Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to

Di-isononylphthalate (DINP) for use in food contact materials

Question N° EFSA-Q-2003-194

Adopted on 30 July 2005

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) has been asked to re-evaluate di-isononylphthalate for use in the manufacture of food contact materials.

Di-isononylphthalate (DINP) is a mixture of esters of o-phthalic acid with C8-C10 (C9 rich) alkyl alcohols. These alcohols can be obtained by different processes, yielding different ratios of chain length and branching distribution, which result in different DINP types. Presently, there are 2 different DINP types being used (CAS 68515-48-0 and CAS 28553-12-0). These DINP mixtures have in common many of their constituents, and differ by isomeric distribution curves. Therefore, in this document they are considered together.

Previously, a group Tolerable Daily Intake (g-TDI) of 0.15 mg/kg bw (with di-isodecylphthalate – (DIDP)) was set by the Scientific Committee for Food (SCF), based on the endpoint of peroxisome proliferation in rodent liver. There is now a scientific consensus that liver peroxisome proliferation in rodents is not relevant for human risk assessment. The usual critical effects of phthalates relate to liver, testicular and reproduction toxicities. From the several studies available on DINP, the critical observations were as follows.

No overt toxicity was observed on reproductive organs in rats. No observed adverse effect levels (NOAEL) of 500 mg/kg bw/day and 622 mg/kg bw/day were established for minor developmental effects and decreases in live birth and survival indices, respectively. Maternal toxicity was limited to lower mean body weight and hepatic changes with a lowest observed adverse effect level (LOAEL) of 114 mg/kg bw/day.

The pivotal toxicological effect for DINP is considered to be the hepatic changes seen in various studies. In a two-year chronic toxicity study in rats, there was an increased incidence of spongiosis hepatis, accompanied by increased serum levels of liver enzymes and increases in absolute and relative liver and kidney weights in both sexes. The Panel agreed to use the NOAEL of 15 mg/kg bw/day for non-peroxisomal proliferation-related chronic hepatic and renal effects in establishing a TDI. Making use of this NOAEL and of an uncertainty factor of 100, a TDI of 0.15 mg/kg bw is derived.

The limited available data on DINP concentration in foods and diets in UK (1996, 1998) and Denmark (2003) were used to provide an estimation of the dietary exposure. In the UK, potential exposure to DINP from dietary sources was based on the method detection limit and estimated to be less than 0.17 µg/kg bw/day. For newborns (0-6 months) and for infants (>6 months), the potential exposure to DINP

derived from infant formulae consumption corresponded to 2.4 µg/kg bw/day and 1.8 µg/kg bw/day, respectively.

In Denmark, the total oral exposure was estimated to be 5 µg/kg bw/day for adults. Higher values for total oral exposure (216, 63 and 10 µg/kg bw/day) were reported for infants (6-12 months), children (1-6 years) and children (7-14 years), respectively. However, the two highest values for young children derived mainly from the contribution of the estimated oral exposure related to toys. DINP use in toys is provisionally banned in EU since 1999. Furthermore; the computer modeling program (EUSES) which was used for these intake estimates is a conservative one and the obtained values are not representative of the possible exposure via food contact materials. However, the value of 10 µg/kg bw/day from this study has been taken as a worst case estimate of dietary exposure to DINP.

The Panel noted that the above estimated exposure via the diet of around 10 µg/kg bw/day is well below the TDI. However, there are some indications that DINP levels in food may be increasing in recent years, and so, more updated estimations of exposure from the diet are desirable.

The Panel also noted that DINP and DIDP (phthalic acid, diester with primary saturated C9-C11 branched alcohols, C10 rich, CAS n° 26761-40-0 and 68515-49-1, REF No 75105) are mixtures that overlap chemically with each other and cannot analytically be distinguished clearly if present in a mixture. For this reason, it is proposed that for DINP and DIDP a group restriction is established for migration from food contact materials.

KEY WORDS

1,2-Benzenedicarboxilic acid, di-C8-10-branched alkyl esters, C9 rich (CAS n° 68515-48-0); Di-isononylphthalate, DINP, (CAS n°28553-12-0), REF No. 75100, food contact materials

BACKGROUND

DINP may be present in food, either due to migration from food contact materials containing DINP or due to its widespread presence as an environmental contaminant which can be found in air, water, soil and food. DINP was evaluated by the Scientific Committee for Food (SCF) in 1999 when a group TDI (with DIDP) for use in food contact materials was established based on the then most sensitive end-point of peroxisome proliferation in rodent liver (SCF, 1999). There is a scientific consensus that liver peroxisome proliferation in rodents is not a relevant endpoint for human risk assessment (IARC, 1995). The Panel has therefore been asked to re-evaluate DINP for use in food contact materials.

TERMS OF REFERENCE

The Commission asks EFSA to re-evaluate di-isononylphthalate (DINP) for use in the manufacture of food contact materials.

ASSESSMENT

1. Chemistry

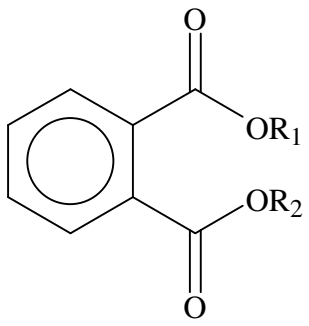
Identification of the substance

Di-isononylphthalate (DINP) is a mixture of esters of o-phthalic acid with C8-C10 (C9 rich) alkyl alcohols. These alcohols can be obtained by different processes, yielding different ratios of chain length and branching distribution, which result into different DINP types. Presently, there are 2 different DINP types being used (CAS 68515-48-0 and CAS 28553-12-0). These DINP mixtures have in common many of their constituents, and differ by isomeric distribution curves.

CAS Nr	68515-48-0 (DINP 1)	28553-12-0 (DINP 2)
EINECS Nr	271-090-9	249-079-5
Substance name (IUPAC)	1,2-Benzenedicarboxylic acid, di-C8-10 branched alkylesters, C9rich	1,2-Benzenedicarboxylic acid, di-C8-10 branched alkylesters, C9rich (Di-isononyl phthalate)
Molecular formula	C8+2xH6+4xO4with x = 8 to 10 (x = 9 as main constituent), average C26H42O4	
Molecular weight	Average 420.6	

FCM Ref N° 75100

Structural formula:



where R1 and R2 = C8-C10, C9 rich, linear and branched

The correct structures can only be estimated. Using data on the repartition of alcohols used for the manufacture of the DINPs, European Council for Plasticisers and Intermediates has made an estimation of the different chain structures that may be present in DINPs (ECPI, 1997).

	DINP 1 (%)	**DINP 2 (%)**
Methyl ethyl hexanols	5 - 10	5 - 10
Dimethyl heptanols	45 - 55	40 - 45
Methyl octanols	5 - 20	35 - 40
n-Nonanol	0 - 1	0 - 10
Isodecanol	15 - 25	-

Purity/impurities, additives

Phthalates are produced with a high degree of purity (> 99.5%), in terms of ester content. Trace impurities have been summarised from producers' data.

i-nonanol	ca. 0.04%
isononylbenzoate	ca. 0.03%
n-butylisononyl phthalate	ca. 0.1%
Water	0.02 - 0.03%

Physico-chemical properties

Property	Value
Melting point	ca. -50°C
Boiling point	> 400°C
Density	ca. 0.975 at 20°C
Vapour pressure	6.10-5 Pa at 20°C
Water solubility	0.6 µg/l at 20°C
Log Kow	8.8

2.Use

Di-isononylphthalate is mainly (approximately 95%) used as a plasticiser in PVC applications. The remaining 5% is used in non-PVC applications (ECPI, 1997). More than half of the DINP used in non-PVC applications involves polymer related-uses (e.g. rubbers). The remaining DINP is used in other applications including inks and pigments, adhesives, sealants, paints and lacquers and lubricants (Legrand, 1996).

3. Exposure via food

No data on the levels of DINP in food in the EU attributable to migration from food contact materials have been submitted by the industry.

In 1996, MAFF's Food Safety Directorate carried out a survey of the levels of DINP and total phthalates in samples of composite fatty foods (MAFF, 1996a). DINP was not detected in the conditions of analysis (limit of detection = 0.01 mg/kg of food). Based on the detection limit, and assuming a food intake per day of 1 kg for an adult of 60 kg, the potential daily exposure to DINP from food would be < 0.17 µg/kg bw/day.
For newborns (0-6 months) and for infants (>6 months), the potential exposure to DINP derived from infant formulae consumption, based on the detection limit, corresponds to 2.4 µg/kg bw/day and 1.8 µg/kg bw/day, respectively (MAFF, 1996b and 1998).

A Danish DINP total oral exposure was reported recently (Müller et al, 2003) and was estimated to be 5 µg/kg bw/day for adults. Higher values for total oral exposure (216, 63 and 10 µg/kg bw/day) were reported for infants (6-12 months), children (1-6 years) and children (7-14 years), respectively. But

these two highest values for young children derive mainly from the contribution due to the estimated oral exposure related to toys that is included in the above values. DINP use in toys is provisionally banned in the EU since 1999 (Commission Decision, 1999 and 2004). Furthermore, the computer modeling program (EUSES) which has been used for these intake estimates is a conservative one and the obtained values are not representative of the possible exposure via food contact materials. However; the value of 10 µg/kg bw/day from this study has been taken as a worst case estimate of dietary exposure to DINP.

There are some indications that DINP levels in food are increasing in recent years, and so, more updated estimations of exposure from the diet are desirable.

4. Toxicological evaluation

Introduction

The Panel did not carry out a new extensive risk assessment but took cognisance of the previous evaluations by the SCF and in particular considered the more recent 2001 DINP Risk Assessment Report (RAR), prepared for the European Union Existing Substances Regulation 793/93, (Annex 1), and the comments of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on this RAR (Annex 2), in order to determine the most significant toxicological end-point for risk assessment. Based on this information, the Panel focused on the most sensitive toxicological end-points for the evaluation of DINP.

The SCF expressed its opinion on DINP in December 1999 (SCF, 1999) based on the following statement in the safety data sheet:

> "For calculating the TDI, the NOAEL (lowest overall NOAEL) of 300 mg/kg (equivalent to 15 mg/kg bw/day) from the 2-year rat study is used.
> Making use of an uncertainty factor of 100 (10 for intraspecies and 10 for interspecies) a TDI of 0.15 mg/kg bw/day is derived.
> DINP and DIDP (phthalic acid, diester with primary saturated C9-C11 branched alcohols, more than 90% C10) being mixtures overlapping chemically each other, it is decided to establish a group-TDI of 0.15 mg/kg bw/day, together with DIDP (CAS n° 26761-40-0, PM/REF. 75105)".

Studies considered by the AFC Panel

The following studies on genotoxicity, carcinogenicity, and liver toxicity, reproduction and development toxicities considered by the Panel for the determination of a NOAEL which could be used as a basis for a TDI calculation are summarized below. Further details on these studies are given in Annex 1 (Risk Assessment Report) and Annex 2 (CSTEE opinion).

> DINP is not mutagenic *in vitro* in bacterial mutation assays or mammalian gene mutation assay (with and without metabolic activation) and is not clastogenic in one cytogenetic assay *in vitro* on CHO cells and in one *in vivo* assay on bone marrow cell of Fisher 344 rats. This suggests that DINP is not genotoxic *in vivo* or *in vitro*.
>
> Following oral exposure, the carcinogenicity of DINP has been investigated in several animal studies. (Exxon Biomedical Sciences (1986), Covance (1998), Aristech Chemical Corporation (1995a), Aristech Chemical Corporation (1995b), Aristech Chemical Corporation (1995c)). The carcinogenic effects in the liver of rodent species are linked to peroxisome proliferation and are no longer considered as relevant to humans.

A NOAEL of 88 mg/kg bw/day has been derived from a chronic / carcinogenicity rat study (Aristech Chemical Corporation, 1994).
At higher dose levels, effects on kidneys and liver were observed. The effects on the latter organ were not related to (rodent specific) peroxisome proliferation and are therefore also relevant for human risk assessment

In relation to reproductive toxicity, a NOAEL of 622 mg/kg bw/day was established for decreases in live birth and survival indices in a one-generation range-finding study in rats. In the two-generation study involving dietary administration at 0.2, 0.4 and 0.8%, parental toxicity was limited to lower mean body weight and hepatic changes with a LOAEL of 114 mg/kg bw/day (Exxon, 1996 a and b). A decrease of mean offspring body weight was observed following parental administration of DINP in the one- and two-generation studies with an estimated LOAEL of 0.2% in diet (159 mg/kg bw/day).

In a developmental study in rats at dose levels up to 1000 mg/kg bw/day, visceral variations (dilated renal pelvis and hydroureter) were observed with a NOAEL of 500 mg/kg bw/day for both maternal toxicity and developmental effects (Exxon, 1994). In another study in rats (BASF, 1995a), skeletal variations (rudimentary cervical and accessory 14th ribs) were observed with a NOAEL of 200 mg/kg bw/day.

In some subacute and subchronic studies with Fischer 344 rats at dose levels of up to 2% in the diet (Biodynamics 1982a, b, c and Hazleton, 1991a), or in a 2-year study with Fischer 344 rats at dose levels of up to 0.6% in the diet (Exxon, 1986) relative testis weights were statistically significantly increased with or without concurrent increase of absolute testis weights and decrease of body weights at high doses.

In mice, a NOAEL of 1,500 mg/kg (276 mg/kg bw/day) can be derived from a 104-week study (Aristech, 1995c) based on testicular weight decrease observed at 4,000 mg/kg (742 mg/kg bw/day).

In the above two-year chronic toxicity study of Exxon (Exxon, 1986) using Fischer 344 rats, there was a dose-related increase in relative organ weights of liver and kidney in both males and females with a clear NOAEL of 15 and 18 mg/kg bw/day for males and females respectively. In addition to the increased liver and kidney weights at the LOAEL of 152 and 184 mg/kg bw/day for females and males, respectively, the males had increased incidences of spongiosis hepatis and serum levels of alkaline phosphatase and transaminases. Spongiosis hepatis was also seen in 5 males in a two-year chronic toxicity/oncogenicity study with rats. (Covance, 1998). The NOAEL/LOAEL for spongiosis hepatis are the same in the two studies as for the increases in liver and kidney weights.

CONCLUSIONS

Based on all the available toxicological evidence, the Panel concluded that effects on liver, reproduction and development are the end-points on which to base the risk assessment. Previous reviews on phthalates have identified as pivotal several rat reproduction studies conducted in the last decade, which gave NOAELs or LOAELs in the region of 15-150 mg/kg bw/day.

No overt toxicity was observed on reproductive organs in rats. NOAELs of 500 mg/kg bw/day and 622 mg/kg bw/day were established for minor developmental effects and decreases in live birth and survival indices, respectively. Maternal toxicity was limited to lower mean body weight and hepatic changes with a LOAEL of 114 mg/kg bw/day.

The pivotal toxicological effect for DINP is considered to be the hepatic changes seen in various studies. In the two-year chronic toxicity study in rats of Exxon (Exxon, 1986), there was an increased incidence of spongiosis hepatis, accompanied by increased serum levels of liver enzymes and increases in absolute and relative liver and kidney weights in both sexes. The Panel agreed to use the NOAEL of 15 mg/kg bw/day for non-peroxisomal proliferation-related chronic hepatic and renal effects in establishing a TDI. Making use of this NOAEL and of an uncertainty factor of 100, a TDI of 0.15 mg/kg bw is derived.

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The Panel noted that the above estimated exposure via the diet of around 10 µg/kg bw/day is well below the TDI. However, there are some indications that DINP levels in food are increasing in recent years, and so, more updated estimations of exposure from the diet are desirable.

The Panel also noted that DINP and DIDP (phthalic acid, diester with primary saturated C9-C11 branched alcohols, C10 rich, CAS n° 26761-40-0 and 68515-49-1, REF No 75105) are mixtures that overlap chemically with each other and cannot analytically be distinguished clearly if present in a mixture. For this reason, it is proposed that for DINP and DIDP a group restriction is established for migration from food contact materials

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ANNEX 1

Extracts from the Risk Assessment Report on DINP
Consolidated Final Report (dated May 2001)

Summary on mutagenicity and carcinogenicity

DINP is not mutagenic *in vitro* in bacterial mutation assays or mammalian gene mutation assay (with and without metabolic activation) and is not clastogenic in one cytogenetic assay *in vitro* on CHO cells and in one *in vivo* assay on bone marrow cell of Fisher 344 rats. This suggests that DINP is not genotoxic *in vivo* or *in vitro*.

The carcinogenicity studies are summarised in the following table

Test	Species	Protocol/doses	Results NOAEL	Test substance	Validity	References
Chronic toxicity/ oncogenicity study Retrospective evaluation of α2u globulin accumulation in male rat kidneys	Fischer 344 rats Male Fischer 344 rats	0-0.03-0.3-0.6% in diet for 2 years	NOAEL = 0.03% (15-18 mg/kg bw/day) From 0.3% (152-184 mg/kg bw/day): ↑MNCL No neoplasic lesion.	CAS 68515-48-0 MRD 83-260	Yes GLP	Exxon Biomedical Sciences (1986) Caldwell et al. (1999a)
Oncongenicity study	Fischer 344 rats	0-500-1,500-6,000-12,000 ppm in diet for 2 years	NOAEL =1 500 ppm (88-103 mg/kg bw/day) 6,000 ppm (359-442 mg/kg bw/day): ↑MNCL (liver and kidney toxicity) 12,000 ppm (733-885 mg/kg bw/day): hepatocellular neoplasia, renal tubule cell carcinomas	CAS 68515-48-0	Yes GLP	Covance (1998); Aristech Chemical Corporation (1995b); Aristech Chemical Corporation (1995a)
Chronic toxicity carcinogenicity feeding study	Sprague Dawley rats	0-500-5,000-10,000 ppm in diet for 2 years	NOAEL = 500 ppm (27-33 mg/kg bw/day) from 5,000 ppm (271-333 mg/kg bw/day) hepatocellular carcinomas (No MNCL)	CAS 71549-78-5 Sancitizer 900	DINP never produced commer-cially	Bio/dynamics (1986)
Oncongenicity study	B6C3F1 mice	0-500-1,500-4,000-8,000 ppm in diet for 2 years	NOAEL = 500 ppm for females (112 mg/kg bw/day) 1,500 ppm (335 mg/kg bw/day) increase of total hepatocellular	CAS 68515-48-0	Yes GLP	Aristech Chemical Corporation (1995c); Covance (1998)

			neoplasms NOAEL = 1,500 ppm (275 mg/kg bw/day) for males 4,000 ppm (742 mg/kg bw/day) increase of both liver adenoma and liver carcinoma)			

Repeated-dose toxicity

The repeated-dose studies are summarised in the following table.

Test	Species	Protocol	NOAEL	LOAEL and Effects observed	References
Oral					
One-week prechronic oral study	rat Fischer 344	CAS 68515-48-0 0-2% in diet		2% (1,700 mg/kg) increased kidney, liver weights, macroscopic liver changes decreased cholesterol, triglycerides at 2%	Bio/dynamics (1982a)
2-week study	rat Fischer 344 females	CAS 68515-48-0 25-75-150-1,500 mg/kg bw/day gavage		25 mg/kg DOS activity (peroxisome proliferation) 1,500 mg/kg liver weight increased	Hüls (1992)
2-week study	rat Fischer 344 females	CAS 28553-12-0 25 - 75 - 150 - 1,500 mg/kg bw/day gavage	25 mg/kg bw/day DOS activity	75 mg/kg bw/day DOS activity peroxisome proliferation 1,500 mg/kg bw/day increased liver weights	Hüls (1992)
2-week or 4-week studies	male Fischer 344 rats;	CAS not specified 0-1,000 -12,000 ppm (rat) in diet	1,000 ppm in rats	12,000 ppm increased in liver weight, PBOx, DNA synthesis. Inhibition GJIC	Smith et al. (1999; 2000)
3-week study	rat Fischer 344	CAS 68515-48-0 0-0.6-1.2-2.5% in diet		0.6% (607-639 mg/kg bw/day) increased liver weights, lauric acid 11 and 12-hydroxylase, decreased cholesterol, triglycerides	Bibra (1985)
4-week study	rat Fischer 344	CAS 28553-12-0 0.2-0.67-2% in diet		0.2% (125 mg/kg bw/day) increased catalase at 0.2% increased CAT activity at 0.2%	Midwest Res. Inst. (1981a)
13-week study	rat	CAS 68515-48-0 0-50-150-500 mg/kg bw/day in diet	150 mg/kg bw/day	500 mg/kg bw/day increased kidney, liver weights with hepatocytic hypertrophy	Hazleton (1971b)
	rat Fischer 344	CAS 68515-48-0 0-0.1-0.3-0.6-1-2% in diet	0.1% (77 mg/kg bw/day)	227 mg/kg bw/day increased kidney, liver weights decreased cholesterol levels from 0.3%	Bio/dynamics (1982b)

	rat Sprague Dawley	CAS 68515-48-0 0-0.3-1% diet		0,3% (201 – 251 mg/kg bw/day) increased kidney, liver weights, decreased triglycerides and urine chemistry changes	Bio/dynamics (1982c)
13-week study	Rat Wistar	CAS 28553-12-0 OECD n°408 0-3,000- 10,000-30,000 ppm in diet		152 – 200 mg/kg bw/day decreased triglyceride levels at 3,000 decreased alimentary peripheral fat deposits in hepatocytes at 3,000 ppm	BASF (1987f)
	rat Fischer 344	CAS 28553-12-0 EPA 0-2,500-5,000-10,000-20,000 ppm in diet		176-218 mg/kg bw/day increased liver and kidney weight at 2,500 ppm	Hazleton (1991a)

13-week study	rat Sprague Dawley	CAS not specified 1,000-3,000-10,000 ppm in the diet	1,000 ppm (60 mg/kg bw/day)	1,000 ppm (60 mg/kg bw/day) increased incidence of mononuclear cell infiltration and mineralisation of the kidneys in male 3,000 ppm (180 mg/kg bw/day) slight signs of anemia in males, increased relative kidney weight and slight slight decreased of globulin in females	Hazleton (1981a)
Chronic toxicity 2-year study	rat Fischer 344	CAS 68515-48-0 0-0.03-0.3-0.6% in diet	0.03% (15-18 mg/kg bw/day)	0.3% (152-184 mg/kg bw/day) increased liver and kidney weights increased incidence of non-neoplasic changes	Exxon Biomedical Sciences (1986)
2-year study	rat Fischer 344	CAS 68515-48-0 0-500-1,500-6,000-12,000 ppm in diet	1,500 ppm (88-103 mg/kg bw/day	6,000 ppm (358-442mg/kg bw/day) increased kidney weights in both sexes; histopathological findings in males; liver toxicity (increased ALT, AST values, liver weights and histopathological findings)	Covance (1998) Aristech Chemical Corporation (1995b) Aristech Chemical Corporation (1994)
2-year study	rat Sprague Dawley	CAS 71549-78-5 0-500-5,000-10,000 ppm in diet		500 ppm (27-33 mg/kg bw/day) minimal to slight focal hepatocellular necrosis in treated males.	Bio/dynamics (1986)
2-week or 4-week studies	male B6C3F1 mice	CAS not specified 0-500-6,000 ppm in diet	500 ppm	6,000 ppm hepatic changes increased in liver weight, PBOx, DNA synthesis; Inhibition GJIC	Smith et al. (1999; 2000)
4-week study	mouse B6C3F1	CAS 28553-12-0 EPA 0-3,000-6,000-12,000-25,000 ppm in diet	3,000 ppm (635 mg/kg bw/day)	635-780 mg/kg bw/day increased liver weight (absolute and relative) at all doses 6,000 ppm (1,300 mg/kg bw/day) decreased absolute/relative testes weight	Hazleton (1991b)

13-week study	mouse B6C3F1	CAS 28553-12-0 EPA 0-1,500-4,000-10,000-20,000 ppm in diet	for liver effect 1,500 ppm (365 mg/kg bw/day) 4,000 ppm (972 mg/kg bw/day)	4,000 ppm (972 mg/kg bw/day). Enlarged liver increased absolute and relative liver weight 10,000 ppm (2,600 mg/kg bw/day) decreased (absolute) epididymis and testes weight	Hazleton (1992)
2-year study	B6C3F1 mice	CAS 68515-48-0 0-500-1,500-4,000-8,000 ppm in diet	500 ppm (90.3 mg/kg bw/day) 1500 ppm (276 mg/kg bw/day)	1,500 ppm (275-335 mg/kg bw/day) increased kidney and liver weights 4,000 ppm (742 mg/kg bw/day) decreased absolute and relative (to brain weight) testis weight	Aristech Chemical Corporation (1995c) Covance (1998)
13-week study	dog beagle	CAS 68515-48-0 0.125-0.5-2% (37-160-2,000 mg/kg bw/day)in diet		37 mg/kg bw/day increased AST in females. increased liver weight.	Hazleton (1971a)

Test	Species	Protocol	NOAEL	LOAEL Effects observed	References
2-week study	adult male cynomolgus monkeys	CAS not specified gavage 0-500 mg/kg bw/day	500 mg/kg bw/dayay.	No changes in body weight, organ weights, urinalysis, hematology, clinical chemistry, no inflammation or necrosis in the liver, kidney and testes, no change in hepatic peroxisomal βoxidation or replicative DNA synthesis. No effect on GIJC *in vitro*.	Pugh et al. (1999; 2000)
13-week study	marmoset monkeys (16-25-month old)	CAS not specified 0-100-500-2,500 mg/kg bw/day	500 mg/kg bw/day	2,500 mg/kg bw/day minor changes: decreased body weight, decreased body weight gain.	Huntington Life Sciences (1998)

In conclusion, for effects on the liver and kidneys, a NOAEL of 88 mg/kg bw/day is determined in rats regarding results found in a chronic / carcinogenic study (Aristech, 1994). For reproductive organs, a NOAEL of 276 mg/kg bw/day can be derived from a mouse study. These NOAELs will be used for the risk characterisation.

Summary on toxicity for reproduction and development

The developmental studies are summarized in the following table.

Species	Protocol/doses	Results NOAEL/LOAEL	Test substance	References
One-generation studies				
Rat Crl: CDBR	0.5-1-1.5%	LOAEL Parents, offspring 0.5%	CAS 68515-48-0 MRD 92-455	Exxon Biomedical Sciences (1996i)
Two-generation studies (oral)				
Rat Crl: CDBR	diet 0-0.2-0.4-0.8%	LOAEL parents, offspring 0.2% (159 mg/kg	CAS 68515-48-0 MRD 92-455	Exxon Biomedical Sciences (1996j) Nikiforov et al. (1995)

		bw/day)		
Developmental toxicity studies				
Rat Sprague Dawley	gavage 0-100-500-1,000 mg/kg bw/day	NOAEL (F, dams) 500 mg/kg bw/day	CAS 68515-48-0 MRD 92-455	Exxon Biomedical Sciences (1994)
Rat Crl: CDBR	range finding study by gavage 0-40-200-500-1,000 mg/kg bw/day	NOAEL (F, dams) 1,000 mg/kg bw/day	CAS 68515-48-0	Nikiforov and Koehler (1994)
Rat Wistar	screening study 0-40-200-1,000 mg/kg bw/day	NOAEL (F, dams) 200 mg/kg bw/day	DINP1 CAS 68515-48-0	Hellwig et al. (1997b)
Rat Wistar	screening study 0-40-200-1,000 mg/kg bw/day	NOAEL (F, dams) 200 mg/kg bw/day	CAS 28553-12-0 DINP 2, Palatinol N (91/26), purity: 99.8%	BASF (1995b) Hellwig et al. (1997b)
Rat Wistar	screening study 0-40-200-1,000 mg/kg bw/day	NOAEL (F, dams) 200 mg/kg bw/day	CAS 28553-12-0 DINP 3, Palatinol DN (92/64) purity: >99.9%	BASF (1995a) Hellwig et al. (1997b)
Rat Sprague Dawley	gavage 0-10-500-1,000 mg/kg bw/day	NOAEL (F, dams) 1,000 mg/kg bw/day	DINP	Hazleton (1981)

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

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

In the two-generation study no changes in reproductive indices are observed. From those assays, no adverse effects on fertility may be anticipated. In regard with offspring survival in rats, at 1.5% (corresponding to a range of 966-2,246 mg/kg bw/day), a decrease of life birth and survival indices was observed in the one generation range-finding study but not observed in the two-generation study, conducted up to 0.8%. For decrease in life birth and survival indices a NOAEL of 622 mg/kg bw/day (the lowest dose of the estimated range) is determined and is taken into account in the risk characterisation.

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

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

ANNEX 2

Extract from CSTEE opinion on the results of the Risk Assessment of DINP

Opinion expressed at the 27th CSTEE plenary meeting, 30 October 2001.

Repeated dose toxicity

A number of repeated dose toxicity studies using rats, mice, rabbits, primates and the dog have been reviewed. In the conclusion for repeated dose toxicity the RAR states the following: "...for effects on the liver and kidneys, a NOAEL of 88 mg/kg bw/day is determined in rats regarding results found in a chronic/carcinogenic study (Aristech, 1994)". The RAR uses this NOAEL for risk characterisation purposes because liver pathology unrelated to peroxisome proliferation was seen in this study. However, in the Exxon study (Lington et al., 1997) using Fischer 344 rats, there was a dose-related increase in relative organ weights of liver and kidney in both males and females with a clear NOAEL of 15(males)-18(females) mg/kg bw/day. In addition to the increased liver and kidney weights at the LOAEL of 152(females)-184(males) mg/kg bw/day, males had increased incidences of spongiosis hepatis and serum levels of alkaline phosphatase and transaminases. Spongiosis hepatis, which is a focal degeneration of parasinusoidal cells, presumably not related to peroxisome proliferation, was also seen in 5 males in the Aristech study (Moore, 1998). The NOAEL/LOAEL for spongiosis hepatis are the same in the two studies as for the increases in liver and kidney weights. The RAR does not use the NOAEL/LOAELs for *spongiosis hepatis* for risk characterisation.
After the RAR was finalised, the Chronic Hazard Advisory Panel on DINP of the US Consumer Product Safety Commission has reported its risk characterisation using *spongiosis hepatis* as the critical endpoint [CSTEE/2001/12-Add. 3 - Report to the U.S. Consumer Product Safety Commission by the Chronic hazard advisory panel on di(isononyl) phthalate (DINP) – June 2001]. The CPSC have calculated the benchmark dose corresponding to a 5% response for this effect to be 12 mg/kg bw/day based on the Exxon study and 15 mg/kg bw/day on the Aristech study. The CSTEE finds the approach applied being scientifically sound and supports the use of the benchmark dose for *spongiosis hepatis* as the starting point of the risk characterisation.

Mutagenicity

DINP has been tested for gene mutations in bacteria and mammalian cells *in vitro,* for unscheduled DNA synthesis in hepatocytes, and for chromosomal aberrations *in vitro* and *in vivo*. DINP has also been studied for cell transforming activity in seven experiments with Balb/c-T3 cells. It was recorded as positive in one experiment, had non-significant doubtful activity in three experiments and was negative in three experiments. The CSTEE supports the conclusion of the RAR which suggests that DINP is not genotoxic *in vivo* or *in vitro*.

Carcinogenicity

In chronic/carcinogenicity studies with DINP, significant increases of liver tumours were seen in rats and mice. However, it was demonstrated that DINP induced hepatic peroxisome proliferation in rodents, but not in monkeys. Further evidence for species differences in the hepatic peroxisome proliferator response is presented by Hasmall et al. (Arch. Toxicol., 73, 451-456, 1999; not included in the RAR). *In vitro*, DINP induced beta-oxidation, DNA synthesis and suppression of apoptosis in

cultured rat hepatocytes, but had no effect on these parameters in cultured human hepatocytes. Thus, the CSTEE agrees with the conclusion of the RAR that the carcinogenic responses in rats and mice have little relevance for humans. In two studies using Fischer rats there were clear increases in the incidences of mononuclear cell leukaemia, but the RAR argues that these should not be considered as relevant to humans.
IARC has categorised MNCL as "an unclassified leukaemia with no known human counterpart" and substances which increase MNCL frequency as "not classifiable as to carcinogenicity in humans" (IARC, 1990). The CSTEE supports this view.
In the Exxon combined chronic toxicity/carcinogenicity study (Lington et al., 1997), malignant tubule cell carcinomas were seen in 2 and 4 males of the high dose and high dose recovery groups, respectively. Non-neoplastic histopathological findings in the male kidneys were consistent with hyaline droplet nephropathy. A retrospective study of these changes identified a dose-dependent increase in the accumulation of α2u-globulin in specific regions of male rat kidneys only (Caldwell et al., 1999). Thus, there are good reasons to regard these kidney tumours to be caused by the species and sex-specific α2u-globulin mechanism which is not relevant for humans.

Reproductive toxicity

The NOAEL/LOAEL for reproductive toxicity have been identified in the report to be as follows: Two generation study in rats (oral) – parents and offspring LOAEL 0.2% (159 6 mg/kg bw/day). Developmental toxicity in rats - 500 mg/kg bw/day for both maternal toxicity and developmental toxicity, and in another study 200 mg/kg bw/day for skeletal variations and 200 mg/kg bw/day for maternal toxicity. The CSTEE agrees with the conclusions of the RAR that the effects observed in the available studies do not justify classification for effects on fertility and development according to the EU classification criteria.
Regarding possible endocrine disrupting properties of DINP the report points out that investigations on possible mechanism of endocrine disruption for androgenic function are currently being conducted by investigating *in vitro* androgen receptor binding for a number of phthalates and an adipate including DBP, DEHP, DIDP, DINP, DEHA and DNOP.
Furthermore, a recent study by Gray et al. (2000) investigating the effects of several phthalates on neonatal rats indicated that DINP might have anti-androgenic potency. However, the reported changes (occurrence of female-like areolas/nipples in infant males) were slight and was only seen at a very high dose (750 mg/kg from gestational day 14 to postnatal day 3). In this respect DINP was about an order of magnitude less active than DEHP and BBP. There has been a proposal by the US National Toxicology Program that further testing be carried out in this area.