and the rats were sacrificed on day 7, 9 and 11 of pregnancy (6 pregnant rats/group). No effect on pre-implantation the loss/litter was reported on exposure day 0-7, 0-9 or 0-11. A marked increase in post-implantation loss per litter was reported on exposure day 0-11**,** only due to the method of quantifying post implantation loss (the presence of a heartbeat on day 11 of pregnancy). Furthermore, compared to control animals a significant decrease in the uterine and ovarian weight and plasma progesterone levels in all groups except for the ovarian weight on day 7 were reported. No significant differences in pre- and post-implantation loss, uterine weight, and plasma progesterone levels were reported between the control and pair fed rats. The post-implantation embryonic loss due to BBP exposure during early pregnancy therefore, may seem to be mediated via the reduction in plasma progesterone levels, and impairment of luteal function.

To further elucidate the effect of BBP on early embryolethality this effect of BBP was investigated in pregnant (10-14/group, 218-240 g) and pseudopregnant (11-13/group, 216-263 g) Wistar rats. Decidualized pseudopregnant rats were used to study the direct effect of BBP on maternal reproductive physiology. Rats were given BBP by gastric intubation at 0, 250, 500, 750 or 1,000 mg/kg bw/day on days 0-8 of pregnancy and the pregnancy outcome was determined on day 20 of pregnancy. The same doses of BBP were given to pseudopregnant rats, and on day 9 of pseudopregnancy the rats were sacrificed and blood samples were collected for progesterone measurements, and the uterus and ovaries were weighted and served as an index for uterine decidualisation. *Results pregnant rats*; Maternal; at 1,000 mg/kg bw/day of BBP two deaths were reported. A significant decrease in body weight gain was observed on day 0-9 in all BBP-treated groups, with a full recovery on day 9-20, except for the 1,000 mg/kg bw/day group. A significant reduction in food consumption was also reported in all exposed groups on day 0 – 9, however, no differences were reported on day 9 – 20. No effects were seen in the adjusted body weight gain between BBP-treated animals and control animals, possible due to recovery of maternal animals from gd 9-20. The female rats in the BBP-treated groups at higher doses showed a reddish staining of the facial fur and/or piloerection. *Foetal effects*; BBP caused a significant increase in the incidence of pre-implantation loss per litter at 1,000 mg/kg bw/day, and of postimplantation

loss per litter at 750 and 1,000 mg/kg bw/day. A significant decrease in body weight of live foetus was observed at 500, 750 and 1,000 mg/kg bw/day. *Results pseudopregnant rats:* At 1,000 mg/kg bw/day two deaths were reported. A significant decrease in body weight gain was reported on day 0-9 at 500, 750 and 1,000 mg/kg bw/day together with a decreased food intake. A significant lower ovarian weight was reported in the 750 and 1,000 mg/kg bw/day group. No differences in the number of corpora lutea were reported in the BBP treated groups compared to the control group. A dose-dependent decrease in uterine weight (statistically significant at 750 and 1,000 mg/kg bw/day) and a trend towards decreased serum progesterone levels at doses ≥ 500 mg/kg bw/day were reported. The results in the pregnant and pseudopregnant rats may indicate a correlation between suppression of the responsiveness of the uterus and the early embryonic loss after administration of relatively high doses of BBP. The LOAEL maternal was 250 mg/kg bw/day, NOAEL offspring was 250 mg/kg bw/day and NOAEL pseudopregnant rats was 250 mg/kg bw/day in this study (Ema et al., 1998a).

The effects of butyl benzyl phthalate (BBP) on the development of the reproductive system in male offspring were studied in Wistar rats (Ema et al., 2002). In this study pregnant rats (16/group) were given BBP by gastric intubation at doses of 250, 500 or 1,000 mg/kg on days 15 to 17 of pregnancy. No death was found in any groups. Maternal effects included a statistically significant decrease in maternal body weight gain at 500 mg/kg (23 ± 6) and 1,000 mg/kg (23 ± 9) compared to 31 ± 5 in the control group. However, no effect was found on the adjusted maternal body weight gain (maternal weight gain excluding the gravide uterus). A statistically significant decrease in maternal food consumption was reported at 500 mg/kg (34 ± 6) and at 1,000 mg/kg (33 ± 6) compared to 45 ± 6 in the control group. A statistically significant decrease in the number of live foetuses per litter was found at 1,000 mg/kg (12.6 ± 1.9 compared to 14.6 ± 1.5 in the control group). The weight of the male and female foetuses was signficantly decreased at 1,000 mg/kg (male 3.82 ± 0.65 compared to 4.58 ± 0.32 in controls, female 3.67 ± 0.56 compared to 4.27 ± 00.31 in controls). A significant increase in the incidece of foetuses/litter with undescended testes was found at 500 (54/14) and 1,000 (97/16) mg/kg compared to 0/16 in the control group. Furthermore, a statistically significant decrease in the anogenital distance (AGD) of male foetuses was observed at 500 and 1,000 mg/kg. The AGD/cube root of body weight ratio in male foetuses was also significantly reduced from 500 mg/kg. The AGD/cube root of body weight ratio in female foetuses in the BBP treated groups were comparable to those in the control group. It was concluded by the authors of the study that BBP given to pregnant rats during gestation day 15-17 produced adverse effects on the development of the reproductive system in male offspring. The NOAEL for maternal toxicity was 250 mg/kg BBP and the NOAEL for developmental toxicity 250 mg/kg.

The effect of BBP on development, and on maternal and embryonic zinc metabolism was studied in pregnant rats (Uriu-Adams et al., 2001), since different chemicals may induce the synthesis of maternal metallothionein (Mt) in the rat. Metallothionein can bind zinc, which may lead to a reduction in the transfer of zinc to the embryo (Taubeneck et al., 1994; Daston et al., 1991) and thus, embryonic zinc deficiency-induced abnormal development of the conceptuses. In this study female Wistar rats (180-200 g, 9-16 rats/group) were administered BBP diluted in corn oil by gavage at doses of 0, 250, 1,000, 1,500 or 2,000 mg/kg bw/day once daily on gestation day (gd) 11, 12 and 13. The animals were killed on gd 20. At doses ≥ 1,000 mg/kg bw/day signs of marked maternal toxicity were reported including diarrhoea, blood in faeces, bloody discharge from the nose and eyes, and reduced activity. In addition, two deaths were reported at 2,000 mg/kg bw/day. The body weight on gd 20 was significant lower in the 2,000 mg/kg bw/day group. Food intake was lowest in the 2,000 mg/kg bw bw/day group from gd 12 to 16 compared to the other BBP dosed groups and the control group, however, food intake was similar in all groups from gd 17 to 19. No effects on maternal haematocrits, liver or

kidney weights were observed. Increased placental weights were seen at doses ≥ 1,500 mg/kg bw/day of BBP. At 2,000 mg/kg bw/day some of the placentas had light green patches. BBP affected reproductive outcome in a dose-dependent manner. Reduced foetal weights were observed at doses ≥ 1,500 mg/kg bw/day and at 2,000 mg/kg bw/day fewer live foetuses and higher percentages of resorptions were seen in the litters compared to the other groups. Gross anomalies were reported in the pups at 1,000, 1,500 and 2,000 mg/kg bw/day upon visual inspections. Effects of BBP on skeletal ossification on gd 20 were significantly changed at 1,500 and 2,000 mg/kg bw/day. A higher incidence of skeletal anomalies (rudimentary ribs and cleft palate) were reported from 1,000 mg/kg bw/day compared to the control and 250 mg/kg bw/day group. Maternal liver Mt was determined in all dose groups. A dose dependent tendency towards increased concentration of maternal liver Mt was seen (0.78 ± 0.13, 0.63 ± 0.11, 0.89 ± 0.15, 1.67 ± 0.23, 2.82 ± 0.76 in the control group, 250, 1,000, 1,500 and 2,000 mg/kg bw/day), however, not statistically significant. At 2,000 mg/kg bw/day of BBP two dams had a six fold higher liver Mt concentration compared to controls, and in these two dams 93-100% resorptions were reported. Maternal plasma concentrations of Zn was not statistically significantly increased among the exposure groups (11.88 ± 0.77, 11.38 ± 0.67, 12.88 ± 0.62, 11.25 ± 0.71 and 14.46 ± 1.29 in the control group, 250, 1,000, 1,500 and 2,000 mg/kg bw/day exposure groups). It was concluded from the study that BBP was not a strong inducer of Mt, and that the teratogenicity of BBP does not appear to be due to alterations in maternal and/or embryonic Zn metabolism (Uriu-Adams et al., 2001).

Sharpe and coworkers (1995) studied whether exposure of male Wistar rats to BBP during gestation or during the first 21 days of postnatal life, affected testicular size or spermatogenesis in adulthood (90-95 days of age). BBP was administered in the drinking water at a concentration of 1,000 μg/l. Estimated intake ranged from approximately 125 μg/kg bw/day the two first days after birth to approximately 370 μg/kg bw/day just before weaning based on intended concentrations in drinking water. No analytical confirmation of these estimates was performed. Diethylstilbestrol (DES, 100 μg/l) and an octylphenol polyethoxylate (OPP, 1,000 μg/l) were used as a positive and negative control. The number of treated dams per group ranged from 5 to 6; the males from these litters resulted in approximately 26 to 36 male offspring examined at post-natal day 90-95 for each treatment group. Two studies were performed where the mothers were treated for approximately 8-9 weeks, covering a 2-week period before mating, throughout gestation and up to until 22 days post partum (spanning the whole period of Sertoli cell proliferation). In study 1 exposure to BBP resulted in a small but significant (from 2,014 mg to 1,809 mg) reduction in mean absolute testicular weight and in the mean relative testicular weight (from 4.12 to 3.81 mg/g body weight). In study 2 (which was identical to study 1) the corresponding values were 1,954 to 1,819 mg (absolute testis weight) and 4.09 to 3.82 mg/g bw (relative testis weight). The values using DES was 2,014 to 1,750 mg (study 1) and 1,954 to 1,847 mg (study 2) regarding absolute testis weights, and 4.12 to 3.94 mg/g bw (study 1) and 4.09 to 3.99 mg/g bw (study 2). BBP also caused a reduction (approximately 20%) in daily sperm production (homogenization-resistant spermatids) corresponding to an expected theoretical (not measured) reduction at 2-4% in the number of Sertoli cells. However, in later comments to the study from Sharpe and Turner (1998), they commented that biological variability in these types of studies may have a greater influence on the test results than the test compound tested, however, they still considered the results of the initial study as valid. Due to the negative results in the two later studies (Ashby et al., 1997 and TNO, 1998a,b, see below) the results of the Sharpe et al. (1995) study seems of limited importance for the risk assessment of BBP for developmental effects.

Ashby and coworkers (1997) performed a similar (but not identical) study as Sharpe and coworkers (1995). Ashby et al. (1997) used a greater number of animals in each group, glass bottles and Alpk:Ap$_f$SD rats whereas Sharpe et al., 1995 used smaller number of animals in each group; plastic bottles and Wistar rats. This study was performed to further study the effect on development of the reproductive system after exposure *in utero* and during lactation to low doses of BBP. In this study BBP (purity 98%) was administered in drinking water (1,000 μg/l) to pregnant Alpk:Ap$_f$SD (AP) rats (age between 10 to 12 weeks; 19 rats) during gestation and lactation (the pre-mating exposure period used by Sharpe was omitted). The diet used in this study contained almost half the amount of soya compared to the Sharpe study. The stability of BBP in water feeding bottles was assessed using LC-MS. Controls were given drinking water (19 rats). The sexual development of the pups were monitored until their termination at postnatal day 90 (pnd 90). The BBP dose corresponded to 182.6 μg/kg/day. Pups derived from animals (6 rats) exposed to diethylstilbestrol (DES) in drinking water (50 μg/l, corresponding to 8.6 μg/kg /day) was used as a positive control. The sexual development of the both sexes of pups were monitored. The body weights of the DES pups were significantly reduced at birth, an effect that persisted until pnd 90. The body weight of BBP pups were marginally increased at birth, but no difference was found at pnd 90. DES affected the sexual development of the pups for all endpoints assessed: anogenital distance (AGD) on pnd 2; average day of vaginal opening and prepuce separation; uterus, testes and accessory sex gland weight; cauda epididymis sperm count, and homogenization resistant testicular sperm count at pnd 90. BBP did not affect any of these parameters, with the exception of a 1.1 day advance in the average day of vaginal opening and a small increase in AGD in female pups on pnd 2. These last two effects were considered related to the increased weight of the BBP pups. The incidence of FSH containing cells in the pituitary gland of animals from each dose group was unaffected at pnd 90 (reduced release of FSH during testicular development may cause a reduced testicular growth). The effects observed in the DES pups are consistent with the results of earlier studies by Sharpe and coworkers (1995). However, the absence of an effect of BBP administration on pup testis weight and testicular sperm count on pnd 90 is in contrast to reductions in these parameters reported by Sharpe (1995). The Sharpe (1995) and Ashby (1997) studies differ in the strain of rats used, in the lack of a pre-mating BBP exposure period in the Ashby study, and use of glass feeding bottles in the Ashby and coworkers (1997) study, however, the impact they may have had on the validity and interpretation of the results is probably minor. The report concluded that the small change in the average day of vaginal opening in BBP pups can not alone be taken as evidence of endocrine disruption (Ashby et al., 1997).

A recent TNO study (TNO, 1998a) was performed according to GLP compliance to investigate the effect on the development of male reproductive organs in male F1 pups. The study was conducted under the same protocol as Sharpe and coworkers (1995) with a few enhancements to increase the overall strength of the study. These included larger number of doses, larger sample size, more extensive evaluation of male pups, evaluation of female pups, and analytical characterisation of dosing solutions (stability data of BBP is included in the end of the TNO studies). BBP was given to Wistar rats (28/group) in drinking water at concentrations of 100, 1,000 and 3,000 μg/L.The measured concentrations of BBP analysed in the drinking water were 80, 84 and 86% of the intended level of the low, mid and high dose. BBP was given to F0 females (parents) during the premating period (2 weeks), gestation and lactational period up to weaning on postnatal day (pnd) 21. During these periods the BBP intake ranged from 10-22, 115-229 and 340-674 μg/kg bw/day for the low, mid and high dose group. During the third week of the lactation period the pups started to drink from the water bottles, therefore, the BBP intake in this period may not be correct, since the BBP intake is calculated on the basis of the water consumption and body weights of the dams. Parental females were sacrificed after pnd 23, and

F1 pups were sacrificed when males and females were 89-97 and 91-101 days old. A positive control group was given DES 50 μg/L for the same period, however, between gestation day 13 and lactation day 3 the concentration of DES was reduced to 10 μg/L due to animal welfare. No mortality or clinical changes were reported during the study. The body weight, body weight change, food and water consumption of the BBP treated groups was comparable to the control animals for the F0 generation. For the DES group a significant reduction in body weight, body weight change, and food and water consumption was reported during the pre-mating, gestation and lactation period of the F0 females. For the F1 generation body weight, body weight change and food consumption of the males and females of the BBP treatd groups and the females of the DES treated group were comparable to the control group. However, for the F1 males the body weight in the DES treated group was reduced from weaning until sacrifice. No effects on the mating index, female fecundity or fertility were reported. Furthermore, no post-implantation loss was reported. Stillborn pups were reported in 4, 5 and 3 litters of the control, high-dose BBP group and the DES group. The number of stillborn pups was 15, 0, 0, 7 and 13 for the control, low-, mid-, high-dose BBP group and DES group. However, on a litter basis the mean number of live born pups was comparable for all groups, except for the DES group which showed a statistically significant decrease in the number of live born pups per litter. The number of pups found dead or missing (cannibalized by the mother) between pnd 1-4 was 2, 2, 30, 29 and 39 for the control, low-, mid-, high-dose BBP group and the DES group. On a pup basis the increase in pup-mortality from pnd 1-4 in the mid- and high-dose BBP group and DES group was statistically significant compared to control. However, on a litter basis the reduction in the number of live pups was only statistically significant in the DES group. Necropsy of the stillborn and dead pups did not reveal any treatment related effects. The sex-ratio of the pups was comparable in all dose groups. No changes were reported in pup weights compared to controls in the BBP dosed groups, whereas pup weights in the DES group were statistically decreased. Sexual maturation was not effected in BBP dosed animals, whereas a delay in time of onset of preputial separation was reported in the DES group. Absolute and relative organ weights in the BBP groups were comparable to control animals, whereas absolute testis weight were significantly decreased, and relative weight of liver in males and females and kidney weight in males were increased in the DES group. In the F1 generation analysis of the epididymal sperm production revealed a lower number of sperm in the DES group ($50.9 \cdot 10^6$/3 ml versus $70 \cdot 10^6$/3 ml in control animals), and a slight decrease in the number of normal sperm in the low dose BBP (197.3 versus 198.4 in control animals) and DES group (195.4 versus 198.4 in control animals). However, the authors concluded that the decrease in the number of normal sperm was not considered to be treatment related. No differences in daily sperm production in the testes were reported between the control, BBP and DES groups. The author concluded that the study did not reproduce any of the effects observed by Sharpe and coworkers (1995) at any dose level tested. Specially, no effect was reported on testis weight and daily sperm production.

Due to the stillborn pups and the pups found dead between pnd 1-4 in the control, mid- and high-dose BBP groups and the DES group, a follow-up study was performed (TNO, 1998b). In this study BBP was given to Wistar rats at concentrations of 1,000 and 3,000 μg/L. The measured concentrations of BBP analysed in the drinking water were 86 and 77% of the intended level of the mid- and high dose. The female rats were exposed to BBP in the drinking water in the pre-mating period (2 weeks), gestation period and up to sacrifice on pnd 7. The intake dose in the premating period was lower in the follow-up study (109 and 280 μg/kg bw/day for the mid- and high- dose group), compared to the initial study (115 and 340 μg/kg bw/day for the mid- and high-dose group). In the follow-up study the number of stillborn pups was 13, 8 and 28 for the control, mid- and high-dose BBP group. On a pup basis the increased number of stillborn pups was only statistically significant in the high-dose BBP group compared to controls. Furthermore,

the number of pups found dead between pnd 1-4 was significantly decreased in the mid-dose BBP group (11 versus 29 in control) and increased in the high-dose BBP group (42 versus 29 in control). However, on a litter basis no effects on pnd 4 were reported. Other effects reported were comparable to the initial study. Necropsy of the stillborn and dead pups did not reveal any treatment related effects. Since the pup mortality in the initial and follow-up study was higher than pup mortality reported in previous dietary study with BBP performed at the same institute, and in studies performed by others (drinking water and diet) this was not considered to be treatment related. Furthermore, pup mortality reported in the control animals in the initial and follow-up study was reported to be higher than the Institute's historical control data. To further evaluate the pupmortality observed in the initial study and follow-up study in control animals and after low dose exposure to BBP in drinking water, a third one-generation study with low exposure to BBP in diet and drinking water was performed.

In this one generation study performed in compliance with GLP and according to OECD Guideline No. 416 Wistar rats (28 per group) were exposed to 1 or 3 ppm BBP (purity 99.2%) in drinking water or in the diet. In females the daily intake of BBP was as following based on daily water or fed intake and body weights; in drinking water; 1 ppm, 0.12 mg/kg bw/day at study start and 0.24 mg/kg bw/day at the end of the study, 3 ppm, 0.35 mg/kg bw/day at study start and 0.80 mg/kg bw/day at end of the study. In diet; at 1 ppm from 0.09 mg/kg bw/day at study start and 0.16 mg/kg bw/day at the end of the study, and at 3 ppm from 0.28 mg/kg bw/day at study start and 0.49 mg/kg bw/day at end of the study. The chemical stability of BBP was assured for a period at least 7 days. The control groups received untreated drinking-water or diet. Females received BBP during the pre-mating (2 weeks), mating (up to 3 weeks) and gestation and lactation period (3 weeks). Males received BBP during the co-housing period only. No clinical or gross pathological findings due to the treatment and no increase in mortality were reported in the parental animals exposed to BBP. Furthermore, no changes in body weigh or food consumption in the BBP exposed parental animals compared to controls were reported. As regards effects on reproduction parameters, no effects on these parameters were reported in BBP exposed animals (insamination index, fertilisation performance, fertility index, gestation length, number of pups born, birth and pup weight development, litter size, stillbirth, sex ratio, viability and lactation). No enhanced peri-natal pup mortality, or test-substance-related clinical or gross pathological findings were reported in the BBP exposed pups (Bayer AG, 1998).

4.1.2.9.6 Stability of BBP in drinking water

The stability of the BBP in drinking water dosing solutions was determined by UV spectra in a separate study (Monsanto, 1997b). Concentrations of BBP dosing solutions were examined 8 hourrs and 7 days after preparation of a 1 or 3 ppm BBP solution. After 8 hours a 30% or 11% reduction in BBP concentration was reported in the 1 or 3 ppm BBP dosing solution. After 7 days a 40% or 62% reduction was reported in the 1 or 3 ppm BBP dosing solution. In the 1899 initial TNO study the dosing solutions were changed daily, and in the 1975-follow up TNO study every four days. The instability of BBP in the dosing solutions is a problem in all BBP drinking water studies, and has to bee reflected when the results from the studies are evaluated.

4.1.2.9.7 Developmental studies, BBP metabolites, animals

The major metabolites of BBP are mono-*n*-butyl phthalate (MBuP) and mono-*n*-benzyl phthalate (MBeP). Larger quantities of MBuP are formed in rats (44% MBuP versus 16% MBeP) (Eigenberg et al., 1986; Mikuriya et al., 1988). Di-*n*-butyl phthalate (DBP) is metabolised to

MBuP (Albro and Moore, 1974; Williams and Blanchfield, 1975; Tanaka et al., 1978). The pattern of malformations produced by MBuP and MBeP were similar (Ema et al., 1995a; Ema et al., 2003) to that produced by BBP (Ema et al., 1992a; 1992b; 1993a) and DBP (Ema et al., 1993b; Ema et al., 1994b). In this section the toxicity of MBuP and MBeP are presented. The section is also supplemented with the more recent developmental toxicity studies performed with DBP. DBP administered during the organogenic and/or late gestation period (Mylchreest et al., 1998) induced an almost similar pattern of malformations in the male reproductive system as were reported after exposure to BBP (Gray et al., 2000; Parks et al., 1999). The supplementations of the DBP studies in the BBP report have not affected the overall conclusion of this section.

The BBP metabolites mono-*n*-butyl phthalate (MBuP, 0, 250, 500 or 625 mg/kg bw/day, Ema et al., 1995a) and mono-*n*-benzyl phthalate (MBeP, 0, 250, 313, 375, 438 or 500 mg/kg bw/day, Ema et al., 1996a) were evaluated for developmental toxicity in Wistar rats (11-15 pregnant rats/group). Rats were exposed once daily to MBuP or MBeP by gastric intubation on day 7-15 of pregnancy and killed on day 20 of pregnancy. After exposure to MBuP a significant decrease in food consumption and a significant reduction in body weight gain in dams was reported from 500 mg/kg bw/day, however, no effects were reported on adjusted body weight gain at 500 and 625 mg/kg bw/day. After exposure to MBeP a significant reduction in food consumption was reported from 250 mg/kg bw/day at pregnancy day 7-15. A significant reduction in weight gain was reported from 313 mg/kg bw/day on pregnancy day 7-15, however, adjusted weight gain was only significantly reduced at 500 mg/kg bw/day. Furthermore, at doses ≥ 313 mg/kg bw/day of MBeP a reddish-brown staining of the facial fur, piloerection and spasticity were reported in the dams. Embryotoxicity was reported after exposure to doses ≥ 500 mg/kg bw/day of MBuP. These included a significant increase in resorption and dead foetuses per litter, a significant increase in postimplantation loss per litter and live foetuses per litter, and a significant decrease in body weight of live foetuses. For MBeP embryotoxicity was reported at doses ≥ 438 mg/kg bw/day of MBeP. These included a significant increase in postimplantaion loss per litter and resorption and dead foetuses per litter, and a significant decrease in body weight of live foetuses. Teratogenicity was reported after exposure to doses ≥ 500 mg/kg bw/day of MBuP. These included a significant increase in foetuses per litter with external malformations (cleft palate), skeletal malformations, and internal malformations (dilatation of renal pelvis). For MBeP teratogenicity was reported at doses ≥ 313 mg/kg bw/day of MBeP. These included a significant increase in foetuses per litter with skeletal malformations, and internal malformations (dilatation of renal pelvis from 375 mg/k bw/day). Maternal and developmental NOAEL was 250 mg/kg bw/day for MBuP, and the maternal LOAEL and developmental NOAEL was 250 mg/kg bw/day for MBeP. The pattern of malformations produced by MBuP and MBeP were similar to that produced by BBP, suggesting that MBuP or MBeP and/or its possible metabolic products may be responsible, at least in part, for the teratogenic effect of BBP.

The developmental toxicity of MBeP was studied in Wistar rats (12 weeks of age) (Ema et al., 1996b). Pregnant rats (10 to 17 rats per group) were given MBeP by gastric intubation at 375, 500 or 625 mg/kg on days 7-9, or 250, 375, 500 or 625 mg/kg on days 10-12 or 13-15 of pregnancy. The pregnant rats were killed on day 20 of pregnancy. *Results after administration on pregnancy day 7-9*; Three of 15 pregnant rats died in the 625 mg/kg dose group. The maternal body weight gain from day 7-10 in the 375, 500 and 625 mg/kg dose groups and on day 10-20 in the 625 mg/kg dose group was significantly reduced, compared to the control group. A decreased food consumption was reported in the same dose groups as well. The adjusted weight gain was only reduced in the 375 and 625 mg/kg dose groups, compared to control animals. Complete resorption of all implanted embryos was reported in 9 of the 12 litters in the 625 mg/kg dose

group. The incidence of postimplantation loss per litter in the 500 and 625 mg/kg dose group was significantly higher than in the control group. A significantly reduction in female offspring body weight was reported from 375 mg/kg, and in male offspring from 500 mg/kg. No external malformations were reported. Skeletal examination revealed a significantly increased incidence of skeletal malformations in the 625 mg/kg dose group on a per litter basis compared to control animals. These malformations included fusion of cervical or thoracic vertebral arches, fusion of thoracic vertebral bodies, absence of lumbar vertebral arches and fusion or absence of ribs. No increased incidence of internal malformations was reported. *Results after administration on pregnancy day 10-12*; Two of the 12 pregnant rats died in the 625 mg/kg dose group. The maternal body weight gain on days 10-13 and days 13-20 in the 500 and 625 mg/kg dose groups was significantly reduced, compared to the control group. The adjusted weight gain was significantly lower in the 250, 500 and 625 mg/kg dose groups compared to control animals. The food consumption on days 10-13 in the 250, 375, 500 and 625 mg/kg dose groups and on days 13-20 in the 250, 500 and 625 mg/kg dose groups was significantly reduced compared to the control group. Complete resorption of all implanted embryos was reported in 3 of the 11 litters in the 500 mg/kg dose group and in 7 of 10 litters in the 625 mg/kg dose group. The incidence of postimplantation loss per litter in the 500 and 625 mg/kg dose group was significantly higher than in the control group. A significantly reduction in female offspring body weight was reported from 500 mg/kg, and in male offspring at 625 mg/kg. There was no significant difference in the incidence of external, skeletal, and internal malformations between the groups exposed to MBeP or the control group. *Results after administration on pregnancy day 13-15;* Four of 17 pregnant rats died in the 625 mg/kg dose group. The maternal body weight gain on day 13-16 and days 16-20 in the 500 and 625 mg/kg dose groups was significantly reduced, compared to the control group. The adjusted weight gain was significantly lower in the 250, 375, 500 and 625 mg/kg dose groups compared to control animals. The food consumption on days 13–16 in all dose groups and on days 16—20 in the 625 mg/kg dose group was significantly reduced compared to control animals. Complete resorption of all implanted embryos was reported in 5 of the 14 litters at 500 mg/kg, and 11 of 13 litters at 625 mg/kg. The incidence of postimplantation loss per litter in the 500 and 625 mg/kg dose group was significantly higher than in the control group. No significant reduction in male or female offspring body weight was reported. The incidence of foetuses with external malformations in the 500 mg/kg dose group was significantly higher compared to control animals, and all of these foetuses had cleft palate. A significantly increased incidence of foetuses with skeletal malformations was reported from 375 mg/kg, and all of these foetues had fusion of the sternebrae. No significantly increased incidence of foetuses with internal malformations was reported. These results indicate that the susceptibility and spectrum of the developmental toxicity of MBeP vary with the developmental stage at the time of administration, as was also reported after administration of BBP during different days of pregnancy.

Ema et al. (2003) studied the effect of monobenzyl phthalate MBeP, a major metabolite of BBP on the development of the reproductive system in rats. He also looked into the role of MBeP in the antiandrogenic effects of BBP. In this study pregnant Wistar rats (16/dose group) were given MBeP by gavage at doses of 167, 250 and 375 mg/kg bw/day on gestation day (gd) 15 to 17 Foetuses were examined on gd 21. Maternal body weight gain on gd 15-18 was significantly decreased from 167 mg/kg bw/day (31, 24, 23 and 15g in the control, 167, 250 and 375 mg/kg bw/day dose group). Maternal food consumption was significantly decreased on gd 15-18 from 167 mg/kg bw/day (54, 46, 40 and 33 in the control, 167, 250 and 375 mg/kg bw/day dose group). The adjusted maternal weight gain was significantly decreased from 250 mg/kg bw/day. Foetal weight was significantly decreased at 375 mg/kg bw/day. A significant increase in the incidence of undescended testes/litter was reported from 250 mg/kg bw/day [2(2), 1(1), 21(12) and 79(16) in the control, 167, 250 and 375 mg/kg bw/day

dose group]. A decrease in anogenital distance (AGD) and ratio of AGD on the cube root of body weight was reported in male foetuses at 250 mg/kg bw/day. No effect on AGD was found in female foetuses. The study indicated that MBeP produced adverse effects on the development of the reproductive system in male offspring and suggested that MBeP may be responsible for the antiandrogenic effects of BBP.

The effect of prenatally exposure to Monobutyl phthalate (MBuP) on testicular descent was studied in Wistar-King A rats (Imajima et al., 1997). Pregnant rats (7 per group) were exposed to MBuP (300 mg/day equivalent to approximately 1,000 mg/kg bw/day) or solvent (sesame oil) by gavage from gestation day (gd) 15 to 18. Male offspring was evaluated on gd 20 and on postnatal day (pnd) 30-40 to determine the position of the testes. On gd 20 all the testes were located in the lower abdominal cavity near the bladder neck in the controls (n = 19 from three litters), however, in offspring treated *in utero* to MBuP the testes were located significantly higher in the abdominal cavity, and some were located near the kidney (n = 15 from three litters). On pnd 30-40, in the control group, all testes descended into the scrotum and the incidence of cryptorchidism was 0% (n = 15 from three litters), whereas, in the MBuP treated offspring (n = 26 from five litters), 22 rats showed cryptorchidism (14 unilateral and 8 bilateral undescended testes), and the incidence of cryptorchidism was 84.6%. Twenty six of the total 30 undescended testes (86.7%) were located in the abdominal cavity and the remaining four (13%) were located at the external inguinal ring. This may indicate that MBuP act in an anti-androgenic manner, since testis descent is under androgenic control.

The time-specific effects of monobutyl phthalate (MBuP) on the transabdominal migration of the testis were studied in fetal rats (Shono et al., 2000). In this study three groups of pregnant Wistar-King A rats were administered MBuP by stomach feed tubing (0.3 g/day, corresponding to approximately 1,000 mg/kg bw/day), group 1 (2 rats) from gestation day (gd) 7-10, group 2 (2 rats) from gd 11-14, and group 3 (6 rats) from gd 15-18. The control group (group 4, 5 rats) was given vehicle (sesame oil) only from gd 7-18. At gd 20 the fetuses were obtained by Caesarean section, and the position of the testes were determined in all groups. Furthermore, in group 3 and 4 the testis, epididymis, cranial suspensory ligament and gubernaculum were examined under a dissecting microscope, and the levels of testosterone were measured. The degree of the transabdominal testicular migration was determined by measuring the distance from the bladder neck to the lower pole of the testis. The results from this study showed that in the control group all 30 testes were anchored at the bottom of the abdominal cavity near the bladder neck by a swollen gubernaculum, whereas in group 3 (exposure to MBuP from gd 15-18) the testes were high in the abdominal cavity and associated with both an elongated gubernaculum and a hypertrophic cranial suspensory ligament. The mean transabdominal testicular migration values in group 1, 2, 3 and 4 were 12.3 ± 5.9 (10 testes), 24.5 ± 5.2 (10 testes), 57.9 ± 2.6 (38 testes) and 9.3 ± 1.9 (30 testes). Values were significantly higher in group 2 and 3 compared to the control group. Histopathologic examination showed a poorly developed epididymis in group 3, with a small thin ductus deferens, although there were no remarkable changes in the morphological features of Sertoli and Leydig cells. The mean testosterone levels were 50.9 ± 3.8 pg/testis in MBuP treated fetuses (25 testes) and 852± 80.3 pg/testis in the control fetuses (30 testis), the levels was significantly lower in the MBuP treated rats compared to the controls.

A comparative developmental study was performed with BBP and Di-*n*-butyl phthalate (DBP) in Wistar rats. Pregnant rats were given either BBP or DBP by gastric intubation at a dose of 750, 1,000 and 1,250 mg/kg bw/day on days 7-9, days 10-12, and days 13-15 of pregnancy (12-13 pregnant rats/group). No information was provided regarding the evaluation of maternal effects. Regardless of the days of treatment, a significantly increased incidence of

post-implantation loss per litter, and decreased number of live foetuses per litter was found at all doses of BBP and DBP. A significant decrease in foetal weight was reported in all dose groups after exposure on gd 7-9. After exposure on gd 10-12 a significant decrease in foetal weight was reported at 1,000 mg/kg bw/day of BBP and 1,250 mg/kg bw/day of DBP, whereas no decrease in foetal weight was reported after exposure on gd 13-15. While treatment with BBP and DBP at doses of 750 mg/kg bw/day and above on days 7-9 or days 13-15 resulted in a significant increase in the incidence of malformations, no increase in the incidence of malformed foetuses was found after treatment with BBP or DBP on days 10-12. These malformations included external malformations (cleft palate) after exposure on gd 13-15, and skeletal malformations (fusion of sternebrae, fusion and absence of cervical vertebral arches, thoracic vertebral arches and bodies, or fusion and absence of ribs) after exposure on gd 7-9 or 13-15. The author concluded from this study that the similarity in the dependence of the gestations days of treatment on the manifestation of developmental toxicity, and on the spectrum of foetal malformations caused by BBP and DBP, they may act by the same mechanism, possibly via a common metabolite of these two parent compounds. The developmental NOAEL was 750 mg/kg bw/day of BBP in this study (Ema et al., 1995b).

The effects of DBP on prenatal and early neonatal development of the reproductive tract in rats were studied *in vivo* (Mylchreest et al., 1998). In this study a markedly disturbed development of the male reproductive tract (internal and external) in rat offspring exposed via their mothers during gestation and lactation, was observed at all dose levels (250, 500 or 750 mg/kg bw/day) in the absence of significant maternal toxicity. In female offspring sporadic cases of reproductive tract malformations were observed at 500 and 750 mg/kg bw/day. Age at vaginal opening and estrus cyclicity were not affected. The results of this study suggested that DBP does not possess estrogenic activity but rather shows anti-androgenic-like activity at these dose levels. The results reported in the Mylchreest et al. (1998) study were confirmed in a study by Ema et al. (1998b). In this study Wistar rats were exposed to DBP, 331, 555 or 661 mg/kg bw/day at day 11-21 of pregnancy.

Comparative embryotoxicities of butyl benzyl phthalate, mono-*n*-butyl phthalate and mono-*n*-benzyl phthalate in mice and rats: *in vivo* and *in vitro* was studied by Saillenfait et al. (2003). For study description and results see Section 4.1.2.9.

4.1.2.9.8 Developmental studies, BBP, humans

In a study by Swan et al. (2005) they examined the anogenital distance (AGD) and other genital measurements (smaller genitalia and undescended testis) in 85 boys 2-30 month of age in relation to prenatal phthalate exposure in humans. The anogenital index (AGI) was defined (AGD divided by weight examinations) and the age adjusted AGI was calculated by regression analysis. *Results:* The urinary concentration of four phthalate metabolites MEP (mono-ethyl-phthalate, reflecting exposure to DEP), MBuP (mono-n-butyl-phthalate, reflecting exposure to DBP), MBeP (mono-benzyl-phthalate, reflecting exposure to BBP) and MiBP (mono-isobutyl-phthalate, reflecting exposure to DiBP) were inversely related to AGI. When comparing boys with prenatal MBeP concentrations the odds ratio for a shorter than expected AGI was 3.8. For the other phthalate metabolites the odds ratios were as following: 10.2 for MBuP, 4.7 for MEP, and 9.1 for MiBP (all p-values < 0.05). The degree of testicular descent was associated with AGD. The proportions of boys with one or both testicles incompletely descended were 20%, 9.5% and 5.9% for boys classified as having short, intermediate and long AGI. A short AGI (25 boys) was defined as an AGI below the 25th percentile for age. This group had an AGI that was on agerage 18.3% (range 10-32%) shorter than expected. Boys (n = 17) with AGI

≥ 75th percentile of expected were classified as having a long AGI, and boys (n = 43) with AGI between the 25th and 75th percentile of expected were considered intermediate. The boys age and weight did not differ appreciably among these groups. AGD was also significantly associated with penile volume; R = 0.27 (p = 0.001) and penile volume divided by weight was correlated with AGI (R = 0.43, p = 0.001). The summary phthalate score was also defines for the quantification of joint exposure to these four phthalate metabolites. The age adjusted AGI decreased significantly with increasing phthalate score (*p*-value for slope = 0.009). The median concentrations of phthalate metabolites that were associated with a shorter AGI and incomplete testis descent were found to be below those phthalate concentrations measured in urine in one-quarter of the female populations in USA (CDC, 2003). These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to the small sample size, only 85 boys, further studies with larger sample size have to be performed before clear conclusions can be drawn from this study.

In a study by Main et al. (2005) they investigated whether phthalate monoester metabolite contamination of human breast milk had any influence on the postnatal surge of reproductive hormones in newborn boys as a sign of testicular dysgenesis. Biological samples were obtained from a prospective Danish-Finnish cohort study on cryptorchidism 1997-2001. The individual breast milk samples were collected 1-3 month postnatally (n = 130, 62 cryptorchid/68 healthy boys), and were analysed for phthalate monoester metabolites; MBeP (mono-benzyl phthalate, reflecting exposure to BBP), MME (mono-methyl phthalate, reflecting exposure to DMP), MEP (mono-ethyl-phthalate, reflecting exposure to DEP), MBuP (mono-n-butyl phthalate, reflecting exposure to DBP), MEHP (mono-2-ethylhexyl-phthalate, reflecting exposure to DEHP), and MINP (mono-isononyl phthalate, reflecting exposure to DINP). Serum samples (obtained in 74% of all boys) were analysed for gonadotropins, sex-hormone binding globuline (SHBG), testosterone, and inhibin B. *Results:* All phthalate monoester metabolites were found in breast milk samples with large variations. The medians (minimum – maximum) levels in µg/L were; MBeP 1.2 (0.2-2.6), MME 0.10 (< 0.01-5.53), MEP 0.95 (0.07-41.4), MBuP 9.6 (0.6-10,900), MEHP 11 (1.5-1,410), and MINP 95 (27-469). No association was found between phthalate monoester levels in breast milk and cryptorchidism. However, MEP and MBuP showed positive correlations with SHBG (r=0.323, p 0.002 and r=0.272, p=0.01, the value for MBeP was r=0.188, p=0.074), MMP, MEP and MBuP with LH/free testosterone ration (r=0.21 to 0.323, p 0.002 to 0.044, the value for MBeP was r=0.06, p=0.57), and MINP with LH (r=0.243, p 0.019, the value for MBeP was r=0.049, p=0.643). MBuP was negatively correlated with free testosterone (r=0.22, p=0.033, the value for MBeP was r=-0.07, p=0.951). The other phthalate monoesters including MBeP showed similar (as indicated above), however, not significant tendencies.

4.1.2.9.9 Summary developmental studies, BBP and BBP metabolites, animals

In the developmental toxicity studies in rats and mice after exposure to BBP or its major metabolites (MBuP or MBeP) developmental toxicity in offspring included prenatal mortality, reduced fetal weight, and malformed foetuses. Maternal toxicity was characterised as reduced body weight gain and increased liver weight, accompanied by a decreased food consumption. The determined NOEL/NOAEL/LOAEL values for maternal toxicity and developmental toxicity derived from the various studies are given in **Table 4.28**.

Table 4.28 Development

Study Design	Effect Level	Critical Effect	Reference
BBP			
Swiss DC-1 mice and Sprague-Dawley rats; 27-30/group; Administration in diet on gd 6-15; 0.1, 0.5, and 1.25% mice (182, 910, 2,330 mg/kg/day mice) and 0.5, 1.25 and 2.0% rats (419, 1,102 and 1,641 mg/kg/day rats)	NOAEL mice maternal 182 mg/kg bw/day and NOAEL offspring 182 mg/kg/day NOAEL rat maternal and offspring 419 mg/kg/day	Mice: At 910 mg/kg/day a slight reduction in dam weight gain (15%), no reduction in adjusted body weight gain, prenatal mortality and malformed fetuses at doses ≥ 910 mg/kg/day. Rat: At 1,102 mg/kg/day reduced dam weight gain. At 1,641 mg/kg/day reduced dam and foetal weight gain, and increased resorption and malformations.	NTP (1990) (mice). NTP (1989) (rats).
Cpb-WU pregnant rats; Administration of BBP by gavage gd 6-15 or 6-20; 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg/day; 25/group in the 0, 450, 750 and 1,250 mg/kg/day dose group, 10/group in the 270, 350, 580, 970, 1,600 and 2,100 g/kg/day dose group	NOAEL maternal 450 mg/kg/day (exp. gd 6-20) and 580 mg/kg/day (exp gd 6-15) NOAEL offspring 270 mg/kg/day (exp gd 6-20) and 350 mg/kg/day (exp.gd 6-15)	Maternal; increased liver weight at 580 mg/kg/day (exp. gd 6-20) and at 750 mg/kg/day (exp. gd 6-15). Offspring; decreased relative testis weight at 270 mg/kg/day (exp. gd 6-20). Effects on testicular migration at 580 mg/kg/day (more pronounced after long exp.), reduced fetal weight from 350 mg/kg/day (exp. gd 6-20) and from 450 mg/kg/day (exp. gd 6-15).	Piersma et al. (2000)
CD Sprague-Dawley rats; 2-generation study; 30/sex/group; administration in feed; 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day.	NOAEL for developmental effects: 50 mg/kg bw/day based on reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring. NOAEL for maternal toxicity: 250 mg/kg bw/day	Development: reduced anogenital distance (absolute and adjusted) from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day. Maternal toxicity: organ weight changes, and histopahological lesions in the liver graded as minimal in females at 750 mg/kg bw/day.	Tyl et al. (2004)
Pregnant Sprague-Dawley rats; (no information about number); administration by gavage; 0 or 750 mg/kg bw/day from gd 14 through pnd 3.		Maternal toxicity: no information. Offspring: on pnd 2 AGD and testis weight was decreased, and on pnd 13 the incidences of areolas were increased for pups exposed *in utero* to BBP.	Parks et al. (1999)

Table 4.28 continued overleaf

Table 4.28 continued Development

Study Design	Effect Level	Critical Effect	Reference
BBP			
Sprague-Dawley rats; two-generation study; 25/sex/group; administration by gavage; 0, 20, 100 and 500 mg/kg bw/day	NOAEL: 20 mg/kg bw/day for developmental effects based on decreased body weight in F1 offspring from 100 mg/kg bw/day.	F_0; decrease i body weight gain in males at 500 mg/kg/day. A dose-dependent increase in kidney weight of both sexes, (significant from 100 mg/kg/day in females and at 500 mg/kg/day in males), a significant increase in liver weight in males at 500 mg/kg/day, and a significant decrease in ovaryweight in females at 500 mg/kg/day. A decrease in testosterone (significant at 500 mg/kg/day), and increase in FSH (significant from 100 mg/kg/day) in males. F1: significantly decreased body weight at birth from 100 mg/kg/day, and at 500 mg/kg/day throughout the study. AGD (absolute) was decreased and preputial separation delayed in males at 500 mg/kg/day. Macroscopic and microscopic changes of testis, and decreased testosterone levels at 500 mg/kg/day after puberty. Significantly decreased testis, epididymis, and seminal vesicle weight at 500 mg/kg/day. Decreased number of germ cells in the seminiferous tubules, and sperm in the epididymis at 500 mg/kg/day. BBP did not affect reproductive ability, including delivery and lactation. F2: no significant effects related to BBP exposure up to pnd 21.	Nagao et al. (2000)
Pregnant 0F1 mice 15-23/group, single oral dose on gd 8 of BBP: 0, 280, 560, 1,120, 1,690 mg/kg. MBuP: 0; 200, 400, 800, 1,200 mg/kg. MBeP: 0, 230, 460, 920, 1,380 mg/kg. PregnantSprague-Dawley rats 7-13/group, single oral dose on gd 10 of BBP: 0, 280, 560, 1,120, 1,690 mg/kg. MBuP: 0;200, 400, 800, 1,200 mg/kg. MBeP: 0, 230, 460, 920, 1,380 mg/kg		Mice; BBP, MBuP and MBeP: Decreased body weight gain at the highest dose levels, however, no changes in corrected body weight gain. A reduction in live foetuses/litter was reported in the higehst dose groups. An increase in resorptions/litter in the highest dose groups. A dose-depended increase in malformed foetuses/litter was reported from 560 mg/kg BBP, 200 mg/kg MBuP and 920 mg/kg MBeP. Rat; BBP, MBuP and MBeP: No decrease in maternal body weight gain. No effects on live foetuses/litter and on post-implantation loss/litter were reported. A slight increase in malformed foetuses/litter was reported from 1,120 mg/kg BBP.	Saillenfait et al. (2003)

Table 4.28 continued overleaf

Table 4.28 continued Development

Study Design	Effect Level	Critical Effect	Reference
BBP			
Pregnant Sprague-Dawley rats 5/group; administration by gavage; corn oil or 750 mg/kg/day from gd 14 through postnatal day 3.		Maternal toxicity: no information. Offspring; 84% showed malformations in the testis, epididymis, accessory reproductive organs and external genitalia at 3-4 month of age. Reduced anogenital distance, decreased testis, seminal vesicle, ventral prostate and epididymis weight at day 2 of age, and males with areolas at day 13 of age.	Gray et al. (2000)
Wistar rats; Administration in diet on day 0-20 of pregnancy; 0.25, 0.5, 1.0 and 2.0% (185, 375, 654 and 974 mg/kg bw/day).	NOAEL maternal: 375 mg/kg bw/day NOAEL offspring: 185 mg/kg bw/day	At doses ≥ 375 mg/kg bw/day reduced body weight gain and at doses ≥ 654 mg/kg/day reduced adjusted weigth gain in dams. At 375 mg/kg/day reduced number of foetuses per litter. At 645 mg/kg /day reduced body weight in foetuses. At 974 mg/kg bw/day complete resorption.	Ema et al. (1990)
Wistar rats; Administration in diet on day 0-20 of pregnancy; 2% (974 mg/kg/day): Pair-feed pregnant rats.		At 974 mg/kg/day complete resorption and reduced body weight and adjusted body weight gain in dams. Pair fed rats showed the same reduction in body weight gain. No other effects in pair-feed pregnant rats.	Ema et al. (1991)
Wistar rats: Gastric intubation on day 7-15 of pregnancy; 500, 750 and 1,000 mg/kg/day.	LOAEL maternal: 500 mg/kg bw/day NOAEL offspring: 500 mg/kg bw/day	At 500 mg/kg/day reduced food consumption in dams. At 750 mg/kg/day reduced food consumption and body weight gain in dams, complete resorption in some dams, decreased foetal weight, malformations. At 1,000 mg/kg/day reduced adjusted body weight gain, high maternal mortality, complete resorption in all dams.	Ema et al. (1992a)
Wistar rats; Administration in diet on day 0-20, 0-7, 7-16 and 16-20 of pregnancy; 2% in the diet (974 mg/kg bw/day); Pair-feed pregnant rats.		Postimplantation loss was increased after exposure on day 0-20, 0-7, 7-16. Teratogenicity was reported after exposure on day-16-20. No effects in pair-feed pregnant rats.	Ema et al. (1992b)
Wistar rats; Administration through diet on day 0-20, 0-11 and 11-20 of pregnancy; 2% in the diet (974 mg/kg bw/day); Pair-feed pregnant rats.		Reduced body weight gain and adjusted body weight gain in dams in all groups. Complete resorption after exposure on day 0-20, 0-11. Teratogenic effects after exposure on day 11-20. No effects in pair-feed pregnant rats.	Ema et al. (1992c)

Table 4.28 continued overleaf

Table 4.28 continued Development

Study Design	Effect Level	Critical Effect	Reference
BBP			
Wistar rats; Administration once daily by gastric intubation on days 7-9, 10-12 and 13-15 of pregnancy; 600, 750 and 1,000 mg/kg bw/day.	NOAEL offspring: 600 mg/kg bw/day	At doses ≥ 750 mg/kg bw/day on day 7-9 or at 1,000 mg/kg bw/day on day 10-12 foetus weight decrease. At 1,000 mg/kg on exposure day 10-12 litters totally resorbed was increased. At doses ≥ 750 mg/kg bw/day on exposure day 7-9 and 13-15 increased malformations.	Ema et al. (1993a)
Wistar rats; Administration in diet on day 0 through sacrifice on day 7, 9 or 11 of pregnancy; 2% (974 mg/kg bw/day); Pair fed pregnant rats.		Increased postimplantation loss in rats killed on day 11. Regardless of day of sacrifice the ovarian and uterine weights and plasma progesterone levels were decreased in BBP treated rats. No effects in pair-feed pregnant rats.	Ema et al. (1994a)
Wistar rats; 10-14 pregnant rats/groups, 11-13 pseudopregnant rats/group; Gastric intubation on day 0-8 of pregnancy; 250, 500, 750 and 1,000 mg/kg bw/day.	LOAEL maternal: 250 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day	At doses ≥ 250 mg/kg bw/day decreased maternal body weight gain. At doses ≥ 500 mg/kg bw/day decreased foetal body weight. At ≥ 750 mg/kg bw/day decrease in implantations per rat. At doses ≥ 500 mg/kg bw/day decreased uterine growth and serum progesterone levels in pseudopregnant rats. No effects on adjusted body weight gain.	Ema et al. (1998a)
Pregnant Wistar rats 16/group; administration by gavage; olive oil, 250, 500 or 1,000 mg/kg from gd 15 to 17.	NOAEL maternal: 250 mg/kg NOAEL offspring: 250 mg/kg	Maternal toxicity: Reduced body weight gain and food consumption from 500 mg/kg. No effect on adjusted body weight gain. Decrease in the number of live foetus/litter, and decreased foetal body weight at 1,000mg/kg. Decrease in AGD in male offspring, and increase in the incidence of undescended testis from 500 mg/kg.	Ema et al. (2002)
Wistar rats; 9-16 pregnant rats/group; Administration by gavage on gd 11,12 and 13, killed on gd 20; 0, 250, 1,000, 1,500 and 2,000 mg/kg/day.		At doses ≥ 1,000 mg/kg/day maternal toxicity. Decreased foetal weight at doses ≥ 1,500 mg/kg/day, gross anomalies at 1,500 and 2,000 mg/kg/day; skeletal anomalies at 1,000 mg/kg/day. A tendency to increased maternal metallothionein at 2,000 mg/kg/day. No measurements of plasma and tissue zinc concentrations.	Keen (1998), Draft

Table 4.28 continued overleaf

Table 4.28 continued Development

Study Design	Effect Level	Critical Effect	Reference
BBP			
Wistar rats; 26-34 female/group; exposure 2 weeks before mating, throught gestation, and 22 days post partum; Administration in drinking water; 1mg/L (0.125 mg/kg bw/day first day - 0.370 mg/kg bw/day before weaning).		Small reduction in absolute and relative testes weight, reduced daily sperm production	Sharpe et al. (1995)
Alpk:ApfSD (AP-rats) 19 female/group; exposure through gestation and up to post natal day 90; Administration in drinking water; 1 mg/L (0.186 mg/kg bw/day).		No critical effects in pups on testicular weights and testicular sperm counts.	Ashby et al. (1997)
Wistar rats (28 female/group); exposure through gestation and up to post natal day 21; Administration in drinking water, 100, 1,000 and 3,000 μg/L (0.01-0.022, 0.115-0.229 and 0.340-0.674 mg/kg bw/day).		No effects on pups regarding the reproductive system. No maternal toxicity. High incidence in pup-mortality in control and BBP exposed pups.	TNO (1998a)
Wistar rats (28 females/group) exposure through gestation and up to post natal day 7; Administration in drinking water, 1,000 and 3,000 μg/L (0.109 and 0.28 mg/kg bw/day).		No effects on pups regarding the reproductive system. No maternal toxicity. High incidence of pup-mortality in control and BBP exposed pups.	TNO (1998b)
Wistar rats (28 females/group), exposure through premating (2 weeks), mating (3 weeks), gestation and lactation (3 weeks). Administration in drinking water, 1 and 3 ppm (0.12-0.24 and 0.35 to 0.8 mg/kg/bw/day); in diet 1 and 3 ppm (0.09-0.16 and 0.28-0.49 mg/kg bw/day).		No effects on pups regarding the reporductive system. No maternal toxicity. No increase in pup-mortality in control animals compared to historical data or in BBP exposed pups.	Bayer (1998)

Table 4.28 continued overleaf

Table 4.28 continued Development

Study Design	Effect Level	Critical Effect	Reference
BBP metabolites, MBuP and MbeP			
Wistar rats; MBuP; Gastric intubation on pregnancy day 7-15; 250, 500 and 625 mg/kg bw/day.	NOAEL maternal: 250 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day	At doses ≥ 500 mg/kg bw/day reduced food consumption and weight gain in dams, increased resorption, dead foetus and postimplantation loss per litter. Increased malformations.	Ema et al. (1995a)
Wistar-King A rats; MBuP; Administration by gavage on gd 7-10 (2 pregnant rats), on gd 11-14 (2 preganant rats) on gd 15-18 (6 pregnant rats); Approx. 1,000 mg/kg bw/day, control rats (5 pregnant rats) received sesame oil from gd 7-18.		Maternal toxicity: No information. Offspring: on gd 20 the testis was located significantly higher in the abdominal cavity after exposure on gd 11-14 and 15-18, compared to controls. The testosterone levels were significantly lower in MBuP treated fetuses compared to control fetuses.	Shono et al. (2000)
Wistar-King A rats; MBuP; 7/group; Administration by gavage on gd 15-18; 0 and approx. 1,000 mg/kg bw/day		Maternal toxicity: no information. Offspring: on gd 20 testis were located higher in the abdominal cavity compared to control pups. On pnd 30-40 the incidence of cryptorchidism was 84.6% in exposed animals and 0% in the control group.	Imajima et al. (1997)
Wistar rats; MBeP; Gastric intubation on pregnancy day 7-15; 250, 313, 375, 438 and 500 mg/kg bw/day.	LOAEL maternal: 250 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day	At doses ≥ 250 mg/kg bw/day reduced food consumption. From 313 mg/kg bw/day reduced weight gain, at 500 mg/kg bw/day reduced adjusted weight gain and maternal toxicity. At doses ≥ 313 mg/kg bw/day malformations. At doses ≥ 438 mg/kg bw/day embryotoxic effects.	Ema et al. (1996a)
Wistar rats; MBeP; Gastric intubation on pregnancy day 7-9, 10-12 or 13-15; 250, 375, 500 and 625 mg/kg bw/day		Significantly increased incidence of postimplantation loss at 500 mg/kg regardless of days of treatment. From 375 mg/kg a significantly increased incidence of teratogenic effects at exposure on pregnancy day 7-9 or13-15. No teratogenic effects on exposure day 10-12.	Ema et al. (1996b)
Wistar rats; MBeP; Gastric intubation on pregnancy day 15-17.; 167, 250 and 375 mg/kg bw/day.	LOAEL maternal: 167 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day	Significantly decreased foetal weight gain at 375 mg/kg bw/day. Significant increase in the incidence of undescended testis from 250 mg/kg bw/day. Decreased AGD from 250 mg/kg bw/day. Significantly decreased maternal food consumption and weight gain from 167 mg/kg bw/day. Significantly decreased adjusted maternal weight gain from 250 mg/kg bw/day.	Ema et al. (2003)

The developmental toxicity of BBP and its major metabolites MBuP and MBeP have evaluated whether or not embryotoxicity (lethality) or teratogenicity was observed in the presence or absence of maternal toxicity. In several developmental toxicity studies in rats (NTP, 1989; Ema and coworkers, 1990 and the following Ema et al., studies; Gray et al., 2000; Parks et al., 1999; Piersma et al., 2000; Nagao et al., 2000; Tyl et al., 2004) indications of maternal effects, when reported, such as reduced weight gain, increased liver or kidney weight, and reduced food consumption, were observed at doses higher, equal or below BBP doses that produced developmental toxicity. In a new 2-generation study (Tyl et al., 2004) the NOAEL for effects in offspring was 50 mg/kg bw/day based on a dose-related significant reduction in anogenital distance (AGD) in both the F1 and F2 male offspring from 250 mg/kg bw/day. When the AGD values were adjusted for individual body weights (by analysis of covariance, ANCOVA with body weight as the covariate) the AGD was still significantly reduced compared to the control group. A reduction in AGD at birth is one of the most sensitive indicators of anti-androgenic activity. At 750 mg/kg bw/day a significant increase in F1 and F2 male pups with one or more nipples and/or areolae were reported. Furthermore, at 750 mg/kg bw/day in the F1 generation a reduction in reproudctive organ weights (testis, epididymis, prostate, and seminal vesicle), and a significant increase in the number of rats with histopathological changes in the reproductive organs were reported. In the F2 male offsprings at weanling necropsy reduced testis weight, and a significant increase in gross lesions in the reproductive organs was reported at 750 mg/kg bw/day. The NOAEL for maternal toxicity was 250 mg/kg bw/day based on organ weight changes (liver and kidney) and histopathological lesions graded as minimal in the liver at 750 mg/kg bw/day. In this 2-generation study BBP was administered in the feed to CD (Sprague-Dawley) rats 30/sex/group at doses of 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day. In the four recently published studies (Piersma et al., 2000; Gray et al., 2000; Parks et al., 1999; Ema et al., 2002) pregnant rats were exposed to BBP by gavage during the organogenic period and/or the late prenatal early postnatal period. In the study performed by Piersma and coworkers (2000) pregnant rats were exposed to BBP *in utero* from gestation day 6 to 20 (long exposure) or 6 to 15 (short exposure), and necropsied on day 21. BBP induced reduced testicular weight in the offspring from 270 mg/kg bw/day, and reduced fetal weight from 350 mg/kg bw/day with a NOAEL at 270 mg/kg bw/day (exposure gd 6-20). Retarded transdominal descent of testis was reported with a NOAEL of 450 mg/kg bw/day. This effect was more pronounced after exposure on gd 6-20. The maternal NOAEL (exposure gd 6-20) was 450 mg/kg bw/day and the NOAEL (exposure gd 6-15) was 580 mg/kg bw/day based on increased liver weight. In the study performed by Gray and coworkers (2000) pregnant rats were given 750 mg/kg bw/day of BBP from gestation day 14 through postnatal day 3. This dose regime induced malformations in the testis, accessory reproductive organs and genitalia in 84% of male offspring at 3-4 month of age. Furthermore, reduced AGD on post-natal day 2 and areolas on post-natal day 13 were additionally seen in male offspring. The study by Parks et al. (1999) used the same dose regime as Gray and coworkers. In this study reduced AGD and testis weight was reported on post-natal day 2, and areolas on post-natal day 13. In the study by Ema et al. (2002) a decrease in AGD and an increase in the incidence of undescended testis was reported in male offspring exposed to 500 or 1,000 mg/kg BBP from gd 15-17. In the recent two-generation study (Nagao et al., 2000) BBP was administered by gavage (0, 20, 100 and 500 mg/kg bw/day). In this study a significant reduction in fetal body weight was reported at 100 and 500 mg/kg bw/day on pnd 0. Furthermore, in male offspring (preweanling rats) a reduction in AGD (absolute), tesist weight, epididymis weight, decreased FSH level and number of spermatogonia and spermatocytes in the seminiferous tubules was reported at 500 mg/kg bw/day. Since birth weight was reduced at 100 mg/kg bw/day in this study, it is unclear whether analysis of adjusted AGD would have shown decreased AGD at 100 mg/kg bw/day. In postweanling rats at 500 mg/kg bw/day a

decreased body, testis and epididymis weight were reported. Furthermore, at 500 mg/kg bw/day, a delay in preputial separation in males, decreased testosterone and LH levels and increased incidence of testicular athrophy with decreased number of germ cells in the seminiferous tubules and decreased number of sperm in the epididymis were reported. The only maternal effects reported in this study was a significant increase in kidney weight (relative and absolute) at 100 and 500 mg/kg bw/day, and a decrease in ovaries weight (absolute and relative) at 500 mg/kg bw/day. In pregnant rats exposed to BBP from gestation day 6 to 15, maternal toxicity evident as reduced weight gain, and/or increased liver weight, and reduced food consumption was reported in the presence of developmental toxicity evident as reduced foetal weight and malformed foetuses (NTP, 1989 (administration in diet); Ema et a., 1992a (administration by gavage). However, in the recent studies performed by Tyl et al. (2004) (administration in diet), and Piersma and co-workers (2000) (administration by gavage) developmental toxicity evident as reduced absolute and adjusted AGD in F1 and F2 offspring (Tyl et al., 2004), and reduced fetal and testicular weight in offspring (Piersma et al., 2000) were reported in the absence of maternal toxicity.

In mice BBP induced malformed foetuses at a dose level (910 mg/kg bw/day) which only induced maternal effects in the form of a slightly reduced (15%) absolute body weight gain (NTP, 1990). No effects on the body weight gain were observed when the weight of the dams was adjusted for the weight of the gravid uterus.

To further study the embryolethality/teratogenic effects of BBP in rats, Ema and co-workers (1992b; 1992c; 1993a; 1994a; 1998a, administration of BBP both by gavage and in the diet) performed studies in which BBP was administered on different days of gestation or in pseudopregnant rats. In most of these studies pair-fed rats were included as reference groups. In rats exposed to BBP on gestation day 7-16 and 11-20, but not in pair-fed rats, an increased incidence of malformations was reported. Furthermore, increased post-implantation loss was observed after exposure on gestation days 0-20, 0-7, 0-8, 0-11, 7-9, 10-12, 13-15 and 7-16 but not after exposure in the later stages of pregnancy (i.e., 16-20 and day 11-20) as compared to control animals and pair-fed rats. In pseudopregnant rats exposed to BBP on day 0-8 of pseudopregnancy uterine decidual growth was decreased at dose levels of 750 mg/kg bw/day and higher, indicating that early embryonic loss may at least in part be mediated via a suppression of uterine decidualisation. These results indicate that the teratogenic effect reported after oral administration of BBP during the organogenic period is primarily the result of BBP exposure, and not a result of the reduced body weight gain observed in dams. BBP or one of its major metabolites are reported to readily cross the placenta barrier, (Kluwe, 1982; Thomas et al., 1986), and is reported in the foetuses (Saillenfait et al., 1998). Maternal toxicity in the studies performed by Ema and co-workers was evaluated in experiments with a long dosing period, but not after a short dosing period. However, maternal toxicity when measured, were present when developmental toxicity was reported.

The effect of low concentration exposure to BBP in drinking water during gestation and early postnatal life on reproductive performance in offspring was evaluated in various rat studies (Sharpe et al., 1995; Ashby et al., 1997; TNO, 1998a and b; Bayer, 1998). A problem with these studies is the instability of BBP in drinking water. The main purpose of the studies was to evaluate a possible estrogenic effect of BBP. However, the results of the studies varied. In the study by Sharpe and coworkers (1995) BBP was shown to affect testicular size and spermatogenesis in offspring after administration of 1,000 µg/l in drinking water corresponding to 0.126 to 0.366 mg/kg bw/day. However, Sharpe and coworkers (1995) considered the biological variance to have a greater influence on the test results than the test compound tested. In a similar (but not identical) study performed by Ashby and coworkers (1997) no effects on

testis weight and spermatogenesis were observed in the offspring. Due to the variable results observed in these studies, two TNO studies, (TNO, 1998a and b) were performed which indicated the same results as Ashby and coworkers (1997). However, in these studies there were a high incidence of pup-mortality in both the control animals (higher than historical control data), and in the animals exposed to low concentrations of BBP (0.01 to 0.674 mg/kg/bw/day). No increase in reproductive parameters in BBP exposed animals was reported compared to control animals. A new study was performed to further evaluate pup-mortality (Bayer, 1998). In this study no effects on reproductive parameters were reported after exposure to low concentrations of BBP (0.09 to 0.8 mg/kg/bw/day), and no increase in pup-mortality was reported in the control animals compared to historical control data, or in the BBP exposed animals. Overall, these studies clearly indicate that no impairment of the reproductive system in the offspring are observed in rats exposed to very low concentrations of BBP during the gestation and lactational period.

The developmental effects of the major BBP metabolites were also investigated in several studies by Ema and co-workers, since BBP is readily hydrolysed after oral administration in the gastro-intestinal tract and the liver to the corresponding monophthalate-esters (MBuP and MBeP). In these studies the developmental toxicity of MBuP and MBeP were evaluated after oral administration in rats on pregnancy day 7-15, or after oral administration of MBeP on pregnancy day 7-9, 10-12, 13-15 or 15-17. The pattern of developmental toxicity observed after exposure to MBuP or MBeP was almost similar to the effects observed after exposure to BBP, suggesting that MBuP and MBeP may be responsible for the embryotoxic and/or teratogenic effect of BBP. In the study bu Imajima et al. (1997) testicular descendent was studied, which is under androgic control. In this study the testis were located significantly higher in the abdominal cavity on gd 20 in rats exposed *in utero* to MBuP (approximately 1,000 mg/kg bw/day) from gd 15-18 compared to control rats, furthermore, on pnd 30-40 cryptorchidism was reported in 84.6% of the exposed offspring, compared to 0% in the control group. Furthermore, Shono et al. (2000) studied the time-specific effects of MBuP on the transabdominal migration of the testis in foetal rats. The foetuses were exposed to MBuP (approximately 1,000 mg/kg bw/day) *in utero* from gd 7-10, 11-14 or 15-18. The study showed that on gd 20 the testis was located significantly higher in the abdominal cavity after exposure on gd 11-14 or 15-18, the effect was more pronounced after exposure on gd 15-18. Furthermore, the testosterone levels were significantly lower in MBuP treated foetuses compared to control foetuses. No information from the Imajima et al. (1997) study or Shono et al. (2000) study was available regarding maternal toxicity. In the study by Ema et al. (2003) *in utero* exposure to MBeP on gd 15-17 was shown to induce a significant decrease in AGD and a significant increase in the incidence of undescended testis.

MBuP is a major metabolite of DBP. In studies where DBP was administered during the organogenesis in rats (Ema et al., 1993b; 1994b; 1998b) malformations in the foetuses were reported. DBP administration during the organogenic and/or late gestation period (Mylchreest et al., 1998) induced a similar pattern of malformations in the male reproductive system as were reported after exposure to BBP (Gray et al., 2000; Parks et al., 1999). However, it should be emphasised that the results of the DBP studies have not been used as the basis for the conclusions and classification proposal for BBP.

The National Toxicology Program (NTP) Center for the evaluation of risk to human reproduction has used a phthalate expert panel to evaluate the reproductive and developmental toxicity of BBP and other phthalates. This expert panel has concluded that the database on developmental toxicity is suffucient to judge that oral exposure to BBP can cause developmental toxicity in rats and mice (Kavlock et al., 2002). Developmental toxicity was reported in rats and

mice exposed *in utero* to BBP in the absence of marked maternal toxicity. In a new 2-generation study in rats (Tyl et al., 2004) the NOAEL for developmental effects in offspring was 50 mg/kg bw/day and the NOAEL for maternal toxicity was 250 mg/kg bw/day. In a recent study in rats exposed to BBP *in utero* from gestation day 6 to 20 the NOAEL for developmental effects was 270 mg/kg bw/day, whereas the maternal LOAEL was 580 mg/kg bw/day. Furthermore, exposure to BBP in late gestation (from gd 15-20) was shown to be important in the determination of developmental effects on the reproductive organs in male offspring. In studies performed to determine if periods of exposure to BBP during pregnancy would modify the developmental toxicity of BBP, teratogenic effects reported after oral administration of BBP during the organogenic period were shown to be a result of BBP exposure. Furthermore, a potential anti-androgen-like activity of BBP has been demonstrated in different *in vitro* and *in vivo* studies. It is concluded that BBP affects development, and is proposed classified with T R61 Repro. Cat. 2, according to EU criteria.In the risk characterisation for developmental effects the NOAEL at 50 mg/kg bw/day from a 2-generation study (Tyl et al., 2004) is used based on a dose-related significant reduction in absolute and adjusted AGD in both F1 and F2 offspring from 250 mg/kg bw/day in the absence of maternal toxicity. In the Nagao et al. (2000) study a decerase in absolute AGD was reported at 500 mg/kg bw/day. In this study the adjusted AGD was not analysed. Since birth weight was reduced at 100 mg/kg bw/day in this study, it is unclear whether analysis of adjusted AGD would have shown decreased AGD at 100 mg/kg bw/day.

4.1.2.9.10 Summary developmental studies, BBP, humans

In a study by Swan et al. (2005) an association between maternal exposures to BBP as well as other phthalates and AGI in boys was reported. When comparing boys with prenatal MBeP (monobenzyl phthalate, reflecting exposure to BBP) exposure the odds ratio for a shorter AGI was 3.8. For the other monoester phthalates the odds ratio were 10.2 for MBuP (reflecting exposure to DBP), 4.7 for MEP (reflecting exposure to DEP), and 9.1 for MiBP (reflecting exposure to DINP) (all p-values < 0.05).

In a study by Main et al. (2005) no association was found between phthalate monoester levels (MEP, MMP, MBuP, MBeP, MINP and MEHP) in breast milk and cryptorchidism in newborn boys. However, a significant association was found between intake of contaminated milk with phthalates (MEP, MBuP, MMP and MINP) and postnatal surge of reproductive hormones (SHBG, LH, testosterone and inhibin B) in newborn boys. As regards the monoester metabolite of BBP, MBeP the tendencies were similar, however, they were not statistically significant.

These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to the small sample size, (85 boys in Swan et al., 2005 and 130 boys in Main et al., 2005), further studies with larger sample size have to be performed before clear conclusions can be drawn from these studies.

4.1.2.9.11 Endocrine activity of BBP and BBP metabolites *in vitro* and *in vivo*

Estrogen activity of BBP in vitro

A potential estrogen activity of BBP and the major metabolites MBuP and MBeP have been assessed in both *in vitro* and *in vivo* studies.

BBP was tested in a recombinant yeast screen for estrogenic activity, where the yeast cells expressed the human estrogen receptor hER. The study included assays to investigate the ability

of BBP to interact both as an agonist or antagonist. 17β-estradiol was used as positive control and ethanol served as negative control. To determine whether BBP possessed an anti-estrogenic activcity, the natural ligand (17β-estradiol ($2.5 \cdot 10^{-10}$ M) was added to the medium at a concentration that produced a sub-maximal response (65%). The estrogen, and anti-estrogenic activity of BBP was tested at BBP concentrations from 10^{-8} to 10^{-4} M. The most potent chemical in each screen was assigned a potency of four plus (++++), and the potency of the chemicals was expressed relative to this. BBP showed a very week estrogenic activity (+), and no anti-estrogenic (-) activity. 17β-estradiol showed a high relative potency (++++) in the hER assay (Sohoni and Sumpter, 1998).

The estrogenic activities of several phthalate esters, including BBP, were investigated *in vitro* using estrogen receptor (ER) competitive ligand binding, mammalian- and yeast-based gene expression, and MCF-7 human breast cancer cell proliferation assays. It was concluded that BBP exhibited weak ER-mediated estrogenic activity based on the results in the studies summarised below in **Table 4.29** (Zacharewski et al., 1998).

Estrogen receptor (ER) competitive ligand binding assay, measuring specific binding of [^{3}H]-E_2 (17β-estradiol) to the rat (Sprague-Dawley) uterine ER, was used to detect estrogenic activity of BBP. ER was isolated from rat uterine. The concentrations of BBP and E_2 were 1-1000 μM and 0.001-100 nM. The competitor was either (i) unlabelled E_2, (ii) BBP or (iii) DMSO solvent alone. Incubations were carried out at 30 ^{o}C for 30 min. BBP weakly competed with E_2 for the binding to the ER. Unlabelled E_2 exhibited an IC_{50} of 1.3 nM (which is within the range of previously reported IC_{50} values), whereas the IC_{50} value for BBP was approximately 36 μM. In this study E_2 was approximately $3 \cdot 10^4$ more potent than BBP (Zacharewski et al., 1998).

In the second study, BBP-induced reporter gene expression in recombinant receptor/reporter gene assay using MCF-7 human breast cancer cells or HeLa human cervical carcinoma cells transfected with Gal4-human estrogen receptor chimera (Gal4-HEGO) and the Gal4-regulated luciferase reporter gene (17m5-Glob-Luc) were studied. The concentrations used were: 0.1, 1 and 10 μM of BBP and 1 pM to 10 nM E_2 as a positive control. BBP was found to significantly induce luciferase activity. 10 μM of BBP induced an increase in luciferase activity of 46 ± 14% in MCF-7 cells, when compared to the 100 ± 27% response induced by 10 nM E_2. 10 μM of BBP induced an increase in luciferase activity in transiently transfected MCF-7 cells of 34 ± 16%, as compared to the maximum 100 ± 20% response following treatment with 10 nM E_2 (Zacharewski et al., 1998).

BBP was also tested in the yeast estrogen receptor-mediated growth assay. The PL3 *S. cerevisiae* was transformed with HEG0 (human estrogen receptor) for the determination of ER-mediated growth on selective media. The concentrations used were: 10 μM of BBP or 1 nM E_2 as control. 10 μM of BBP was able to weakly support ER-mediated growth of PL3 cells (Zacharewski et al., 1998).

The ability of BBP to act as an estrogen and promote estrogen-dependent cell proliferation was examined using the estrogen-dependent MCF-7BUS human breast cancer cell line. Cell proliferation was evaluated as fold-induction growth relative to the DMSO control. Concentrations used were: 0.1, 1 or 10 μM of BBP or 1 pM to 10 nM of E_2 as a positive control. 10 μM of BBP showed significant induction of growth relative to 1 nM E_2. The consistency of the assay was extremely variable (Zacharewski et al., 1998).

The recombinant yeast screen test was used to assess possible estrogenic activity of BBP. Concentrations used were: 10^{-3} M to $4.8 \cdot 10^{-7}$ M of BBP or 10^{-8} M to $4.8 \cdot 10^{-12}$ M of E_2

(positive control). The yeast cells were incubated in medium for up to 13 days. After 6 days of incubation, BBP possessed estrogenic activity in the screen assay, but approximately one millionfold less than E_2. This makes BBP considerably less potent than other environmental estrogens such as bisphenol-A and nonylphenol. The response with BBP reached a plateau at approximately 50% of the maximum response achieved with E_2. To determine whether BBP is only a partial estrogen agonist, or whether other explanations account for the sub-maximal response observed, a yeast screen containing BBP was incubated for longer time than usual, and the response monitored daily. On day 4 (the usual incubation time for this yeast assay), the BBP response was weak. On day 13 however, the highest concentration of BBP produced the maximal response possible. Thus the potency of BBP increased with time (Harris et al., 1997).

In the same study (Harris et al., 1997) BBP was also tested for estrogenic activity in two estrogen-responsive human breast cancer cell lines, MCF-7 and ZR-75. The concentrations used in the MCF-7 assay were: 10^{-5} M of BBP and 10^{-8} M of E_2 as a positive control. Cells were exposed for up to 12 days. For the ZR-75 cells the treatment was: 10^{-5} M, 10^{-6} M, and 10^{-7} M for BBP and 10^{-8} M, 10^{-10} M, and 10^{-12} M for E_2. BBP exhibited estrogenic activity in these assays. Cells were counted at a single end point on day 11. At a concentration of 10^{-5} M, BBP was approximately as potent as 10^{-10} M of E_2 in the ZR-75 cells (Harris et al., 1997).

Proliferative potency of BBP was tested in the human breast cancer cells ZR-75. The cells were exposed to 10^{-5} M of BBP and to 10^{-9} M of E_2 as a positive control. Cell densities were counted on days 0, 3, 6, 8 and 10. BBP was found to have a potent effect on cell growth at 10^{-5} M, although the growth response was less than the maximal response shown by E_2. In the same study, the stimulatory effect of BBP on the transcriptional activity of the estrogen receptor directly was examined on transiently transfected MCF7 cells using the reporter plasmids pTKLUC and pERE-TKLUC. BBP stimulated transcription at concentrations in the range 10^{-6} to 10^{-4} M. At a concentration of 10^{-5} M BBP stimulated transcription of the reporter genes to a similar extent as 10^{-11} M of E_2 (Jobling et al., 1995).

The estrogenic activity of BBP was assessed in the E-SCREEN Test, which measures the growth of human breast MCF-7 cells. Cells were exposed for 6 days, to a range of concentrations of BBP and E_2 (concentrations not given). Relative proliferative potencies (RPP, %) were determined, which measures: (the ratio between the minimal concentration of E_2 needed for maximal cell yield and the minimal dose of BBP needed to achieve a similar effect) · 100. E_2 induced maximal cell yields at 30 pM, whereas BBP was needed at a concentration of 10 μM for maximal cell yield. RPP for BBP was 0.0003% as compared to 100% for E_2. BBP was thus weakly positive in the E-SCREEN test (Soto et al., 1995).

The recombinant yeast screen was used to study if DBP or E_2 had a synergistic effect on the estrogenic potential of BBP (10^{-4} M or 10^{-5} M BBP; 10^{-4} M or 10^{-5} M DBP; or 10^{-11} M E_2). The concentration of E_2 used produced only a small response above background, so that, if additive effects or synergism occurred, they could be observed within the range of the assay. In all cases, the response obtained was very close to what was expected if an additive effect had occurred, i.e. no evidence of synergism was observed (Harris et al., 1997).

Estrogen activity of BBP metabolites in vitro

The recombinant yeast screen, in which the human estrogen receptor has been integrated in a form capable of binding to estrogen response elements, and controlling the expression of the reporter gene lac-Z was used to assess the estrogenic potential of MBuP and MBeP from 10^{-3} M to $4.8 \cdot 10^{-7}$ M. None of the metabolites exhibited estrogenic activity in this assay (Harris et al., 1997).

Estrogen activity of BBP in vivo

The potential of BBP (purity > 98.5%) to promote uterine growth in immature (20-22 days old) female Alkp:APfSD rats following oral exposure was evaluated using a standard *in vivo* uterotrophic assay. Groups of six rats were administered BBP at dose levels of 0, 56, 280, 1,120, and 2,240 mg/kg bw by gavage. 2,240 mg/kg was the maximum tolerated dose. All doses were administered daily for three days and animals were killed 24 hours after the final dose. The uteri were excised and weighed. 0.01 mg of oestradiol benzoate/kg bw served as a positive control. Following each daily dose, animals at the two highest dose levels remained subdued for several hours, but had recovered before the next dosing. Mean body weight gain was significantly reduced in the high dose group. There were statistically significant reductions in both absolute and relative uterine weights in the 1,120 mg/kg/day group (79%, $p < 0.05$ and 81%, $p < 0.05$). No reduction in uterine weight was noted in the high dose group. The positive control increased the absolute uterine weight 3.44-fold ($p < 0.001$) and the uterine body weight ratio was increased 3.67-fold ($p < 0.001$). The report concluded that BBP does not possess the potential to promote uterine growth in immature female rats when dosed orally (Monsanto, 1996b).

The potential of BBP (purity > 98.5%) to promote uterine growth in immature (20-22 days old) female Alkp:APfSD rats following subcutaneous exposure was evaluated using a standard *in vivo* uterotrophic assay. Groups of six rats were administered BBP at dose levels of 0, 0.5, 5, 50, 500 and 5,000 mg/kg bw by subcutaneous injections. All doses were given daily for three days and animals were killed 24 hours after the final dose. The uteri were excised and weighed. 0.01 mg/kg bw/day of oestradiol benzoate was used as a positive control. There were no treatment-related effects or clinical signs in the BBP treatment groups. There were statistically significant reductions in both absolute and relative uterine weights in the 5 mg/kg bw/day BBP group (76% and 77%). None of the other BBP groups were affected. Absolute uterine weight in the positive control group increased 3.91-fold ($p < 0.01$) and 4.00-fold based on the uterine:body weight ratio. The report concluded that BBP does not possess the potential to promote uterine growth in immature female rats when dosed subcutaneously (Monsanto, 1996a).

BBP was tested for its ability to induce increased uterine wet weight (animal weight 50-55 g) and vaginal cell cornification (animal weight 175-200 g) in OXV Sprague-Dawley rats. The rats were dosed by gavage, once daily for a period of 4 days. The dose levels were 0, 20, 200, and 2,000 mg BBP/kg bw/day. 1 mg/kg bw/day of ethynyl oestradiol (EE) was used as positive control. In the uterine and cell vaginal cornification assay animals (groups of 10) were killed on day 5. EE exposure resulted in a 7-fold increase in uterine wet weight. No significant increase in uterine weight was noted with BBP. Oral treatment with EE induced vaginal cell cornification in all animals by day 3 and this was sustained through days 4 and 5 as determined by vaginal smears. 1 mg/kg bw/day EE was scored as 100% efficacious in inducing the keratinisation of vaginal cells. The results with BBP were 3%, 0% and 0% for 20, 200 and 2,000 mg/kg bw/day of BBP. The numbers of positive smears were: control 0/10, positive control 10/10, and for BBP 20 mg/kg bw/day 2/10, 200 mg/kg bw/day 0/10 and 2,000 mg/kg bw/day 0/9 (Zacharewski et al., 1998).

BBP was tested for its ability to induce increased uterine vascular permeability in female Swiss albino ovariectomized mice (3 month of age) 4 hours after a single subcutaneous (sc) administration of 10^{-4} mol BBP in 0.1 ml saline. The permeability of the uterine vasculature was measured from the leakage of intravenously administered [^{125}I]-labelled human serum albumin. In this acute *in vivo* assay BBP produced no significant effect on uterine vascular permeability (Mulligan et al., 1998).

The effect of BBP on gene expression in the adult hypothalamus of female rats was studied by examining the effect of BBP and 17β-estradiol (positive control) on the expression of estrogen regulated mRNAs, i.e. progesterone receptor (PR) mRNA, preproenkephalin (PPE) mRNA, and neurotensin (NT) mRNA, in the hypothalamus and pituitary of adult female Wistar rats. Female rats 7-8 weeks of age were ovariectomised (OVX).Two weeks after OVX the rats were subcutaneously injected with 10 mg BBP or 10 μg 17β-estradiol in sesame oil, or with sesame oil alone as negative control. Twenty four hours after injection, tissues including the preoptic area (POA) mediobasal hypothalamus (MBH) and anterior pituitary were collected. Nothern blot revealed that injection of 17β-estradiol resulted in expected changes, i.e. significant increase in PR mRNA in the POA, MBH and anterior pituitary, and in PPE mRNA in the MBH. Injection of BBP increased PR mRNA in the POA and anterior pituitary, although the increase in the anterior pituitary was not significant. BBP failed to induce changes in either NT mRNA in the POA or PPE mRNA in the MBH. The explanation given by the authors was that the estrogenic activity of BBP is weak, and thus revealed only on genes strongly regulated by estrogens, or in tissues highly sensitive to estrogens (Funabashi et al., 2001).

Estrogen activity of BBP metabolites in vivo

The major metabolites of BBP are monobutyl phthalate (MBuP) and monobenzyl phthalate (MBeP). Larger quantities of MBuP than MBeP are formed from BBP (44% MBuP versus 16% MBeP) (Eigenberg et al., 1986; Mikuriya et al., 1988). In this section the estrogen activity of MBuP and MBeP *in vivo* are presented. The potential of MBuP (purity > 99%) to promote uterine growth in immature (20-22 days old) female Alkp:APfSD rats following oral exposure was evaluated using a standard *in vivo* uterotrophic assay. Groups of six rats were administered MBuP at dose levels of 0, 1, 10, 100 and 1,000 mg/kg bw by gavage. All doses were given daily for three days and animals were killed 24 hours after the final dose. The uteri were excised and weighed. 0.01 mg/kg bw/day of oestradiol benzoate served as a positive control. There were no treatment-related effects or clinical signs in MBuP exposed rats and no significant differences in the group mean terminal body weights or body weight gains. MBuP caused no significant effects on either the absolute uterine weight or relative uterine:body weight ratio. In the positive control group the absolute uterine weight was increased 3.91-fold ($p < 0.01$) and the uterine:body weight ratio was increased 4.00-fold ($p < 0.01$) (Monsanto, 1996c).

The potential of monobenzyl phthalate (MBeP; purity > 99%) to promote uterine growth in immature (20-22 days old) female Alkp:APfSD rats following oral exposure was evaluated using a standard *in vivo* uterotrophic assay. Groups of six rats were administered MBeP at dose levels of 0, 50, 250, 500, 1,000 and 1,500 mg/kg bw by gavage. All doses were given daily for three days and animals were killed 24 hours after the final dose. The uteri were excised and weighed. 0.01 mg/kg bw/day of oestradiol benzoate served as a positive control. After a single dose of MBeP, animals in the 1,000 and 1,500 mg/kg groups became subdued and exhibited piloerection. The severity of these effects was dose-dependent. One animal in the 1,500 mg/kg group died and another animal in this group remained subdued. Rest of the animals in this group was killed. Post-mortem examination of the animals killed prior to scheduled termination revealed distension of the bladder. In two of the remaining animals, the kidneys were slightly pale in colour. MBeP caused a significant decrease in absolute uterine weight in both the 500 and 1,000 mg/kg bw/day groups (79% and 69%). Statistically significant reduction in relative uterine weight was also noted for the 500 and 1,000 mg/kg bw/day MBeP groups (80% and 71%). No significant effects on uterine weight were observed in the 50 or 250 mg/k bw/day treatment groups. In the positive control group the absolute uterine weight was increased 3.44-fold ($p < 0.01$) and the uterine:body weight ratio was increased 3.67-fold ($p < 0.01$) (Monsanto, 1996d).

Summary estrogen activity of BBP and BBP metabolites in vitro and in vivo

A potential estrogen activity of BBP and the major BBP metabolites MBuP and MBeP have been assessed in both *in vitro* and *in vivo* studies. The main results from the various studies are given in **Table 4.29**.

Table 4.29 Estrogen activity of BBP and BBP metabolites

Study Design	Critical Effect	Reference
In vitro, BBP		
ER competetive ligand binding assay; 1-1,000 µM BBP; 30 minutes incubation.	BBP weakly competed with E2 for binding to ER. E2 was approx. $3 \cdot 10^4$ more potent than BBP.	Zacharewski et al. (1998)
Gene expression in recombinant receptor/reporter (luciferase) gene assay; 0.1, 1, 10 µM BBP.	10 µM BBP increased reporter gen (luciferase) activity.	Zacharewski et al. (1998)
ER-mediated growth of yeast assay; 10 µM.	10 µM of BBP weakly supported ER-mediated growth of yeast cells.	Zacharewski et al. (1998)
Estrogen-dependent cell proliferation assay; 0.1, 1 and 10 µM BBP.	10 µM showed induction of cell growth.	Zacharewski et al. (1998)
Gene expression in recombinant receptor/reporter (ß-galactosidase) gene assay; 13 days incubation; 48-1,000 µM BBP.	After 6 days BBP possessed weak estrogenic activity. The activity was one millionfold less than E2.	Harris et al. (1997)
Estrogen-dependent cell proliferation assay; 12 days incubation; 10 µM BBP.	10 µM BBP exhibited estrogenic activity measured as induced cell growth. E2 was approx. 10^4 more potent than BBP.	Harris et al. (1997)
Estrogen-dependent cell proliferation assay (E-SCREEN); 6 days.	10 µM BBP was needed for maximal cell yield, whereas 0.00003 µM of E2 was needed.	Soto et al. (1995)
Estrogen-dependent cell proliferation assay; 100 µM BBP.	A potent effect on cell growth was reported at 10^{-5} M BBP, however this effect was less potent than exposure to 10^{-9} M E2.	Jobling et al. (1995)
Transcriptional activity of ER directly; 0.01-1 µM BBP.	10^{-5} M (0.1 µM) BBP stimulated transcription to similar extent as 10^{-11} M E2.	Jobling et al. (1995)
Yeast cells expressing human estrogen or androgen receptor, agonistic or antagonistic properties studied; BBP cons. from 10^{-8} to 10^{-4} M	BBP was a weak estrogen, negativ androgen and anti-estrogen and a potent anti-androgen.	Sohoni and Sumpter (1998)
In vitro BBP metabolites, MBuP and MbeP		
MBuP and MBeP; Gene expression in recombinant receptor/reporter (ß-galactosidase) gene assay; 48 – 1,000 µM.	No estrogenic activity of MBuP and MBeP.	Harris et al. (1997)

Table 4.29 continued overleaf

Table 4.29 continued Estrogen activity of BBP and BBP metabolites

Study Design	Critical Effect	Reference
In vivo, BBP		
Alkp:ApfSD immature rats; 6 female/group; gavage; 3 days; 56, 280, 1,120 and 2,240 mg/kg bw/day BBP; standard *in vivo* uterotrophic assay.	At 1,120 but not at 2,240 mg/kg bw/day, reduced absolute and relative uterine weight. At 2,240 reduced body weight.	Monsanto (1996b)
Alkp:ApfSD immature rats; 6 female/group/ subcutaneous administration; 3 days; 0.5, 5, 50, 500 and 5,000 mg/kg bw/day BBP; standard *in vivo* uterotrophic assay.	At 5 mg/kg bw/day, but not at the higher doses, reduced absolute and relative uterine weight.	Monsanto (1996a)
Swiss albino ovariectomized mice (3 month); subcutaneous injection in 0.1 ml saline; 4 hours; 10^{-4} mol BBP.	No effect on uterine vascular permeability.	Mulligan et al. (1998)
OXV Sprague-Dawley rats; 10 female/group by gavage; 4 days; 20, 200 and 2,000 mg/kg bw/day; vaginal cell cornification assay.	No increase in uterine weight.	Zacharewski et al. (1998)
OVX Wistar rats; one subcutaneous injection; 10 mg BBP or 10 μg 17β-estradiol (positive control). The expression of estrogen regulated mRNAs were studied i.e. progesterone receptor (PR) mRNA, preproenkephalin (PPE) mRNA, and neurotensin (NT) mRNA, in the hypothalamus and pituitary	17β-estradiol resulted in expected changes, i.e. significant increase in PR mRNA in the preoptic area (POA), mediobasal hypothalamus (MBH) and anterior pituitary, and in PPE mRNA in the MBH. Injection of BBP increased PR mRNA in the POA and anterior pituitary, although the increase in the anterior pituitary was not significant. BBP failed to induce changes in either NT mRNA in the POA or PPE mRNA in the MBH.	Funabashi et al. (2001)
In vivo BBP metabolites, MBuP and MbeP		
MBuP; Alkp:ApfSD rats; 6 female/group; gavage; 3 days; 1, 10, 100 and 1,000 mg/kg bw/day; standard *in vivo* uterotrophic assay.	No increase in uterine weight.	Monsanto (1996c)
MBeP; Alkp:ApfSD rats; 6 female/group; gavage; 50, 250 500, 1,000 and 1,500 mg/kg bw/day; standard *in vivo* uterotrophic assay.	At 500 and 1,000 mg/kg bw/day reduced relative and absolute uterine weight. At 1,000 and 1,500 mg/kg bw/day piloerection.	Monsanto (1996d)

In vitro

The *in vitro* studies includes a recombinant yeast screen assay, an estrogen-receptor (ER) competitive ligand binding assay, mammalian- and yeast-based gene expression assays and an estrogen-dependent cell proliferation assay. In these assays performed to evaluate a possible estrogen activity of BBP, only a weak estrogen activity at high concentrations of BBP (10-100 μM) was reported. In the same assays E_2 (17ß-estradiol) was approximately 10^4 to 10^6 more potent than BBP. The metabolites MBuP and MBeP did not exhibit estrogenic activity in a recombinant yeast screen assay.

In vivo

The estrogenic activity of BBP and its major metabolites were studied in standard *in vivo* uterotrophic assays. In these studies BBP and MBuP did not possess the potential to promote

uterine growth in immature female rats exposed orally to BBP (up to 2,240 mg/kg bw/day) or subcutaneous to BBP (up to 5,000 mg/kg bw/day), whereas, MBeP caused a significant reduction in absolute and relative uterine weight at 500 and 1,000 mg/kg bw/day, however, at 1,000 mg/kg bw/day the animals became subdued, and at 1500 mg/kg bw/day the animals were killed prior to scheduled termination due to systemic toxicity. From the developmental study with MBeP (Ema et al., 1995a) it seems like MBeP is more toxic than BBP (reduced weight gain in dams, reddis stainig of the facial fur, pilo-erection, and spasticity from 313 mg/kg bw/day). Taking these considerations into account, and that no reduction in uterin weight in three other studies (Zacharewski et al., 1998 where both Alpk:ApfSD immature rats and OXV Sprague-Dawley rats were studied and Monsanto 1996c), and that BBP only possessed a very week estrogenic ativity in various *in vitro* studies, the reduction in uterine weight reported in the Monsanto (1996d) study is considered to be of limited importance in the evaluation of a potential estrogenic activity of BBP *in vivo*. The results from the *in vivo* uterotrophic assays are in accordance with the results reported in the low dose drinking water studies (TNO, 1998a and b; Bayer, 1998; Ashby et al., 1997; see developmental section). In these studies no effects on reproductive parameters were reported after exposure to very low concentrations of BBP (0.01-0.674 mg/kg bw/day) *in utero* and during lactation.

In a study by Funabashi et al. (2001) the expression of estrogen regulated mRNAs was studied in the hypothalamus, preoptic area and pituitary in OVX female rats following subcutaneous injection of 10 mg BBP or 10 μg 17β-estradiol (positive control). 17β-estradiol resulted in expected changes in mRNAs. However, injection of BBP increased PR mRNA only in the preoptic area. It was concluded that that the estrogenic activity of BBP was weak, and thus revealed only on genes strongly regulated by estrogens, or in tissues highly sensitive to estrogens.

Anti-androgen activity of BBP in vitro

BBP was tested in a recombinant yeast screen for androgen activity where the yeast cells expressed the human androgen receptor hAR. The study included assays to investigate the ability of BBP to interact both as an agonist or antagonist. Dihydrotestosterone (DHT) was used as a positive control and ethanol served as negative control. To determine whether BBP possessed an anti-androgen activcity, DHT ($1.25 \cdot 10^{-9}$ M) was added to the medium at a concentration that produced a sub-maximal response (65%). The androgen and anti-androgenic activity of BBP was tested at BBP concentrations from 10^{-8} to 10^{-4} M. The most potent chemical in each screen was assigned a potency of four plus (++++), and the potency of the chemicals were expressed relative to this. BBP showed no androgen activity (-), however, was a potent anti-androgen (++++). BBP was as potent as the known anti-androgen flutamide. DHT had a high relative potency (++++) in the hAR assay (Sohoni and Sumpter, 1998).

Anti-androgen activity of BBP, MBuP and MBeP in vivo

In this section the nine recent studies (Tyl et al., 2004; Piersma et a., 2000; Gray et al., 2000; Parks et al., 1999; Imajima et al., 1997; Nagao et al., 2000; Shono et al., 2000; Ema et al., 2002; Ema et al., 2003) are presented since effects, which may be indicative of an anti-androgen-like activity of BBP, MBuP or MBeP were reported. These effects includes reduced testicular weight, reduced ano-genital distance (AGD), and retarded transdominal descent of testis in male offspring exposed to BBP, MBuP or MBeP during the organogenic period and/or the late prenatal early postnatal period. Furthermore, this section is also supplemented with the conclusions from the studies where an anti-androgen-like effect of DBP is proposed by the authors (Mylchreest et al., 1998; Ema et al., 1998b), since one of the major metabolites of DBP

is MBuP (Albro and Moore, 1974; Williams and Blanchfield, 1975; Tanaka et al., 1978). Furthermore, the pattern of malformations reported in foetuses after exposure to MBuP and MBeP were similar (Ema et al., 1995; Ema et al., 2003) to that produced by BBP (Ema et al., 1992a,b,c) and DBP (Ema et al., 1993b; 1994b).

In the Piersma et al. (2000) developmental toxicity study pregnant rats were exposed to BBP by gavage (0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg/day) *in utero* from gestation day (gd) 6 to 15 (short time exposure) or 6 to 20 (long time exposure) and necropsied on post-natal day 21. BBP induced reduced testicular weight in offspring exposed to BBP from gd 6-20 with a LOAEL of 270 mg/kg/day. Retarded transdominal descent of testis was reported with a LOAEL of 580 mg/kg/day. This effect was more pronounced after long time exposure *in utero* to BBP. The maternal LOAEL was 580 mg/kg/day based on increased liver weight. For more study-information see Section 4.1.2.9.

In the Gray et al. (2000) and Parks et al. (1999) studies pregnant Sprague-Dawley rats were given 750 mg/kg/day of BBP by gavage from gestation day (gd) 14 through postnatal day (pnd) 3. Reduced AGD and testis weight was reported on pnd 2, and areolas on pnd 13 were seen in the male offspring in both studies. In the Gray et al. (2000) study male offspring at approximately 90 days of age exposed from gd 14 through pnd 3 was also studied for malformations in the reproductive organs, and 84% of the male offspring were reported to have malformations in the testis, accessory reproductive organs and genitalia. For more study-information see Section 4.1.2.9.

In the Imajima et al. (1997) study the effect of prenatally exposure to Monobutyl phthalate (MBuP) approximately 1,000 mg/kg bw/day on testicular descent was studied in Wistar-King A rats. Pregnant rats were gavaged from gestation day 15 to 18. In the control offspring all the testes were located in the lower abdominal cavity near the bladder neck on gd 20, whereas, in offspring treated *in utero* to MBuP the testes were located significantly higher in the abdominal cavity, and some were located near the kidney. On pnd 30 – 40, in the control group all testes descended into the scrotum and the incidence of cryptorchidism was 0%, whereas, in the MBuP treated offspring 22 rats showed cryptorchidism (14 unilateral and 8 bilateral undescended testes), and the incidence of cryptorchidism was 84.6%. This may indicate that MBuP act in an anti-androgenic manner, since testis descent is under androgenic control. For further study description see Section 4.1.2.9.

In the Shono et al. (2000) study the time-specific effects of monobutyl phthalate (MBuP) on the transabdominal migration of the testis, and the levels of testosterone were studied in foetal rats. Three groups of pregnant Wistar-King A rats were administered MBuP by gavage (2-6 pregnant rats/group, 0.3 g/day, corresponding to approximately 1,000 mg/kg bw/day). Group 1 was exposed from gestation day (gd) 7-10, group 2 from gd 11-14, and group 3 from gd 15-18. The control group (group 4) received vehicle from gd 7-18. At gd 20 the foetuses were obtained by Caesarean section, and the position of the testes were determined in all groups. The results from this study showed that in the control group all 30 testes were anchored at the bottom of the abdominal cavity near the bladder neck by a swollen gubernaculum, whereas in group 3 (exposure to MBuP from gd 15-18) the testes were high in the abdominal cavity and associated with both an elongated gubernaculum and a hypertrophic cranial suspensory ligament. The mean transabdominal testicular migration values (the distance from the bladder neck to the lower pole of the testis) in group 1, 2, 3, and 4 were 12.3 ± 5.9 (10 testes), 24.5 ± 5.2 (10 testes), 57.9 ± 2.6 (38 testes), and 9.3 ± 1.9 (30 testes). Values were significantly higher in group 2 and 3 compared to the control group. The mean testosterone levels were 50.9 ± 3.8 pg/testis in MBuP treated foetuses (25 testes) and 852 ± 80.3 pg/testis in the control foetuses (30 testis), the levels was

significantly lower in the MBuP treated rats compared to the controls. For further study description see Section 4.1.2.9.

Ema et al. (2002) studied he effects of BBP on the development of the reproductive system in male offspring. In this study pregnant Wistar rats (16/group) were given BBP by gastric intubation at doses of 250, 500 or 1,000 mg/kg on days 15 to 17 of pregnancy. A significant increase in the incidece of foetuses/litter with undescended testes was found at 500 (54/14) and 1,000 (97/16) mg/kg compared to 0/16 in the control group. Furthermore, a statistically significant decrease in the AGD of male foetuses was observed at 500 and 1,000 mg/kg. The AGD/cube root of body weight ratio in male foetuses was also significantly reduced from 500 mg/kg. The AGD/cube root of body weight ratio in female foetuses in the BBP treated groups were comparable to those in the control group. It was concluded by the authors of the study that BBP given to pregnant rats during gestation day 15-17 produced adverse effects on the development of the reproductive system in male offspring. For further study description see Section 4.1.2.9.

Ema et al. (2003) studied the effect of monobenzyl phthalate MBeP, a major metabolite of BBP on the development of the reproductive system in rats. He also looked into the role of MBeP in the antiadrogenic effects of BBP. In this study pregnant Wistar rats (16/dose group) were given MBeP by gavage at doses of 167, 250 and 375 mg/kg bw/day on gestation day (gd) 15 to 17. Foetuses were examined on gd 21. Maternal body weight gain on gd 15-18 was significantly decreased from 167 mg/kg bw/day (31, 24, 23 and 15g in the control, 167, 250 and 375 mg/kg bw/day dose group). Maternal food consumption was significantly decreased on gd 15-18 from 167 mg/kg bw/day (54, 46, 40 and 33 in the control, 167, 250 and 375 mg/kg bw/day dose group). The adjusted maternal weight gain was significantly decreased from 250 mg/kg bw/day. Foetal weight was significantly decreased at 375 mg/kg bw/day. A significant increase in the incidence of undescended testes/litter was reported from 250 mg/kg bw/day (2(2), 1(1), 21(12) and 79(16) in the control, 167, 250 and 375 mg/kg bw/day dose group). A decrease in the AGD and ratio of AGD on the cube root of body weight was reported in male foetuses at 250 mg/kg bw/day. No effect on AGD was found in female foetuses. The study indicated that MBeP produced adverse effects on the development of the reproductive system in male offspring and suggested that MBeP may be responsible for the antiandrogenic effects of BBP.

In the 2-generation study (Tyl et al., 2004) a dose related significant reduction in the absolute and adjusted AGD was reported in the F1 and F2 pups from 250 mg/kg bw/day. Reduced AGD at birth is reported to be one of the most sensitive indicators of andorgenic activity (Gray et al., 1997). Furthermore, at 750 mg/kg bw/day a significant increase in F1 and F2 male pups with one or more nipples and/or areolae were reported. At weanling in F1 and F2 offspring a significant reduction in testis weight was reported in the 750 mg/kg bw/day dose group. At post natal day 21 necropsies the percentage of males with reproductive tract malformations (RTM) were significantly increased at 750 mg/kg bw/day in the F1 and F2 offsprings, and at adult necropsies the percentage of males with RTM were significantly increased in the F1 offspring (F2 offspring was not evaluated as adults). In F1 parental male a significant decrease in the testis, epididymis, prostate and seminal vesicle weights were reported (not evaluated in the F2 generation).

In the two-generation study performed by Nagao et al. (2000) a decrease in the weights of testis, epididymis, and seminal vesicle were reported in the F1 generation exposed to 500 mg/kg bw/day BBP *in utero* or via milk, when evaluated at weaning or after puberty. In addition, in the same group, tubular atrophy and decreased germial epithelium was observed. A decrease in AGD was reported in male F1 offspring. For further study description see Section 4.1.2.9.

The effects of DBP on prenatal and early neonatal development of the reproductive tract in rats were studied *in vivo* (Mylchreest et al., 1998). In this study a marked disturbed development of the male reproductive tract (internal and external) in rat offspring exposed via their mothers during gestation and lactation was observed at all dose levels (250, 500 or 750 mg/kg bw/day by gavage) in the absence of significant maternal toxicity. In female offspring sporadic cases of reproductive tract malformations were observed at 500 and 750 mg/kg bw/day. Age at vaginal opening and estrus cyclicity was not affected. The results of this study suggested that DBP does not possess estrogenic activity but rather shows anti-androgenic activity at these dose levels. The results reported in the Mylchreest et al. (1998) study were confirmed in a study by Ema et al. (1998b). In this study Wistar rats were exposed to DBP (331, 555 or 661 mg/kg bw) from gestation day 11-21.

Summary anti-androgen activity of BBP in vitro and in vivo

BBP was shown in one *in vitro* study to be a potent anti-androgen in yeast cells expressing the androgen receptor. Nine *in vivo* studies are available which indicate an anti-androgen-like activity of BBP or its major metabolites in rats, MBuP and MBeP (Piersma et al., 2000; Gray et al., 2000; Parks et al., 1999; Imajima et al., 1997; Shono et al., 2000; Nagao et al., 2000; Tyl et al., 2004; Ema et al., 2002; Ema et al., 2003). Effects reported in the Piersma et al. (2000) study included a reduction in testicular weight in offspring, and effects on testicular migration from 270 mg/kg bw/day and 580 mg/kg bw/day after *in utero* exposure to BBP from gestation day 6 to 20. In the Gray et al. (2000) study malformations in the reproductive organs in 84% of male offspring (approximately 90 days of age) exposed to 750 mg/kg bw/day BBP from gestation day 14 through postnatal day 2 were reported. Furthermore in the Gray et al. (2000) and Parks et al. (1999) studies a reduced AGD and testis weight in males at day 2 of age, and males with areolas at day 13 of age were reported. In the Imajima et al. (1997) study and the Shono et al. (2000) study testicular descendent was studied, which is under androgenic control. In this study the testis were located significantly higher in the abdominal cavity on gd 20 offspring compared to control rats exposed *in utero* to MBuP from gd 15-18. Furthermore, in the Imajima et al. (1997) study, on pnd 30-40 cryptorchidism was reported in 84.6% of the exposed offspring, compared to 0% in the control group. In the study by Ema et al. (2002) *in utero* exposure to 500 and 1,000 mg/kg BBP on gd 15-17 induced a significant decrease in the AGD and a significant increase in the incidence of undescended testis. In the study by Ema et al. (2003) *in utero* exposure to MBeP on gd 15-17 was shown to induce a significant decrease in AGD and a significant increase in the incidence of undescended testis. In the study by Nagao et al. (2000) a decrease in the weight of the testis, epididymis, and seminal vesicle, and tubular atrophy and decreased germinal epithelium was reported in F1 male offspring exposed to 500 mg/kg bw/day BBP during gestation and lactation and evaluated at weaning or after puberty. Furthermore, a decrease in AGD was reported in male offspring in the 500 mg/kg bw/day dose group, which is a sensitive indicator of anti-androgen activity. In the Tyl et al. (2004) study a dose-related decrease in absolute and adjusted AGD was reported in F1 and F2 male pups from 250 mg/kg bw/day. Furthermore, at 750 mg/kg bw/day in F1 and F2 offspring a significant decrease in reproductive organ weights, and a significant increase in the percentage of males with reproductive tract malformations were reported. A potential anti-androgen-like effect of DBP has been indicated in different studies (Mylchreest et al., 1998; Ema et al., 1998b; Gray et al., 1998; Foster et al., 1998). In some of these studies the authors proposed that the major metabolite of DBP; MBuP may elicit an anti-androgen-like effect.

An association between prenatal and postnatal exposure to phthalates and whether the exposure had any influence on reproductive organ development in newborn boys was studied in two epidemiological studies. In the study by Swan et al. (2005) an association between maternal

exposures to BBP as well as other phthalates and AGI in boys was reported. When comparing boys with prenatal MBeP (monobenzyl phthalate, reflecting exposure to BBP) exposure the odds ratio for a shorter AGI was 3.8. For the other monoester phthalates the odds ratio were 10.2 for MBuP (reflecting exposure to DBP), 4.7 for MEP (reflecting exposure to DEP), and 9.1 for MiBP (reflecting exposure to DINP) (all *p*-values < 0.05).

In the study by Main et al. (2005) no association was found between phthalate monoester levels (MEP, MMP, MBuP, MBeP, MINP and MEHP) in breast milk and cryptorchidism in newborn boys. However, a significant association was found between intake of milk contaminated with phthalates (MEP, MBuP, MMP and MINP) and postnatal surge of reproductive hormones (SHBG, LH, testosterone and inhibin B) in newborn boys. As regards the monoester metabolite of BBP, MBeP the tendencies were similar, however, they were not statistically significant.

These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to the small sample size, (85 boys in Swan et al., 2005 and 130 boys in Main et al., 2005) further studies with larger sample size have to be performed before clear conclusions can be drawn from these studies.

4.1.3 Risk characterisation

4.1.3.1 General aspects

The human population may be exposed to BBP at the workplace, from the use of consumer products, and indirectly via the environment (see Section 4.1.1.2, 4.1.1.3, and 4.1.1.4). The main exposure routs for wokers are expected to be inhalation and dermal contact. Ingestion is considered not to be relevant for occupational exposure. For consumers, and humans exposed indirectly via the environment, the main exposure is expected to be from ingestion. In recent studies urinary phthalate metabolites were measured in human reference populations. These studies indicated that human exposure to phthalates including BBP is both higher and more common than previously suspected (Blount et al., 2000a; CDC, 2001; CDC, 2003; Hoppin et al., 2002; Koch et al., 2003; Brock et al., 2002; Adibi et al., 2003). See Section 4.1.1.4 for study description.

In rats, the kinetics of BBP after oral administration was dose-dependent. Excretion of radiolabelled BBP in the urine was between 70% and 80% in the dose-range of 2 mg/kg p.o. and 200 mg/kg p.o. whereas 22.4% were excreted in the urine after administration of 2,000 mg/kg p.o. The excretion of radioactivity in the feces was 20% after intravenous administration which indicates that the absorption in the dose range between 2 mg/kg p.o. and 200 mg/kg p.o. is nearly complete. After dermal application, 30-40% of the applied amount seems to be absorbed and reaches the systemic circulation. The extent of systemic availability of the substance administered by inhalation is not known as specific data are lacking.

BBP is metabolized to monobutyl phthalate (MBuP) or monobenzyl phthalate (MBeP). This metabolism may take place in the gut wall and/or liver. In adult and immature rats, the ratio of monobutyl phthalate to monobenzyl phthalate found in the urine is 3:1. Both metabolites were found in the bile. Reabsorption from gut lumen may take place. There is no evidence of tissue accumulation. The percentage of excreted metabolites (MBuP and MBeP) in the urine in adult rats was shown to be higher compared to immature rats. The excretion of BBP metabolites in urine has also been studied in humans. Contrary to the metabolism of BBP in rats, BBP is mainly

metabolised to MBeP in humans. However, limited data on the metabolism of BBP in humans is available.

No half-life of BBP in the body has been calculated. However, the available data indicate a half-life of less than 24 hours.

In the risk characterisation, 100% absorption is assumed for both inhalation and oral exposure, whereas the absorption for dermal exposure is set at 5%.

None of the acute toxicity studies have been performed according to current guidelines or in compliance with GLP. The acute toxicity of BBP in animals is low. The oral LD_{50} values of BBP ranged from 2,330 – 20,400 mg/kg bw/day in rats and was 4,170 mg/kg bw/day (female) and 6,160 mg/kg bw/day (male) in mice. The dermal LD_{50} value in rabbits was greater than 10,000 mg/kg bw/day, whereas in rats the dermal LD_{50} value was 6700 mg/kg bw/day. The LD_{50} values of BBP from i.p. administration were in the same range as from oral or dermal exposure. No information on acute toxicity after inhalation exposure is identified. The wide range of oral LD_{50} values in rats may be due to the water insolubility of BBP. The lowest LD_{50} value was obtained when BBP was administered in a corn oil vehicle.

With respect to the irritation potential of BBP, animal studies performed according to current standards for both skin and eye irritation were available, whereas in humans only skin irritation was studied. From these studies it appears that BBP is not irritating to the skin, however, a slight eye irritation was reported in rabbits using the Draize procedure. No data on respiratory irritation from animal or human studies are available.

As regards the sensitizing effect of BBP both animal and human studies were located. In an ear swelling test in mice and guinea pigs, BBP was negative. However, the test has not been fully evaluated and no standard protocols are available. In two human studies no sensitisation of BBP was reported. No data on respiratory sensitisation from animal studies are available. In a case-control study an association was found between childeren exposed to BBP in house dust and cases of allergic symptoms. However, in this study very small differences were found in the concentrations of BBP in house dust from controls and cases of allergic symptoms in children, and the children were exposed to other phthalates (DBP, DEHP etc) as well. Furthermore, demographic factors and pet ownership were not considered in this study. Due to the limitations in study design, no clear conclusion can be drawn from the study on the relationship between BBP in house dust and allergic symptoms in children.

With respect to repeated dose toxicity, the data from a well performed 13 week study with oral administration of BBP to rats revealed a NOAEL of 151 mg/kg bw/day (Hammond et al., 1987). This NOAEL value is used in the risk assessment for consumers and indirect exposure via the environment for oral exposure to BBP. A 13 week inhalation study in rats performed in compliance with GLP revealed a NOAEL of 218 mg/m^3 (Monsanto, 1982). This NOAEL value is used in the risk assessment for workers for inhalation exposure to BBP, and for indoor air exposure to BBP for consumers. In the oral repeated dose toxicity study histopathological changes, gross morphological changes, and increased kidney weight and an urinary pH decrease were reported at the next highest BBP dose; 381 mg/kg bw/day in male rats. In the inhalation repeated dose toxicity study a significantly increased kidney and liver weight was reported at 789 mg/m^3 in male and female rats, and a decrease in serum glucose in male rats.

Based on the data available for BBP from a variety of *in vitro* and *in vivo* genotoxicity studies; mutagenicity in *Salmonellea typhimurium* or in mouse lymphoma cells; sister chromatid exchanges (SCE) or chromosomal abberations (CA) in CHO hamster cells; morphological transformation in Syrian hamster embryo cells or BALB/3T3 cells; sex-linked recessive lethals

in *Drosophila melanogaster* or dominant lethal mutations in mice, and taking into consideration the non-genotoxic properties of other phthalate esters, BBP can be considered as a non-genotoxic substance.

Phthalate esters are known to induce peroxisom proliferation in the liver of mice and rats. In general the longer chain dialkylphthalates are more potent inducers than the shorter chains, and branched chain phthalates seemed more potent than straight. Many peroxisome proliferators have been shown to induce hepatocellular tumours when administrated at high dose-levels for long periods to mice and rats despite being non-genotoxic. The mechanisms of induction of carcinogenicity by peroxisome proliferators are considered to have a threshold. Species differences in sensitivity to chemicals that induce peroxisome proliferation are reported. Mice and rats are very sensitive, hamsters have a less marked response, whereas guinea-pigs, primates and humans are rather insensitive or non-responsive. BBP induce peroxisome proliferation in rats, however compared with Di-(ethylhexyl) phthalate BBP appears to be less effective in causing peroxisome proliferation. As regards the carcinogenicity data for BBP no hepatocellular tumours were reported in mice and rats. However, an increased incidence of mononuclear cell leukemias was reported in female rats at high doses (12,000 ppm) of BBP, a marginally increased incidence of pancreatic adenomas and transitional epithelial papilloma of the urinary bladder was found in female rats. No increase in the incidence of tumours was reported in mice. Overall, BBP can be considered as a non-carcinogenic substance.

Regarding toxicity to reproduction, fertility as well as developmental studies are available. When taking the available data base into account a NOAEL at 100 mg/kg bw/day for effects on the reproductive organs/fertility from a 2-generation study in rats is used in the risk assessment (Nagao et al., 2000). The NOAEL is based on atrophy of the testis, epididymis, and seminal vesicle, and reduced reproductive organ weights at 10 or 18 weeks of age in the F1 generation at 500 mg/kg bw/day. In this two-generation study BBP was administered by gavage (0, 20, 100 and 500 mg/kg bw/day) to Sprague-Dawley rats. The results were as following; a significant reduction in fetal body weight was reported at 100 and 500 mg/kg bw/day on pnd 0. Furthermore, in male offspring (preweanling rats) a reduction in AGD (absolute), testis weight, epididymis weight, decreased FSH level and number of spermatogonia and spermatocytes in the seminiferous tubules was reported at 500 mg/kg bw/day. In postweanling rats at 500 mg/kg bw/day a decreased body, testis and epididymis weight was reported. Furhtermore, at 500 mg/kg bw/day, a delay in preputial separation in males, decreased testosterone and LH levels and increased incidence of testicular athrophy with decreased number of germ cells in the seminiferous tubules and decreased number of sperm in the epididymis was reported. In another recent 2-generation study (Tyl et al., 2004) significantly reduced mating and fertility indices were reported in F1 parents to make F2 offspring at 750 mg/kg bw/day. In the same study a significantly reduced relative and absolute paired ovaries and uterus weight was reported in F0 females. In adult F1 males a significant increase in reproductive tract malformations was reported (53.33% compared to 0% in controls). No increases in reproductive tract malformations were reported in females. Systemic toxicity reported at 750 mg/kg bw/day was limited to organ weight changes (liver, kidney) in males and females and histopathological lesions graded as minimal in females. The NOAEL for fertility was 250 mg/kg bw/day from this study.

For development a NOAEL at 50 mg/kg bw/day for offspring is used in the risk assessment (Tyl et al., 2004). This NOAEL value is based on a dose-related significant reduction in absolute and adjusted AGD in both F1 and F2 offspring from 250 mg/kg bw/day. At the next higher dose, 750 mg/kg bw/day a significant increase in F1 and F2 male pups with one or more nipples and/or areolae was reported. At weanling in F1 and F2 offspring a significant reduction in testis weight was reported. At post natal day 21 necropsies the percentage of males with reproductive tract

malformations (RTM) were significantly increased in the F1 and F2 offsprings, and at adult necropsies the percentage of males with RTM were significantly increased in the F1 offspring (F2 offspring was not evaluated as adults). In F1 parental male a significant decrease in the testis, epididymis, prostate and seminal vesicle weight was reported (not evaluated in the F2 generation). The NOAEL for maternal toxicity was 750 mg/kg bw/day and was based on organ weight changes (liver and kidney) and histopathological lesions graded as minimal in the liver at 750 mg/kg bw/day. In this 2-generation study BBP was administered in the feed at doses of 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day. The developmental toxicity was also studied in mice exposed to BBP from gestation day (gd) 6 to 15 and sacrificed on gd 17. The NOAEL for offspring in this study was 182 mg/kg/day and are based on prenatal mortality and malformed foetuses at doses ≥ 910 mg/kg bw/day (NTP report, 1990). The maternal NOAEL value was 182 mg/kg/day. At the next higher dose level (910 mg/kg/day) a reduced dam weight gain (15%) with no reduction in adjusted body weight gain was reported. Due to the great distance between the exposure groups in the mice study, the 2-generation study in rats is used in the risk assessment for developmental effects.

Only one human study is available where the relation between exposure to phthalates and semen quality was evaluated. In this study an association was found between high levels of mono butyl phthalate and/or mono benzyl phthalate in the urine and altered semen quality including semen concentration, semen motality and semen morphology (Duty et al., 2003). Due to the mixed exposure to various phthalates it is difficult to conclude that the effect observed on semen quality is related only to BBP exposure. Furthermore, the phthalates were only measured in a single spot urine sample in a relative small group of men (168) derived from subfertile couples. Due to the limitation of the study the NOAEL value for effects on reproductive organs in experimental animal studies will be used in the risk characterisation of BBP.

An association between prenatal and postnatal exposure to phthalates and whether the exposure had any influence on reproductive organ development in newborn boys was studied in two epidemiological studies. In the study by Swan et al. (2005) an association between maternal exposures to BBP as well as other phthalates and AGI in boys was reported. When comparing boys with prenatal MBeP (monobenzyl phthalate, reflecting exposure to BBP) exposure the odds ratio for a shorter AGI was 3.8. For the other monoester phthalates the odds ratio were 10.2 for MBuP (reflecting exposure to DBP), 4.7 for MEP (reflecting exposure to DEP), and 9.1 for MiBP (reflecting exposure to DINP) (all p-values < 0.05).

In the study by Main et al. (2005) no association was found between phthalate monoester levels (MEP, MMP, MBuP, MBeP, MINP and MEHP) in breast milk and cryptorchidism in newborn boys. However, a significant association was found between intake of milk contaminated with phthalates (MEP, MBuP, MMP and MINP) and postnatal surge of reproductive hormones (SHBG, LH, testosterone and inhibin B) in newborn boys. As regards the monoester metabolite of BBP, MBeP the tendencies were similar, however, they were not statistically significant.

These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to the small sample size, (85 boys in Swan et al., 2005 and 130 boys in Main et al., 2005), further studies with larger sample size would have to be performed before clear conclusions can be drawn from these studies.

In conclusion, BBP is found to adversely affect the reproductive organs in experimental animal studies which may affect fertility. Furthermore, the substance is found to be a developmental toxicant and to possess anti-androgen like properties in experimental animal studies.

Table 4.30 The NOAEL values for the concerned endpoints used in the calculation of the MOS values

Endpoint	Study design	Critical effect	NOAEL/ LOAEL	Reference
Repeated dose toxicity	Wistar rats; 10/sex/group; 3 months; oral administration (in diet): 2500-12,000 (corresp. to approx. 151, 381, 960 mg/kg bw/day	Male rats: At doses ≥ 381 mg/kg bw/day kidney weight increase, gross morphological changes in the liver and histopathological changes in pancreas. At 960 mg/kg bw/day body weight decrease, slight anemia, liver weight increase and histopathologic changes in liver.	NOAEL: 151 mg/kg bw/day in male rats	Hammond et al. (1987)
Repeated dose toxicity	Sprague-Dawley rats; 25/sex/group; 13 weeks; inhalation: 51, 218 and 789 mg/m^3	At 789 mg/m^3 increase in relative liver and kidney weight in male and female rats. Decrease in serum glucose in male rats.	NOAEL: 218 mg/m^3 in male and female rats	Monsanto (1982)
Reproduction toxicity, fertility/effects on the reproductive organs	Sprague-Dawley rats; two-generation study; 25/sex/group; administration by gavage; 0, 20, 100 and 500 mg/kg bw/day BBP	F_O; decrease i body weight gain in males at 500 mg/kg/day. A dose-dependent increase in kidney weight of both sexes, (significant from 100 mg/kg/day in females and at 500 mg/kg/day in males), a significant increase in liver weight in males at 500 mg/kg/day, and a significant decrease in ovary weight in females at 500 mg/kg/day. A decrease in testosterone (significant at 500 mg/kg/day), and increase in FSH (significant from 100 mg/kg/day) in males. F1: significantly decreased body weight at birth from 100 mg/kg/day, and at 500 mg/kg/day throughout the study. AGD was decreased and preputial separation delayed in males at 500 mg/kg/day. Macroscopic and microscopic changes of testis, and decreased testosterone levels at 500 mg/kg/day after puberty. Significantly decreased testis, epididymis, and seminal vesicle weight at 500 mg/kg/day in F1 postweaning. Decreased number of germ cells in the seminiferous tubules, and sperm in the epididymis at 500 mg/kg/day as well. BBP did not affect reproductive ability, including delivery and lactation.	No NOAEL value could be derived for effects on fertility. NOAEL for effects on the reproductive organs: 100 mg/kg bw/day	Nagao et al. (2000)

Table 4.30 continued overleaf

Table 4.30 continued The NOAEL values for the concerned endpoints used in the calculation of the MOS values

Endpoint	Study design	Critical effect	NOAEL/ LOAEL	Reference
Reproduction toxicity, development	CD Sprague-Dawley rats; 2-generation study; 30/sex/group; administration in feed; 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day.	Development: reduced AGD from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day. Maternal toxicity: organ weight changes (liver and kidney), and histopathological lesions in the liver graded as minimal at 750 mg/kg bw/day.	NOAEL for developmental effects: 50 mg/kg bw/day based on reduced AGD from 250 mg/kg bw/day in F1 and F2 offspring. NOAEL for maternal toxicity: 250 mg/kg bw/day	Tyl et al. (2004)

4.1.3.3 Workers

The main routes of exposure for workers are expected to be by inhalation and dermal contact. Ingestion is considered not to be relevant for occupational exposure. The highest exposure levels are expected when performing processes at elevated temperatures and working operations creating aerosols. Based upon the evaluated information on the exposure scenarios presented in Section 4.1.1.2 i.e. production of BBP, industrial use of BBP-containing products and professional end-use of products containing BBP, the values presented in **Table 4.11** are used in the risk characterisation of BBP.

To calculate the MOS-values for the concerned endpoints the NOAEL-value for the respective endpoints (see **Table 4.30**) are divided by the external or internal exposure. The internal exposure from the inhalatory route is estimated by the formula below, where the parameters used is given in **Table 4.31**. Data on absorption after inhalation is lacking. Therefore, 100% respiratory absorption is used as a default value.

$$U_{inh} = \frac{B_{inh} * C * V_{inh}}{BW}$$

The dermal exposure is estimated by EASE for some scenarios (see Section 4.1.1.2). The internal exposure from the dermal route is considered to be 5% of the exposure as a worst case estimate (see below), divided with a bodyweight of 70 kg.

Table 4.31 The parameters used in the formulas for estimating internal exposure by the inhalatory route

Symbol	Description	Value	Unit
U_{inh}	Internal exposure by the inhalatory route		mg/kg bw/day
B_{inh}	Bioavailability for inhalation exposure	1 (100%)	
C	Air concentration	-	mg/m^3
V_{inh}	Inhalation rate	10	m^3/day
BW	Body weight of a worker	70	kg

Table 4.32 Internal exposure calculated from external exposure (taken forward from Section 4.1.1.2)

Exposure scenario	Inhalation		Dermal		Combined route
	External exposure	Internal exposure	External exposure	Internal exposure	Internal exposure
	mg/m³	mg/kg/day	mg/day	mg/kg/day	mg/kg/day
Scenario 1: Production of BBP					
Scenario 1B: Drumming					
Reasonable worst case	1.0	0.14	420	0.3	0.44
Scenario 2: Industrial use of BBP-containing products					
Scenario 2A2: Processing of PVC floats					
Reasonable worst case	< 0.005	< 0.0007	< 840	< 0.6	< 0.6
Scenario 2C1: Flooring with the calendering process					
Typical value	0.4	0.06	420	0.3	0.36
Reasonable worst case	3.0	0.43	420[1)]	0.3	0.73
Scenario 3: Professional end-use of semi- and end-products containing BBP					
Values taken from scenario 2C1					
Typical value	< 0.4	< 0.06	420	0.3	< 0.36
Reasonable worst case	< 3.0	< 0.43	420	0.3	< 0.73
Values taken from scenario 2A2					
Reasonable worst case	<< 0.005	<< 0.0007	<< 840	<< 0.6	<< 0.6

1) Typical value is also taken as a worst case.

MOS-values for the concerned endpoints

As described above, the MOS values for the concerned endpoints are calculated by dividing the NOAEL values for the respective endpoints (see **Table 4.30**) with the internal or external exposure estimates for the various scenarios described in **Table 4.32**.

Acute toxicity

Acute toxicity of BBP in animals is low. The oral LD_{50} values of BBP ranged from 2,330-20,400 mg/kg bw for rats and 4,170-6,160 mg/kg bw in mice. Dermal exposure of rabbits gave an LD_{50} value > 10,000 mg/kg bw where as in rats the LD_{50} value is reported to be 6,700 mg/kg bw. Compared with the anticipated occupational exposure levels it is concluded that BBP is of no concern for workers with respect to acute effects.

Conclusion (ii).

Irritation/corrosivity

In humans, BBP was found to have no skin irritating effect. No human experience indicating eye irritation due to BBP exposure was located. It is concluded that BBP is of no concern for workers with regard to irritation/Corrosivity.

Conclusion (ii).

Sensitisation

As regards the sensitizing effect of BBP both animal and human studies were available. In an ear swelling test in mice and guinea pigs, BBP was negative. No skin sensitisation was reported in two human studies with BBP. In a case-control study an association was found between cases of persistent allergic symptoms in children and the concentration of BBP in dust collected from their homes compared to children without such symptoms. Due to the limitations in study design, no clear conclusion can be drawn from the study. It is concluded that sensitisation is of no concern for workers.

Conclusion (ii).

Repeated dose toxicity

The most relevant study is a 90-day inhalation study with Sprague Dawley rats (Monsanto, 1982). A NOAEL of 218 mg/m^3 was identified in this study based on increased liver and kidney weight in male and female rats and decreased serum glucose in male rats at 789 mg/m^3. This level might be of concern for workers and MOS values are calculated for the different scenarios for the inhalatory route (see **Table 4.33**), the dermal route (see **Table 4.34**) and for the combined route (see **Table 4.35**). The following factors are used in converting the inhalatory exposure dose in rats to an estimated oral route dose (from mg/m^3 to mg/kg bw rats/day): bodyweight of 300g, an inhalatory volume of 14.4 l/hour of rats and exposure-duration of 6 hours/day.

$$NOAEL_{oral} = \frac{218\ mg/m^3 \cdot 0.0144\ m^3/hour \cdot 6hours/day}{0.3\ kg} = 62.8\ mg/kg\ bw/day$$

This gives an estimated oral NOAEL of 62.8 mg/kg bodyweight/day, which is used in calculation of the MOS-value for the dermal and combined route.

Table 4.33 MOSs calculated for the inhalatory route for the endpoint repeated dose toxicity. NOAEL is ≥ 218 mg/m³ (Monsanto, 1982)

Scenario	External exposure (mg/m³)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	1.0	218	(ii)
Scenario 2: Industrial use of BBP-containing products (2C1)			
Typical value	0.4	545	(ii)
Reasonable worst case	3.0	73	(ii)
Scenario 3: Professional end-use of products containing BBP			
Typical value	< 0.4	> 545	(ii)
Reasonable worst case	< 3.0	> 73	(ii)

Table 4.34 MOSs calculated for the dermal route for the endpoint repeated dose toxicity. NOAEL is ≥ 218 mg/m³ or 62.8 mg/kg bw/day (Monsanto, 1982)

Scenario	Internal exposure (mg/kg bw/day)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	0.3	209	(ii)
Scenario 2: Industrial use of BBP-containing products			
Scenario 2A2: Processing of PVC floats			
Reasonable worst case	< 0.6	> 105	(ii)
Scenario 2C1: Flooring with the calendaring process			
Typical value	0.3	209	(ii)
Reasonable worst case	0.3	209	(ii)
Scenario 3: Professional end-use of products containing BBP			
Values taken from scenario 2A2			
Reasonable worst case	<< 0.6	>> 105	(ii)
Values taken from scenario 2C1			
Typical value	0.3	209	(ii)
Reasonable worst case	0.3	209	(ii)

Table 4.35 MOSs calculated for the combined route for the endpoint repeated dose toxicity. NOAEL is 218 mg/m³ or 62.8 mg/kg bw/day (Monsanto, 1982)

Scenario	Internal combined exposure (mg/kg bw/day)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	0.44	143	(ii)
Scenario 2: Industrial use of BBP-Containing products (2C1)			
Typical value	0.36	174	(ii)
Reasonable worst case	0.73	86	(ii)
Scenario 3: Professional end-use of semi- and end-products containing BBP			
Typical value	< 0.36	> 174	(ii)
Reasonable worst case	< 0.73	> 86	(ii)

The MOS-values varies from 73 to > 545 and a **Conclusion (ii)** is drawn for all scenarios and routes for the endpoint repeated dose toxicity.

Mutagenicity

The almost uniformly negative mutagenic effects of BBP in several test systems, indicates that this effect is of no concern for workers.

Conclusion (ii).

Carcinogenicity

There have been some indications of a carcinogenic effect of BBP in rats but not in mice; however no genotoxic effects were evident. BBP is considered to be non-genotoxic and non-carcinogenic substance.The substance is considered to be of no concern for the anticipated occupational exposure levels.

Conclusion (ii).

Reproductive toxicity

Fertility

Regarding toxicity to reproduction, fertility as well as developmental studies is available. When taking the available data base into account a NOAEL at 100 mg/kg bw/day for effects on the reproductive organs/fertility from a 2-generation study in rats is used in the risk assessment (Nagao et al., 2000). The NOAEL is based on atrophy of the testis, epididymis, and seminal vesicle, and reduced reproductive organ weights at 10 or 18 weeks of age in the F1 generation at 500 mg/kg bw/day. This level may be of concern for workers and MOS values for different exposure scenarios have been calculated for the inhalatory route (see **Table 4.36**), the dermal route (see **Table 4.37**) and for the combined route (see **Table 4.38**).

Table 4.36 MOSs calculated for the inhalatory route for the endpoint fertility toxicity. NOAEL is 100 mg/kg bw/day (Nagao et al., 2000)

Occupational scenario	Internal exposure (mg/kg bw/day)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	0.14	714	(ii)
Scenario 2: Industrial use of BBP-containing products (2C1)			
Typical value	0.06	1,667	(ii)
Reasonable worst case	0.43	233	(ii)
Scenario 3: Professional end-use of products containing BBP			
Typical value	< 0.06	> 1,667	(ii)
Reasonable worst case	< 0.43	> 233	(ii)

Table 4.37 MOSs calculated for the dermal route for the endpoint fertility toxicity. NOAEL is 100 mg/kg bw/day (Nagao et al., 2000)

Scenario	Internal exposure (mg/kg bw/day)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	0.3	333	(ii)
Scenario 2: Industrial use of BBP-containing products			
Scenario 2A2: Processing of PVC floats			
Reasonable worst case	< 0.6	> 167	(ii)
Scenario 2C1: Flooring with the calendering process			
Typical value	0.3	333	(ii)
Reasonable worst case	0.3	333	(ii)
Scenario 3: Professional end-use of products containing BBP			
Values taken from scenario 2A2			
Reasonable worst case	<< 0.6	>> 167	(ii)
Values taken from scenario 2C1			
Typical value	0.3	333	(ii)
Reasonable worst case	0.3	333	(ii)

Table 4.38 Calculated MOS values for the combined route for the endpoint fertility toxicity, NOAEL is 100 mg/kg bw/day (Nagao et al., 2000)

Scenario	Internal Internal combined exposure (mg/kg bw/day)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	0.44	227	(ii)
Scenario 2: Industrial use of BBP-Containing products (2C1)			
Typical value	0.36	278	(ii)
Reasonable worst case	0.73	137	(ii)
Scenario 3: Professional end-use of semi-and end-products containing BBP			
Typical value	< 0.36	> 278	(ii)
Reasonable worst case	< 0.73	> 137	(ii)

Developmental toxicity

BBP and its major metabolites have been reported to give potential toxic effects on development following exposure almost exclusively in rats. For development a NOAEL of 50 mg/kg bw/day for offspring is used in the risk assessment (Tyl et al., 2004). This NOAEL value is based on a dose-related significant reduction in absolute and adjusted anogenital distance (AGD) in both F1 and F2 offspring from 250 mg/kg bw/day. MOS values for different exposure scenarios have been calculated for the inhalatory route (see **Table 4.39**), the dermal route (see b) and for the combined route (see **Table 4.13**).

Table 4.39 MOSs calculated for inhalative exposure for the endpoint developmental toxicity. NOAEL is 50mg/kg bw/day (Tyl et al., 2004)

Occupational scenario	Internal exposure (mg/kg bw/day)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	0.14	357	(ii)
Scenario 2: Industrial use of BBP-containing products (2C1)			
Typical value	0.06	833	(ii)
Reasonable worst case	0.43	116	(ii)
Scenario 3: Professional end-use of products containing BBP			
Typical value	< 0.06	> 833	(ii)
Reasonable worst case	< 0.43	> 116	(ii)

Table 4.40 MOSs calculated for the dermal route for the endpoint developmental toxicity. NOAEL is 50 mg/kg bw/day (Tyl et al., 2004)

Scenario	Internal exposure (mg/kg bw/day)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	0.3	167	(ii)
Scenario 2: Industrial use of BBP-containing products			
Scenario 2A2: Processing of PVC floats			
Reasonable worst case	< 0.6	> 83	(ii)
Scenario 2C1: Flooring with the calendering process			
Typical value	0.3	167	(ii)
Reasonable worst case	0.3	167	(ii)
Scenario 3: Professional end-use of products containing BBP			
Values taken from scenario 2A2			
Reasonable worst case	<< 0.6	>> 83	(ii)
Values taken from scenario 2C1			
Typical value	0.3	167	(ii)
Reasonable worst case	0.3	167	(ii)

Table 4.41 Calculated MOS values for the combined route for the endpoint reproductive toxicity (developmental), The NOAEL is 50 mg/kg bodyweight/day (Tyl et al., 2004)

Scenario	Internal combined exposure (mg/kg bw/day)	MOS	Conclusion
Scenario 1: Production of BBP			
Reasonable worst case	0.44	113	(ii)
Scenario 2: Industrial use of BBP-Containing products (2C1)			
Typical value	0.36	139	(ii)
Reasonable worst case	0.73	68	(ii)
Scenario 3: Professional end-use of semi-and end-products containing BBP			
Typical value	< 0.36	> 139	(ii)
Reasonable worst case	< 0.73	> 68	(ii)

The lowest MOS values are calculated for the endpoint reproductive toxicity (developmental). Given the estimated exposure levels for the combined route (see **Table 4.32**), the MOS-values for the different scenarios for this endpoint vary between 68 and 139 (see **Table 4.13**).

The main routes of exposure for workers are expected to be by inhalation or dermal contact. Our knowledge of the toxicokinetics of BBP, especially following these routes, is limited. Also, the assessments of the hazardous properties of BBP are based on animal data, as no significant studies in humans are available. Most likely there are interspecies differences in the sensitivity for BBP, but data on this are also lacking.

The lack of measured exposure data and uncertainty in the descriptions of the available measured data, adds a significant uncertainty to the assessment of the risk related to the exposure of BBP. In the risk assessment of BBP, therefore, worst case exposure scenarios have been used, both for inhalatory and dermal exposure. There is a lack of data on the toxicokinetics of BBP and the absorption of BBP due to inhalation or dermal contact. Therefore, in calculating the internal exposure a 100% bioavailability (uptake) is used as a default value for the inhalatory route, while dermal absorption is considered to be 5% as a worst case estimate. The calculated internal exposures to BBP for the different scenarios are probably overestimated.

Conclusion

Based on an evaluation of available toxicological and exposure data it is concluded that there is no concern for BBP with respect to any of the considered endpoints or scenarios. However it should be noted that BBP in the future might replace other phtalates for industrial uses, and in such cases risk assessments for the new working scenarios should be added to this risk assessment report.

Conclusion (ii) for all scenarios and endpoints.

4.1.3.3 Consumers

Consumers may be exposed to BBP by intake of food that have been wrapped in foodpackaging containing BBP and/or infant formula, from indoor air due to the use of BBP in both PVC and non-PVC polymetric material found in the home, and by the use of baby equipment and children toys. These scenarios of consumer exposure to BBP are described in Section 4.1.1.3. The internal consumer exposure to BBP for the various scenarios is described in **Table 4.42**.

Table 4.42 Internal exposure to BBP for consumers for the various scenarios described in Section 4.1.1.3

Exposure scenario	Adults		Children	
	Inhalation (mg/kg bw/day)	Oral (mg/kg bw/day)	Inhalation (mg/kg bw/day)	Oral (mg/kg bw/day)
Intake of BBP from food and foodpackaging[a]		0.0003		0.00083
Intake of BBP from infant formula and food and foodpackaging [b]				0.00102
Intake of BBP from indoor air[c]	0.000083		0.000083	
Intake of BBP from baby equipment and children toys[d]				0.00095

a) The estimated average intake of BBP based on total diet study was 0.008 mg/person/day, and the high level estimate was 0.02 mg/person/day (MAFF, 1996a). For the risk assessment the MAFF (1996a) estimate of 0.02 mg/person/day, or 0.0003 mg/kg bw/day if the weight is 70 kg is used as a worst case approach. Children are estimated to eat 3 times less than adults and weight 8 kg.

b) The estimated average intake of BBP from infant formula at birth is 0.000187 mg/kg bw/day (MAFF, 1998). The estimated intake of BBP from infant formula and via food for infants is 0.00102 mg/kg/day.

c) Estimates of BBP in indoor air have been performed from 125 homes in Riverside California and is used for risk assessment (California Environmental Protection Agency, 1992). The maximum exposure estimate from this study was 0.000083 mg/kg/day.

d) Risk assessment is only considered for oral exposure of BBP from baby equipment and children toys. A worst case scenario of 0.00095 mg/kg/day is used because additional exposure to BBP may occur by dermal contact, and because more than one phthalate may occur in baby equipment and children toys.

4.1.3.3.1 MOS values for concerned endpoints

The MOS values for the concerned endpoints are calculated by dividing the NOAEL/LOAEL values for the respective critical effects with the internal exposure estimates for the various scenarios described in **Table 4.42**.

4.1.3.3.2 Acute toxicity

Acute toxicity of BBP in animals is low. The oral LD_{50} values of BBP ranged from 2,330-20,400 mg/kg bw for rats and 4,170-6,160 mg/kg bw for mice. Dermal exposure of rabbits gave a LD_{50} value > 10,000 mg/kg bw, whereas in rats the LD_{50} value is reported to be 6,700 mg/kg bw. Compared with the anticipated consumer exposure levels it is concluded that BBP is of no concern for consumers with respect to acute effects of BBP. There is at present no need for further information or testing or for risk reduction measures beyond those which are being applied already.

Conclusion (ii).

4.1.3.3.3 Irritation/corrosivity

In humans BBP was found to have no skin irritating effects. No human experience indicating eye irritation due to BBP exposure was located. It is concluded that BBP is of no concern for consumers with regard to irritation/corrosivity. There is at present no need for further information or testing or for risk reduction measures beyond those which are being applied already.

Conclusion (ii).

4.1.3.3.4 Sensitisation

No skin sensitisation was reported in two human studies with BBP. It is concluded that skin sensitisation is of no concern for consumers. Regarding respiratory sensitisation a case-control study is available. In this study an association was found between children exposed to BBP in house dust and cases of allergic symptoms, however, due to limitations in the study design, and since the children were exposed to other phthalates (DBP, DEHP etc.) in house dust, no clear conclusions can be drawn from this study. There is at present no need for further information or testing or for risk reduction measures beyond those which are being applied already.

Conclusion (ii).

4.1.3.3.5 Repeated dose toxicity

Consumer exposure by intake of food contaminated from foodpackaging and/or infant formula

In the risk assessment the MAFF (1996a) estimate of 0.02 mg/person/day, or 0.0003 mg/kg bw/day if the weight is 70 kg is used as a worst-case approach.

The NOAEL for repeated dose toxicity is 151 mg/kg/day (Hammond et al., 1987). This gives a MOS value for repeated dose toxicity at 503,000, and no concern for consumer exposure from food contaminated from foodpackaging is expected.

Conclusion (ii).

The estimated average intake of BBP from infant formula at birth is 0.000187 mg/kg bw/day (MAFF, 1998). The estimated intake of BBP from infant formula and via food for infants is 0.00102 mg/kg/day. The NOAEL for repeated dose toxicity is 151 mg/kg/day (Hammond et al., 1987). The MOS values from these two exposure scenarios (infant formula and infant formula plus food) are 800,000 and 148,000. No concerns for consumer exposure (infants) from these two scenarios are expected.

Conclusion (ii).

Consumer exposure from indoor air

The maximum exposure estimate from 125 homes in Riverside California was 0.000083 mg/kg/day (California Environmental Protection Agency, 1992). The NOAEL for repeated dose toxicity is 218 mg/m^3 (Monsanto, 1982). The following factors are used in converting the inhalatory exposure dose in rats to an estimated oral route dose in rats (from mg/m^3 to mg/kg bw rats/day): bodyweight rat 300 g, an inhalatory volume of 14.4 l/hour of rats and exposure-duration of 24 hours/day. This gives an estimated NOAEL of 251.14 mg/kg bw/day, which is used in calculating the MOS values. The MOS value from this maximum exposure scenario is 3,000,800. No concern for consumer exposure from indoor air exposure to BBP is expected.

Conclusion (ii).

Consumer exposure from baby equipment and children toys

Children may be exposed to BBP from baby equipment and children toys in different ways, however, for small children the oral exposure is probably the most important route as they suck and chew these products. A worst case scenario of 0.00095 mg/kg/day is used. The NOAEL for repeated dose toxicity is 151 mg/kg/day (Hammond et al., 1987). The MOS value from this scenario is 160,000. No concern for exposure to BBP from the use of baby equipment or toys is expected.

Conclusion (ii).

The conclusion for consumers related to toys and childcare articles reflects the exposure situation at the time of data collection for the RAR. BBP is not intentionally used in toys and childcare articles in EU but may be present as impurities in trace amounts. The possible situation that BBP might be used as a substitute for other phthalates in toys and childcare articles has not been taken into account.

4.1.3.3.6 Mutagenicity

The almost uniformal negative mutagenic effects of BBP in several test systems, indicates that this effect is of no concern for consumers. There is at present no need for further information or testing or for risk reduction measures beyond those which are being applied already.

Conclusion (ii).

4.1.3.3.7 Carcinogenicity

There have been some indications of carcinogenic effects of BBP in rats but not in mice, however, no genotoxic effects were evident. Since no mutagenic effects are reported, the carcinogenic effect is considered to be of no concern for consumers. There is at present no need for further information or testing or for risk reduction measures beyond those which are being applied already.

Conclusion (ii).

4.1.3.3.8 Reproductive toxicity

Consumer exposure by the intake of food contaminated from foodpackaging and/or infant formula

In the risk assessment the MAFF (1996a) estimate of 0.02 mg/person/day, or 0.0003 mg/kg bw/day if the weight is 70 kg is be used as a worst case approach.

The NOAEL for fertility is 100 mg/kg bw/day (Nagao et al., 2000), and the NOAEL for developmental toxicity in offspring is 50 mg/kg bw/day in rats (Tyl et al., 2004). This gives MOS values for fertility and developmental toxicity at 330.000 and 167,000 and no concern for consumer exposure from food contaminated from foodpackaging is expected.

Conclusion (ii).

The estimated average intake of BBP from infant formula at birth is 0.000187 mg/kg bw/day (MAFF, 1998). The estimated intake of BBP from infant formula and via food for infants is 0.00102 mg/kg bw/day. The MOS values from these two exposure scenarios are for fertility 535,000 and 98,000, and for developmental toxicity 260,000 and 49,000, and no concern for consumer exposure from these two exposure scenarios is expected.

Conclusion (ii).

Consumer exposure from indoor air

The maximum exposure estimate from 125 homes in Riverside California was 0.000083 mg/kg bw/day (California Environmental Protection Agency, 1992). The NOAEL for fertility and NOAEL for developmental toxicity are 100 mg/kg bw/day in rats for fertility (Nagao et al., 2000), and 50 mg/kg bw/day in rats for developmental toxicity in offspring (Tyl et al., 2004). The MOS value for fertility is 1,200,000 and for developmental toxicity 600,000. No concern for consumer exposure from indoor air exposure to BBP is expected.

Conclusion (ii).

Consumer exposure from baby equipment and children toys

Children may be exposed to BBP from baby equipment and children toys in different ways, however, for small children the oral exposure is probably the most important route as they suck and chew these products. A worst-case scenario at 0.00095 mg/kg bw/day is used. The NOAEL for fertility and NOAEL for developmental toxicity are 100 mg/kg bw/day in rats for fertility (Nagao et al., 2000), and 50 mg/kg bw/day in rats for developmental toxicity in offspring (Tyl et al., 2004). The MOS value for fertility is 105,000, and for developmental toxicity 53,000. No

concerns for exposure to BBP from the use of baby equipment or children toys are expected. **Conclusion (ii)**.

However it should be noted that possible use of BBP as a replacement of other phthalates in baby equipment and children toys has not been evaluated.

Table 4.43 Summary of the MOS values for the various consumer exposure scenarios

Exposure scenarios	MOS values Repeated dose toxicity	MOS values Fertility	MOS values Development
Food and foodpackaging	503,000	330,000	167,000
Infant formula	790,000	535,000	260,000
Infant formula and food and foodpackaging	148,000	98,000	49,000
Indoor air	3,000,800	1,200,000	600,000
Baby equipment and children toys	160,000	105,000	53,000

4.1.3.3 Humans exposed via the environment

The critical endpoints associated with exposure to BBP are repeated dose toxicity and reproductive toxicity including both fertility and developmental effects in offspring. These effects are therefore considered in the risk characterisation of indirect exposure via the environment, and for combined exposure to BBP.

BBP is distributed in the environment as a consequence of its manufacture, use and disposal. BBP may be released to the environment through waste water and air effluents at the sites where it is produced, processed, formulated and after end use. These indirect exposure scenarios are taken into account in Section 3.

For local BBP exposure assessment production, and processing/formulation are considered and these scenarios are evaluated with the EUSES program. The indirect human exposure values based on EUSES calculations (from **Table 4.16**) and the corresponding MOS values for the critical effects of BPP exposure (repeated dose toxicity, fertility and developmental effects in offspring) are given in **Table 4.44**. The NOAEL for repeated dose toxicity is 151 mg/kg/day (Hammond et al., 1987). The NOAEL for fertility is 100 mg/kg bw/day (Nagao et al., 2000), and the NOAEL for developmental toxicity in offspring is 50 mg/kg bw/day in rats (Tyl et al., 2004), and these values are used in the risk characterisation for indirect exposure via the environment (see **Table 4.44**). For repeated dose toxicity, fertility and developmental effects in offspring the MOS values calculated from indirect human local exposure for scenario IIIa to IIIh-1 (all scenarios) are considered sufficient. There is at present no need for further information or testing or for risk reduction measures beyond those which are being applied already.

Conclusion (ii).

Table 4.44 MOS values for indirect human local exposure to BBP via the environment

Scenario[a]	Human intake mg/kg bw/day[b]	MOS repeated dose toxicity[c]	MOS fertility[d]	MOS development[e]
IIIa large site	0.0189	8,000	5,300	2,600
IIIa small site	0.0295	5,000	3,400	1,700
IIIb-1	0.0007	216,000	143,000	71,000
IIIb-2	0.0002	755,000	500,000	250,000
IIIc	0.0043	35,000	23,000	12,000
IIId	0.0027	56,000	37,000	19,000
IIIe-1	0.0004	378,000	250,000	125,000
IIIe-2	0.0006	252,000	167,000	83,000
IIIf-1	0.0021	72,000	48,000	24,000
IIIf-2	0.0002	755,000	500,000	250,000
IIIg-1	0.0011	137,000	90,000	45,000
IIIg-2	0.0004	378,000	250,000	125,000
IIIh-1	0.0067	23,000	15,000	7,500

a) Refers to the scenarios described in Section 3.1.1
b) Assumed to breath 20 m^3 of air per day, drink 2 L water and weight 70 kg (TGD values)
c) NOAEL value from a 13 week oral study in rats with a NOAEL at 151 mg/kg bw/day (Hammond et al., 1987), see Section 4.1.3.1 for further details.
d) NOAEL value for fertility from a 2-genertaion study in rats with a NOAEL at 100 mg/kg bw/day (Nagao et al., 2000), see Section 4.1.3.1 for further details.
e) NOAEL value for developmental toxicity in offspring in rats from a 2-generation study with a NOAEL at 50 mg/kg bw/day (Tyl et al., 2004), see Section 4.1.3.1 for further details.

For regional BBP exposure assessment production, processing/formulation, and distribution are considered. The $PEC_{regional}$ of BBP in air, water, sediment and soil was based on the TGD and evaluated with the model SIMPLEBOX included in the EUSES program (see Section 3, Section 3.1.4, **Table 3.5**). The indirectly regional exposure in mg/kg bw/day and the corresponding MOS values for repeated dose toxicity, fertility and developmental effects in offspring are presented in **Table 4.45** and range from 385,000 (development) to 1,161,000 (repeated dose toxicity). Accordingly, the MOS values for repeated dose toxicity, fertility and developmental effects in offspring calculated from $PEC_{regional}$ and indirect exposure based on EUSES calculations are considered sufficient, indicating no concern for exposure to BBP from the regional scenario.

Conclusion (ii).

Human exposure to BBP can be calculated from urinary excretion of the BBP metabolite monobenzyl phthalate (MBeP). In these studies the total exposure to BBP, from all sources, and via all exposure routs were measured. The calculation of the exposure level form urinary excretion of MBeP is based on the study by Anderson et al. (2000). However, in this study only 7 volunteers were used per group which limits the value of this study, and leads to an uncertainty in the calculations of the exposure to the phthalaes based on measured urinary monoester metabolites. The level of MBeP measured in the urine was shown to be higher in children compared to adults. Based on the analysis of BBP metabolites in urine of adults and children (1-2 years and 6-11 years) in USA and EU (see Section 4.1.1.4) the daily intake of BBP has been calculated for these 3 groups. For adults the calculated level (95^{th} percentile) was

$3.5 \cdot 10^{-3}$ mg/kg bw/day (Blount et al., 2000a), for children 6-11 years (95th percentile) $5.46 \cdot 10^{-3}$ mg/kg bw/day (CDC, 2003), and for children 1-2 years 4.9 · 10-3 mg/kg bw/day (geometric mean) and 0.0182 mg/kg bw/day (maximum value from 19 children) (Brock et al., 2002). The corrresponding MOS values are shown in **Table 4.45** below. The MOS values are considered sufficient.

Conclusion (ii).

Table 4.45 MOS values for indirect human regional exposure to BBP via the environment

Scenario[a]	Human intake mg/kg bw/day[b]	MOS Repeated dose toxicity[c]	MOS Fertility[d]	MOS development[e]
Regional as estimated by EUSES	$1.3 \cdot 10^{-4}$	1,161,000	770,000	385,000
Calculated from metabolites in urine for adults	3.5 · 10-3	43,000	28,500	14,250
Calculated from metabolites in urine for children 6-11 years	5.46 · 10-3	28,000	18,300	9,150
Calculated from metabolites in urine for children 1-2 years	4.6 · 10-3 (geometric mean) 0.0182 (maximum value)	33,000 (geometric mean) 8,300 (maximum value)	22,000 (geometric mean) 5,500 (maximum value)	11,000 (geometric mean) 2,750 (maximum value)

a) Referes to scenarios described in Section 4.1.1.4.
b) Assumed to breath 20 cm^3 of air per day, drink 2 L water per day, and weight 70 kg (TGD values)
c) NOAEL value from a 13 week oral study in rats with a NOAEL at 151 mg/kg bw/day (Hammond et al., 1987) see Section 4.1.3.1 for further details.
d) NOAEL value for fertility from a 2-generation study in rats with a NOAEL at 100 mg/kg bw/day (Nagao et al., 2000), see Section 4.1.3.1 for further details.
e) NOAEL value for developmental toxicity in offspring in rats from a 2-generation study with a NOAEL at 50 mg/kg bwday (Tyl et al., 2004), see Section 4.1.3.1 for further details.

Table 4.46 Summary of the conclusions for indirect exposure via the environment, local, regional and calculated from metabolites measured in urine

Critical endpoints	Local, (all scenarios)	Regional estimated by EUSES	Calculated from metabolites in urine for adults, children 6-11 years and children 1-2 years
Repeated dose toxicity	(ii)	(ii)	(ii)
Fertility	(ii)	(ii)	(ii)
Development	(ii)	(ii)	(ii)

4.1.3.3 Combined exposure

Due to the use of BBP in flexible PVC-products, and the diffuse emission of BBP from these products, humans may be exposed to BBP from different sources. The combined exposure to BBP is the sum of all the specific sources (occupational exposure, consumer exposure, and indirect exposure via the environment), and by all routes of exposure (oral, dermal or by

inhalation). However, since occupational exposure values will totally dominate the exposure levels for adults, it is not considered relevant to make a separate calculation for combined exposure for adults including occupational exposure.

Children are potentially exposed via many products and sources, combined exposure values have been calculated. And due to the different BBP exposure scenarios for children and adults, two combined exposures estimated are performed, one for children (0 to 2 years) and one for adults:

I Children exposure to BBP from toys, infant formula, indoor air and indirectly via the environment (air, water and food).

II Adult exposure to BBP as a consumer and indirect via the environment (air, water and food).

The critical effects of BBP considered are repeated dose toxicity and reproductive toxicity including both fertility and developmental effects in offspring. In **Table 4.47** the combined exposure to BBP for children with the corresponding MOS values for repeated dose toxicity, fertility and developmental effects in offspring are presented, and in **Table 4.48**, the combined exposure to BBP for adults (minus occupational exposure).

Table 4.47 MOS values for combined exposure to BBP for children (0-2 years)

Scenarios	Daily intake mg/kg bw/day	MOS Repeated dose toxicity[c]	MOS Fertility[d]	MOS developmental[e]
Food and foodpackaging	0.00083			
Infant formula[a]	0.000187			
Indoor air	$8.3 \cdot 10^{-5}$			
Baby equipment and children toys	0.00095			
Indirectly via the environment (local)[b]	0.0295 (IIIa small site)			
Indirectly via the environment (regional[f]) Calculated from urinary concentrations of BBP metabolites[g]	$1.3 \cdot 10^{-4}$[f] 0.0182[g]			
Combined local[b]	0.03155 (IIIa small site)	4,800 (IIIa small site)	3,200 (IIIa small site)	1,600 (IIIa small site)
Combined regional[f] Calculated from urinary concentrations of BBP metabolites[g]	0.0022[f] 0.0182[g]	69,000[f] 8,300[g]	45,500[f] 5,500[g]	23,000[f] 2,700[g]

a) Mean value from birth and 6 month
b) Worst case scenarios from local exposure (scenario IIIa small site)
c) NOAEL value from a 13 week oral study in rats with a NOAEL at 151 mg/kg bw/day (Hammond et al., 1987), see Section 4.1.3.1for further details.
d) NOAEL value for fertility from a 2-generation study in rats with a NOAEL at 100 mg/kg bw/day (Nagao et al., 2000), see Section 4.1.3.1 for further details.
e) NOAEL value for developmental toxicity in offspring in rats from a 2-generation study with a NOAEL at 50 mg/kg bw/day (Tyl et al., 2004), see Section 4.1.3.1 for further details.
f) Regional exposure as estimated by EUSES.
g) Calculation from urianry metabolites of children (1-2 years) by Brock et al. (2002).

The MOS values for repeated dose toxicity, fertility and developmental effects in offspring for combined local exposure (scenario IIIa small site) for children (0-2 years), are considered sufficient, indicating no concern for local exposure to BBP.

Conclusion (ii).

The MOS values for repeated dose toxicity, fertility and developmental effects in offspring for combined regional exposure to BBP as measured by EUSES, and as calculated from urinary excretion of MBeP (Brock et al., 2002; Anderson et al., 2000) for children (0-2 years) are considered sufficient, indicating no concern for exposure to BBP from regional exposure.

Conclusion (ii).

Table 4.48 MOS values for combined exposure to BBP for adults

Scenario	Daily intake mg/kg bw/day	MOS Repeated dose toxicity[b]	MOS Fertility[c]	MOS development[d]
Food and foodpackaging	$3 \cdot 10^{-4}$			
Indoor air	$3.2 \cdot 10^{-5}$			
Indirectly via the environment (local)[a]	0.0295 (IIIa small site) [a]			
Indirectly via the environment (regional[e]) Calculated from urinary excretion of BBP metabolites[f]	$1.3 \cdot 10^{-4}$[e] $3.5 \cdot 10^{-3}$[f]			
Combined local[a]	0,03[a] (IIIa small site)	5000[a] (IIIa small site)	3300[a] (small site)	1700[a] IIIa small site)
Combined regional[e] Calculated from urinary excretion of BBP metabolites[f]	$4.6 \cdot 10^{-4}$[e] 0.0035[f]	328,000[e] 43,000[f]	217,000[e] 28,000[f]	109,000[g] 14,000[f]

a) Worst case scenarios from local exposure (scenario IIIa small site)
b) NOAEL value from a 13 week oral study in rats with a NOAEL at 151 mg/kg bw/day (Hammond et al., 1987), see Section 4.1.3.1 for further details.
c) NOAEL value for fertility from a 2-generation study in rats with a NOAEL at 100 mg/kg bw/day (Nagao et al., 2000), see Section 4.1.3.1 for further details.
d) NOAEL value for developmental toxicity in offspring in rats form a 2-generation study with a NOAEL at 50 mg/kg bw/day (Tyl et al., 2004), see Section 4.1.3.1 for further details.
e) Regional exposure as estimated by EUSES.
f) Calculation from urinary metabolites of non-occupationally exposed adulsts by Blount et al. (2000a).

Table 4.49 Summary of the conclusions for combined exposure to BBP for children

Critical endpoints	Combined local (scenario IIIa small site)	Combined regional estimated by EUSES and Brock et al. (2002)
Repeated dose toxicity	(ii)	(ii)
Fertility	(ii)	(ii)
Development	(ii)	(ii)

Table 4.50 Summary of the conclusions for combined exposure to BBP for adults

Critical endpoints	Combined local (scenario IIIa small site)	Combined regional estimated by EUSES and Blount et al. (2001)
Repeated dose toxicity	(ii)	(ii)
Fertility	(ii)	(ii)
Development	(ii)	(ii)

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

Flammability, explosive properties and oxidising properties are not considered to form a hazard. There is no need for further information and/or testing with regard to physicochemical properties.

Conclusion (ii).

5 RESULTS

5.1 ENVIRONMENT

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 is reached for the following life cycle steps/environmental compartments:

- For the use categories IIIa (flooring large and small site) and IIIh (non polymer use "confidential") at life cycle step III (processing and formulation) for surface water (including sediment)
- For the use categories IIIa (flooring large and small site), IIIc (PVC coated textiles) and IIIh (non polymer use "confidential) at life cycle step III (processing and formulation) for the terrestrial compartment

The exposure assessment for flooring (IIIa) and PVC coated textiles (IIIc) are based on the ESD "Plastics" (OECD, 2004). The recently updated ESD has passed the OECD process and is based on best available information. Further site specific data have not been obtained. The exposure scenario IIIh is based on information from Industry. The PEC/PNEC ratios for the aquatic (including sediment) and the terrestrial compartment are above 1, thus a risk to the aquatic and terrestrial environment has to be expected.

Flooring sites were split into large sites with air treatment facilities in place and small sites without air treatment (in accordance with the ESD on Plastics Additives from 2004). Industry stressed that the estimation of plant size on the basis of BBP consumption may be misleading because BBP is usually not used alone but in a mixture with other plasticisers. Hence, small sites with respect to BBP are not necessarily small sites in terms of plasticiser use and industry has confirmed that the sites are actually not small sites in terms of plasticiser use. However, information from industry has also shown that there are actually sites without air treatment and hence the worst case ESD-scenario for small sites, which do not have air treatment in place, was not omitted even though the sites may not be small sites in terms of the definition of the ESD with respect to total plasticiser use.

According to industry emissions to waste water are an overestimation, both for the large sites and for the small site scenario, but as no site specific emission data have become available emission factors are takes from the ESD.

Conclusion (iii) is based on BBP consumption data from 2004. For 2005 there are only two producers left and industry provided estimations of the expected use volume of BBP for all use categories. These figures are confidential as there are only two producers left.

The total BBP volume used for flooring in 2005 has been further reduced, but the scenarios used in this risk assessment are still relevant. In 2005 it is still valid to use the ESD emission factors for a small site since sites without air treatment have been identified.

Applying the expected use volumes for 2005 to "PVC coated textiles" (IIIc) no risk to soil is to be expected.

Conclusion (i) There is a need for further information and/or testing.

A long-term fish study on reproductive and endocrine effects has to be performed.

Conclusion (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.

This conclusion is reached for the following life cycle steps/environmental compartments

- Production and distribution (Life cycle I and II) for all environmental compartments
- For the use categories IIIb, IIIc, IIId, IIIe IIIf and IIIg at life cycle step III (processing and formulation) for surface water (including sediment)
- For the use categories IIIb, IIId, IIIe IIIf and IIIg at life cycle step III (processing and formulation) for the terrestrial compartment
- For use and disposal (Life cycle IV and V) for all environmental compartments
- For the atmosphere (all life cycle steps)
- For STP at all production, formulation and processing sites
- For secondary poisoning (all life cycle steps)

Conclusions (ii) for surface water (including sediment) and the terrestrial compartment have to be seen as provisional until possible endocrine effects in fish have been resolved.

5.2 HUMAN HEALTH

5.2.1 Human Health (toxicity)

5.2.1.3 Workers

Conclusion (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.

5.2.1.3 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

It should be noted that the conclusion for “consumers” related to toys and childcare articles reflects the exposure situation at the time of data collection for this RAR. BBP is not intentionally used in toys and childcare articles in the EU but may be present as impuritiy in trace amounts. The possible situation that BBP might be used as a substitute for other phtalates in toys and childcare articles has not been taken into account.

5.2.1.3 Humans exposed via the environment:

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

It should be noted that recent epidemiological studies have indicated an association between maternal exposures to BBP as well as to other phthalates and the length of the Anogenital distance (AGD) in newborn boys. These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to small sample size in the studies, this issue will have to be further investigated, and new studies in the future should be taken into account in the risk assessment of BBP.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
AGD	Anogenital distance
AGI	Anogenital index
ALAT	Alanin aminotransferase
ASAT	Aspergine aminotransferase
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BBP	Benzyl butyl phthalate
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CAT	Carnitine acetyltransferase
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
DBP	Dibutyl phthalate
dfi	daily food intake
DG	Directorate General

DEP	Diethyl phthalate
DEHP	Di-(20ethylhexyl)phthalate, Bis(2-ethylhexyl) phthalate
DES	Diethylstilbestrol
DIDP	Di"isodecyl" phthalate
DIN	Deutsche Industrie Norm (German norm)
DINP	Di"isononyl" phthalate
DIOP	Diiso octyl phthalate
DNA	DeoxyriboNucleic Acid
DNOP	Di-n-octyl phthalate
DOC	Dissolved Organic Carbon
DOP	Dioctyl phthalate
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
dwt	Dry weight
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECPI	European Council for Plasticisers and Intermediates
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EM	Electron microscopy
EN	European Norm
EPA	Environmental Protection Agency (USA)
ER	Estrogen receptor
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]

F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
FSH	Follicle stimulating hormone
GD	Gestation day
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 tonnes/annum)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LH	Luteinising hormone
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration

MBuP	Monobutyl phthalate
MBeP	Monobenzyl phthalate
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MPE	micronucleated polychromatic erythrocytes
Mt	metallothionein
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidising (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OPP	Octylphenol poluethoxylate
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PAE	Phthalic acid esters
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PCA	Passive cutanous anaphylaxis
PCoA	Palmitoyl Coenzyme A
PE	polychromatic erythrocytes
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
pnd	Postnatal day

PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
PR	Progesterone receptor
PRL	Prolactin
PVC	Polyvinylchloride
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst-Case
SCAS	Semi Continuous Activate Sludge
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SCHER	Scientific Committee on Health and Environmental Risks
SHBG	Sex-hormone binding globuline
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations

UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organisation
wwt	Wet weight
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Appendix I Leaching of monoesters from Landfill

Primary degradation metabolites of BBP (monoesters) were detected in a landfill simulation study by Ejlertsson (1997) and in a long-term landfill study by Mersiowsky et al (1999). Moreover, concern related to endocrine disruption properties (see human health section) is also attached to BBP's primary degradation metabolites monobutyl phthalate and monobenzyl phthalate.

In the landfill simulation study by Ejlertsson (1997) BBP and its monoesters were monitored. Monoesters were in the ≤ 1-10 mg/l range during the first year, while this was reduced to 20-500 µg/l during the second and third year. High concentrations of monoesters relative to BBP indicate rapid primary degradation of BBP even under landfill situations (both aerobic and anaerobic conditions). Analysis of pieces of a two layered PVC carpet after two years in a soil lysimeter showed that 20% of the BBP in the outer layer was lost, whereas no loss could be detected from the second layer.

Because most of the loss of BBP from PVC in the landfill is degraded to its monoesters under anoxic conditions and the environmental concern regarding risks related to monoesters are comparable with those of BBP, the monoester release should also be estimated. In the study by Mersiowsky et al (1999) monoesters were also monitored in the landfills. Maximum concentration measured was found to be 156 µg/l (sum of monobutyl phthalate and monobenzyl phthalate). Using 156 µg/l as realistic worst case and the same calculation procedure as above results in a monoester release from landfills to WWTP of 40.8 tonnes/year. Seepage from landfill may either be directed to a STP (standard for new landfills) or is released directly to surface water, which may occur for older landfills. Assuming standard TGD defaults for dilution and 91% degradation in STP gives a local PECaquatic of 1.4 µg/l when seepage is processed by STP and 15.6 µg/l when seepage is bypassed a STP but diluted by a factor 10 in receiving waters.

Acute test on invertebrates of metabolites of BBP

An acute test with 3 metabolites of BBP on Daphnia magna was performed according to US. EPA (1975) guidelines (Monsanto 88-9253). The test was performed according to GLP. The metabolites tested were phthalic acid, monobutyl phthalate and monobenzyl phthalate. Nominal concentrations were not verified analytically and water hardness was 60 mg/l. 50% immobilisation was not achieved for any of the compounds. The highest tested concentrations were 640, 320 and 160 mg/l. The NOECs were > 640, 160 and 40 mg/l.

Tentative PNEC derivation for the aquatic environment

As fairly large amounts of monoesters were found in leachates from landfills, a tentative PNEC for these is derived in order to assess if these releases may pose a risk. There is only one test available for each metabolite (Monsanto 88-9253). None of the tests was performed at concentrations giving 50% immobilisation. However some immobilisation was observed for the monobutyl and monobenzylphthalate at the highest tested concentration. Lacking an EC_{50} value the lowest concentration giving significant immobility (80 mg/l) is used. Applying an assessment factor of 1,000 gives a PNECaquatic of 80 µg/l.

PEC/PNEC evaluation

PECsurfacewater was estimated to be 1.4 µg/l for treated seepage and 15.6 µg/l for untreated seepage. With a PNECsurface water of 80 µg/l this results in a PEC/PNEC ratio of 0.0175 and 0.195, both below 1.

Although the PEC/PNEC evaluation for the aquatic environment indicates no risk, data are still missing for chronic effects like those related to endochrine disruption properties, which means that no conclusion can be drawn.

Appendix II Confidential use

European Commission

EUR 22773 EN **European Union Risk Assessment Report**
benzyl butyl phthalate (BBP), **Volume 76**

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The report provides the comprehensive risk assessment of the substance Benzyl Butyl Phthalate. It has been prepared by Norway in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

Part I - Environment

This part of the evaluation considers the emissions and the resulting exposure to the environment in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.

The environmental risk assessment concludes that there is concern for surface water (including sediment) and the terrestrial compartment from some use categories and there is a need for further information and for testing (long-term fish study on reproductive and endocrine effects). There is no concern for the atmosphere and sewage treatment plants.

Part II – Human Health

This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment concludes that there is no concern for workers, consumers humans exposed via the environment and for human health (physico-chemical properties).

The conclusions of this report will lead to risk reduction measures to be proposed by the Commission’s committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.

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European Union Risk Assessment Report

benzyl butyl phthalate (BBP)

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