Rijksinstituut
voor **Volksgezondheid**
**en Milieu**

# Risk assessment non-phthalate plasticizers in toys

PJCM Janssen and HJ Bremmer.

National Institute for Public Health and the Environment (RIVM)
Centre for Substances and Integrated Risk Assessment (SIR)
P.O. Box 1
3720 BA Bilthoven
The Netherlands

Date: 9 November 2009

In compliance with quality assurance procedures within RIVM/SIR, this assessment was reviewed by JD te Biesebeek, JGM van Engelen, AG Schuur.

Dr. Aldert Piersma (RIVM) contributed expert advice on reproductive toxicity of the plasticizer 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB).

[1]Corresponding author; e-mail: paul.janssen@rivm Tel.: 030-2743331.

## 1. Introduction

In a 2008 survey concerning the presence of plasticizers in toys and childcare articles, the Dutch Food and Consumer Product Safety Authority (VWA) investigated compliance with newly introduced European limits for the use of six phthalates in toys and childcare articles (VWA, 2008). In this survey VWA identified several non-phthalate plasticizers in toy materials and pointed out the need for a risk assessment for the use of these compounds in this application. As a follow-up to the 2008 survey new migration measurements were performed for several non-phthalate plasticizers as identified in 12 individual toy items (VWA, 2009).

Non-phthalate plasticizers identified in the toy items sampled were diisononylcyclohexanoate (DINCH), diethylhexylterephthalate (DEHT) and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB). Below in section 2 we review available toxicological data for these compounds. Next, in section 3 we use the migration data as generated by VWA for an exposure assessment for the presence of

these non-phthalate plasticizers in the toys. In section 4 the risk assessment is presented.

## 2. Toxicity data for non-phthalate plasticizers

DINCH and DEHT have increasingly gained attention as alternatives for phthalates. TXIB has a longer history as a plasticizer, already having been submitted to toxicity testing as early as in the 1960's. An overview of available toxicity data for DINCH, DEHT and TXIB is presented in Appendices 1 through 3. These appendices are largely based on summary information expressly provided by industry for the present risk assessment. For TXIB, Eastman Chemicals additionally supplied the full study report of an OECD 421 reproduction developmental screening assay. In the following paragraphs we outline the critical toxic properties for the individual compounds.

### 2.1 DINCH

This compound is produced from diisonylphthalate by complete hydrogenation of its benzene ring structure. DINCH is lipophilic with a calculated $LogK_{ow}$ of 10.0. Its water solubility is low, as is its vapour pressure. A comprehensive toxicological database for DINCH is available including studies on toxicokinetics in rats, acute and subacute toxicity in rats, skin/eye irritation and dermal sensitisation, semichronic toxicity in rats, chronic toxicity and carcinogenicity in rats, genotoxicity, reproduction toxicity and developmental toxicity (rats and rabbits). For the present evaluation BASF, the firm responsible for both manufacture and marketing of DINCH, provided summary information on these studies.

Toxicokinetic data pertaining to rats show excretion in faeces of about 60% of the dose after oral uptake. By far the largest part of faecal excretion is as unchanged parent compound. Biliary excretion of metabolites into faeces amounted to 12% of the dose. The main biliary metabolite was the glucuronic acid conjugate of the monoisononyl ester. Excretion in urine covered about 30% of the dose. Several urinary metabolites were identified, the most important of which was cyclohexanedicarboxylic acid. Overall, oral bioavailability was estimated at 40-49%.

In repeated-dose toxicity studies with DINCH in rats, liver enzyme induction in combination with thyroid hypertrophy and thyroid hyperplasia were observed. Liver enzyme induction is thought to result in increased catabolism of thyroxine, which leads to increased levels of thyroid stimulating hormone (TSH) through a physiological feedback mechanism. Increased TSH levels then induce thyroid follicular hypertrophy. In the chronic rat study this effect led to thyroid tumours. As was also concluded by EFSA (2006), the relevance of this effect for human health is

limited given the much higher sensitivity of rodents to chemical disturbance of thyroid function compared to humans (IARC 1999). Concerning these tumours it is important to note also that genotoxicity studies carried out with DINCH show lack of genotoxic potential by the compound.

The repeated-dose toxicity studies performed with DINCH indicate increased γ-glutamyltransferase and decreased bilirubin (indicators of cholestasis) and increased blood platelet counts as the critical toxic effects. In the chronic rat study the NOAEL for these effects was 200 mg/kg bw/day.

In developmental toxicity studies in rats and rabbits DINCH showed no adverse effect for this endpoint (NOAEL>1200 mg/kg bw/day). In a further rat developmental study with continued dosing up to day 20 after birth, no effect was discernible on sexual development of male and female pups after about 100 days. The studied parameters included sperm motility and testes histopathology. In a 2-generation reproduction study, DINCH showed no adverse effect on reproduction parameters at dose levels up to 1000 mg/kg bw/day. Reproductive organ histopathology carried in $F_1$-animals showed no effect. In parent animals ($F_0$ and $F_1$) toxic effects were noted, notably increased weights of liver and kidneys, increased γ-glutamyltransferase and decreased serum bilirubin. The NOAEL for these effects was 100 mg/kg bw/day (LOAEL 300 mg/kg bw/day).

In its evaluation within the EU regulatory framework of food contact materials, EFSA (2006) selected 100 mg/kg bw/day as the overall NOAEL (taken from the 2-generation rat reproduction study). This level will be used for calculation of the margin of safety in the present risk assessment.

Given the fact of dermal contact with toys, it is relevant to note that DINCH showed only low dermal irritation potential and was negative in available studies on dermal sensitisation.

**2.2 DEHT**

This compound differs from the well-known phthalate DEHP (di-2-ethylhexyl phthalate) in that the ethylhexyl groups in this di-ester are placed in para-position on the benzene ring instead of in ortho-position as with the DEHP di-ester. DEHT is lipophilic ($LogK_{ow}$ 8.39). Its water solubility is low, as is its vapour pressure.

A comprehensive toxicological database for DEHT is available, including studies on toxicokinetics in rats, acute and subacute toxicity in rats, skin/eye irritation and dermal sensitization studies, semichronic toxicity in rats, chronic toxicity and carcinogenicity in rats, genotoxicity, reproduction toxicity and developmental toxicity. Further, its potency for induction of peroxisome proliferation in rats was

determined as was its *in vivo* estrogenicity. For the present evaluation Eastman Chemical Company, the firm responsible for both manufacture and marketing of DEHT, provided summary information on these studies.

A comparative *in vitro* toxicokinetic study in rat intestinal homogenate showed formation of free ethylhexanol to a degree indicating complete hydrolysis of the DEHT di-ester. In this study less free ethylhexanol was formed from DEHP, which indicates the persistence of the mono-ester. The latter is known as the toxic moiety for DEHP. In an *in vivo* toxicokinetic study in rats also only limited formation of the mono-ester of DEHT was observed. In this study at a relatively high dose level of 100 mg/kg bw/day (no lower dose levels tested), about 35% was recovered as unchanged parent compound in faeces and about 50% in urine as unlabeled terephthalic acid. A small part of 3.6% of the $^{14}C$ was exhaled as $CO_2$. Only minor amounts of mono(2-ethylhexyl)terephthalate were found (maximally 9% based on detected amounts of other moieties).

In repeated-dose toxicity studies liver peroxisome proliferation was found at very high dose levels (2.5% in diet) after subacute administration. The study-authors suggest this effect may be related to decreased feed intake but another possible explanation is through the action of the free ethylhexanol formed from DEHT. In a subsequent 90-days study increased liver weight was the only effect (NOAEL 0.5% in diet). In a chronic dietary study in rats toxic responses were confined to slightly decreased growth and food conversion efficiency in males and females at 0.6 and 1.2% and increased incidence of lense opacity in females at 0.6 and 1.2%. No increase in tumour incidence was noted in this study. The NOAEL in this study was 0.15% (equivalent to 79 mg/kg bw/day in males and 102 mg/kg bw/day in females).

Genotoxicity studies carried out with DEHT were negative, indicating lack of potential for this endpoint.

Developmental toxicity was studied in rats. Decreased maternal growth was the only effect found after dietary administration of 1.0% during gestation (NOAEL 0.3%). In this study no effect on fetuses was observed. In a further rat study with gavage dosing of either DEHP or DEHT from gestation day 14 until postnatal day 3, sexual maturation and male mating behaviour were studied, showing clear effects by DEHP (shortened anogenital distance, reduced testis weight and testis abnormalities) but none by DEHT. A developmental toxicity study was also performed in mice. Increased liver weight in dams was the only effect (NOAEL 0.1% equal to 197 mg/kg bw/day). No effect on fetuses occurred (NOAEL >0.7%).

In a 2-generation reproduction study in rats in which DEHT was administered via the diet at test concentrations of 0, 0.3, 0.6 or 1.0% no effect on reproduction or sperm

production was found. Parental body weights were lower at 1.0% and offspring body weights were lower at 0.6 and 1.0%. The NOAEL for toxicity in this study was 0.3% (approximately 150 mg/kg bw/day). Oral estrogenicity of DEHT was studied in the rat uterotrophic assay. The result of this study was negative (no effect).

In its evaluation within the EU regulatory framework of food contact materials, EFSA (2008) selected 79 mg/kg bw/day as the overall NOAEL (taken from the chronic toxicity/carcinogenicity study). This level will be used for calculation of the margin of safety in the present risk assessment.

Given the fact of dermal contact with toys it is relevant to note that DEHT showed only low dermal irritation potential and was negative in available studies on dermal sensitisation.

**2.3 TXIB**

This lipophilic compound has a molecular weight of 286.4 and an estimated $LogK_{ow}$ of >4.1. Its water solubility is low, as is its vapour pressure. The toxicological database for TXIB consists of limited data on toxicokinetics in rats, acute and subacute toxicity in rats, skin/eye irritation and dermal sensitization studies, semichronic toxicity in rats and dogs, genotoxicity, reproduction toxicity and developmental toxicity. For the present evaluation Eastman Chemical Company, the firm responsible for both manufacture and marketing of TXIB, provided summary information on these studies. Upon request the firm supplied the full report of an OECD 421 reproduction developmental screening assay it carried out with TXIB.

Limited toxicokinetic information shows urine as the primary route of excretion. Urinary excretion was as unidentified metabolites with a small proportion of unchanged TXIB. Urinary metabolites were consistent with complete cleavage to the constituent glycol molecule. In faeces up to 31% of the dose was recovered, either as 2,2,4-trimethyl pentanediol or unchanged TXIB, indicating partial esterase cleavage of the two isobutyrates.

A limited early semichronic feeding study in rats showed the liver as the target organ. In a more recent 90-day dietary study in the same species increased cholesterol and bilirubin occurred at the highest dose level of 750 mg/kg bw/day. Kidney effects in this study were seen in the high dose males only, consisting of increased kidney weight and hyaline droplets due to accumulation of alpha-2μ-globulin accompanied by an increased incidence rate of chronic progressive nephropathy. The severity of the nephropathy was comparable to that in the control group. In a neurological evaluation using a functional observation battery that was part of this study, no effect was found. The NOAEL in this study was 150 mg/kg bw/day.

No chronic toxicity studies were carried out with TXIB. Genotoxicity testing *in vitro* showed absence of potential for this endpoint.

Limited data on developmental toxicity and reproduction toxicity are available for TXIB. An OECD 422 reproductive/developmental toxicity screening test was carried out in rats. No effect on reproductive parameters or occurrence of pup abnormalities was observed after gavage dosing of 30, 150 and 750 mg/kg bw/day for 28 days (2 weeks before mating and 2 weeks during mating) followed by 10 additional treatment days in males and throughout gestation and until day 3 of lactation in females. The mean estrous cycle at 750 mg/kg was 4.1 days, which was significantly shorter than the control value (4.6 days). This change, however, was still within the historical control range (4.0 days). In parent animals changes indicating liver and kidney toxicity were observed at 150 and 750 mg/kg. This consisted of increased liver weight (males 150 and 750 mg/kg), increased kidney weight (males 150 mg/kg) increased serum creatinine and bilirubin (males and females 150 and 750 mg/kg), increased blood protein (males 750 mg/kg), brown-coloured livers (males 750 mg/kg), basophilic changes in renal tubular epithelium and hyaline droplet degeneration (males 150 and 750 mg/kg), necrosis and fibrosis of proximal renal tubuli, dilatation of distal renal tubuli (males 750 mg/kg) and decreased fatty changes and swelling of the liver cells (males 750 mg/kg). The NOAEL for parental toxicity was 30 mg/kg bw/day. The only other study performed was a screening study according to OECD 421. TXIB was administered from pre-mating (14 days), mating (1 to 8 days), gestation (21-23 days), through early lactation (4 to 5 days) at dietary test concentrations of 0.15, 0.45 or 1.5 % (daily doses of 91, 276, 905 mg/kg bw in males and 120, 359, 1135 mg/kg bw in females). In the high dose group the numbers of implantation sites and corpora lutea were reduced, as were mean litter weights on days 0 and 4 and number of live pups on days 0 and 4. Other parameters of reproductive performance were not affected. There were no neonatal observations of significance and none of the pups showed any external abnormalities. Reductions of total count of epididymal spermatozoa were found at all dose levels. This reduction, however, did not show a clear dose relation. Additionally in the high dose group reductions in the numbers of sperm/tissue in testes and weight-adjusted testicular spermatids heads were detected. The latter effect was also present in the low dose males but not in the medium-dose group. Weights of testes, epididymis and ovaries were normal. Neither was there an effect on histopathology in these organs (histopathology was done only in controls and the high dose group). Sperm motility was also not affected at any dose level. The observed effects in this study are evaluated as follows. The reductions in implantation sites and corpora lutea as observed in the highest dose group clearly indicate an early effect on reproduction. The biological significance of the observed changes on sperm count parameters, however, is doubtful. The variability in these parameters is known to be high and the observed changes lacked a dose related pattern. Histopathology in testes and

epididymus, which can be regarded as a more stable measure of male reproductive toxicity, showed no effect. In conclusion this study does not provide clear evidence for a specific effect on sperm production. Based on the reductions in implantations and corpora lutea, the NOAEL for reproductive effects from this study is 276 mg/kg bw/day in males and 359 mg/kg bw/day in females.

In its evaluation within the scope of food contact materials, EFSA (2006) did not derive an overall NOAEL. Overall the dataset for this compound is limited. For the present risk assessment from the toxicological experiments as described above, the NOAEL of 30 mg/kg bw/day is selected as point of departure. This value is based on increased bilirubin as indicative of hepatotoxicity as found in parent animals in a 28-days developmental toxicity and reproduction toxicity screening study. The LOAEL in this study was 150 mg/kg bw/day. The limited nature study of this study and of the available data set as a whole make the selected point of departure less reliable than that for the two other compounds. Nevertheless, given the relatively large margin with the LOAEL in this study and with relevant NOAELs from the other TXIB studies an extra factor for data set limitation is not considered necessary.

Given the fact of dermal contact with toys, it is relevant to note that TXIB showed only low dermal irritation potential and was negative in available studies on dermal sensitisation.

## 3. Exposure assessment

### 3.1 Experiments

VWA experimentally determined migration of the three non-phthalate plasticizers, from 12 different types of children's toys. The individual toy items were: ice mug (mouse), ice mug (chicken), duck (5 cm), bathroom toy (sucker), bathroom toy (orange ball), bathroom toy (pink ball), doll, bottle of the doll, inflatable turtle, inflatable swimming jacket, skippy ball, finger doll (shark).

The migration of plasticizers was determined in saliva simulant according to Schakel (2005). The experiments simulate the mouthing of a toy. Circular samples were obtained by punching the toy material. These samples were put in water in a flask and rotated at 60 rpm for 60 minutes at 20 °C. Subsequently the extract was analysed using a gas chromatograph with an UV detector. This method is comparable to Simoneau and Rijk (2001).

The results of the measurements are described in table 1.

*Table 1: Results migration experiments (VWA 2009)*

| Name plasticizer | Abbreviation | CAS nr | Mean migration [µg / (min x 10 $cm^2$)] | Maximum migration [µg / (min x 10 $cm^2$)] |
|---|---|---|---|---|
| 2,2,4-trimethyl-1,3-pentanediol diisobutyrate | TXIB | 6846-50-0 | 0.87 | 2.25 |
| diethylhexyl terephthalate | DEHT | 6422-86-2 | 0.27 | 0.48 |
| diisononyl cyclohexanate | DINCH | 166412-78-8 | 0.41 | 0.86 |

## 3. 2 Scenarios

*3.2.1 Oral exposure*

To describe the oral exposure of a child mouthing a toy, the starting point is a child with a body weight of 8 kg (age: about 10 months), who mouths a toy 3 hours per day. Children of this age show the most frequent mouthing-behaviour and have a low body weight. The exposure due to mouthing by children of this age will be the highest, expressed in mg/ kg bw.

This particular scenario, proposed by the CSTEE (1998, 1998a), is frequently used in the EU, for example in the EU-RAR for bis(2-ethylhexyl)phthalate (DEHP) (2008). It was also used in assessing exposure by children to phthalates via scoubidou strings (Schuur and Baars, 2004). This scenario should be viewed as a worst-case default for mouthing by a young child.

*3.2.2 Dermal contact*

For most of the investigated toys, however, mouthing is of minor importance. Given the size of the toys mouthing is unlikely, which applies for example to the ball and the skippy ball. Usually, for children up to 3 years, during play with the investigated toys, dermal contact is more important.

To describe dermal contact, the scenario is a child with a body weight of 15 kg (age: about 3 years), who has skin contact with the toy during 3 hours a day. It is assumed that the skin contact area with the toy is 100 $cm^2$. This scenario for dermal exposure is also used in the EU-RAR for bis(2-ethylhexyl)phthalate (DEHP) (2008). We assume that, due to hand-to-mouth contact, 10 % of the total amount on the skin will be taken up orally, as described in Bremmer et al. (2006).

In the EU-RAR for bis(2-ethylhexyl)phthalate (DEHP) (2008), wipe-off experiments are used to describe dermal exposure. For the present risk assessment we only have migration experiments with saliva simulant, which simulates mouthing. Saliva simulant is an aqueous solution with a number of salts (Schakel, 2005). It is assumed

that experiments with sweat simulant will give results in the same order of magnitude. Worst-case it is assumed that the child sweats during playing. The migration experiments with saliva simulant can thus be used to describe dermal exposure.

*3.2.3 Inhalation exposure*

Because of the small size of the toys and the low vapour pressure of the three plasticizers, inhalation exposure due to individual toys is considered negligible compared to the oral and dermal exposures.

*3.2.4 Non-toy sources of exposure*

Each of the three investigated plasticizers are authorized for use in food contact materials. For TXIB this authorization is limited to single use gloves in contact with food with a restriction of 5 mg/kg food (EFSA 2006). The authorized uses of the plasticizers will lead to some dietary exposure of consumers. The degree of actual exposure however is unknown. In absence of data on levels in foods or, even more basically, on the extent of actual use in packaging materials for various foodstuffs, no further indication of the resultant dietary exposure is possible.

If large amounts of plastics containing these plasticizers would be present indoors, for example in floor covering, inhalation exposure of the three plasticizers could be relevant. Only for TXIB some information is available on this point from the literature. Cain et al. (2005) review indoor air measurements of TXIB in homes, stating that measured levels generally are quite low. Concentration in air samples from Swedish homes, they point out, generally were below 1 ppb (11.9 $\mu g/m^3$) but were higher (≤10 ppb, 119 $\mu g/m^3$) in homes with recent painting with water-base paint. In one case, with all rooms recently painted and PVC floor covering, concentration reached 370 $\mu g/m^3$. For 800 English homes they report a similar picture, with 95% below 1.2 ppb and a maximum below 10 ppb (119 $\mu g/m^3$) (Cain et al. 2005). An earlier report is by Rosell (1990), who reported indoor air levels above 100 $\mu g/m^3$ in public buildings and office buildings, for which he identified flooring material containing 7 to 8% TXIB as the likely source. For DEHT and DINCH no data are available. Overall the data on possible indoor inhalation exposure are considered too incomplete for inclusion in the present risk assessment.

**3.3. Calculation of the exposure**

The exposure is calculated for a child (8 kg) who mouths a toy and for a child (15 kg) who has dermal contact with the toy. Only the maximal migration values from the VWA measurements are used for the calculation.

| | | |
|---|---|---|
| Maximal migration values: | TXIB | 2.25 µg / (min x 10 $cm^2$) |
| | DEHT | 0.48 µg / (min x 10 $cm^2$) |
| | DINCH | 0.86 µg / (min x 10 $cm^2$) |

Exposure is calculated as the internal dose, taking into account dermal and oral absorption.
For TXIB, toxicokinetic data indicate high oral absorption (see appendix 3). Based on the available data an absorption percentage of 80% is chosen for the oral route for this compound. No information on dermal absorption is available for TXIB. Based on molecular weight and $\log K_{ow}$ dermal absorption may be roughly estimated to be in the 'moderate' category. Accordingly an estimated value of 50% is used in the calculation. We consider this value as a 'reasonable worst case'.
For DEHT a toxicokinetic study in rats suggests a gastro-intestinal absorption of about 60%. In a study in human skin *in vitro*, a very low skin penetration of 0.056% was found. Based on this study dermal absorption is put at 1% for the present risk assessment.
For DINCH, based on oral rat data, gastrointestinal absorption is put at 50%. Dermal penetration was not determined for this compound. Based on high molecular weight and high $\log K_{ow}$ dermal absorption is estimated to be low. Given the chemical similarity of DINCH to diisononylphthalate the dermal penetration as used in the EU risk assessment for the latter compound is adopted for DINCH, i.e. 5%.

| | Dermal absorption [%] | Oral absorption [%] |
|---|---|---|
| TXIB | 50 | 80 |
| DEHT | 1 | 60 |
| DINCH | 5 | 50 |

<u>Scenario 1; exposure due to mouthing</u>
A child of 8 kg mouths a surface of 10 $cm^2$ of the toy during 3 hours per day.

TXIB
External oral exposure:
2.25 µg/(min x 10 $cm^2$) = 2.25 x 180 [min/day] / 8 [kg bw] = 51 µg / (kg bw x day)
Internal oral exposure: 51 µg / (kg bw x day) x 0.8 [g/g] = 41 µg / (kg bw x day)

DEHT
External oral exposure:
0.48 µg /(min x 10 $cm^2$) = 0.48 x 180 [min/day] / 8 [kg bw] = 11 µg / (kg bw x day)
Internal oral exposure: 11 µg / (kg bw x day) x 0.6 [g/g] = 6.6 µg / (kg bw x day)

DINCH
External oral exposure:
0.86 µg /(min x 10 $cm^2$) = 0.86 x 180 [min/day] / 8 [kg bw] = 19 µg / (kg bw x day)
Internal oral exposure: 19 µg / (kg bw x day) x 0.5 [g/g] = 9.5 µg / (kg bw x day)

Scenario 2; exposure due to dermal contact

A child of 15 kg has dermal contact with a surface of 100 $cm^2$ of the toy during 3 hours a day. It is assumed that 10 % of the total amount on the skin is taken in orally, due to hand to mouth contact.

TXIB

Total amount on the skin

2.25 µg/(min x 10$cm^2$) = 2.25 x 180 [min/day] x 100/10 [$cm^2$/$cm^2$] / 15 [kg bw]= 270 µg / (kg bw x day)

Internal dermal exposure: 0.9 [g/g] x 270 [µg / (kg bw x day)] x 0.5 [g/g]= 122 µg / (kg bw x day)

Internal oral exposure: 0.1 [g/g]x 270 [µg / (kg bw/day)] x 0.8[g/g] = 22 µg / (kg bw x day)

Total internal exposure: 140 µg / (kg bw x day)

DEHT

Total amount on the skin

0.48 µg/(min x 10 $cm^2$) = 0.48 x 180 [min/day] x 100/10 [$cm^2$/$cm^2$] / 15 [kg bw]= 57.6 µg / (kg bw x day)

Internal dermal exposure: 0.9 [g/g] x 57.6 [µg / (kg bw x day)] x 0.01 [g/g]= 0.52 µg / (kg bw x day)

Internal oral exposure: 0.1 [g/g]x 57.6 [µg / (kg bwxday)] x 0.6 [g/g] = 3.5 µg / (kg bw x day)

Total internal exposure: 4.0 µg / (kg bw x day)

DINCH

Total amount on the skin

0.86 µg/(min x 10 $cm^2$) = 0.86 x 180 [min/day] x 100/10 [$cm^2$/$cm^2$] / 15 [kg bw]= 103 µg / (kg bw x day)

Internal dermal exposure: 0.9 [g/g] x 103 [µg / (kg bw x day)] x 0.05 [g/g]= 4.6 µg / (kg bw x day)

Internal oral exposure: 0.1 [g/g]x 103 [µg / (kg bw x day)] x 0.5 [g/g] = 5.2 µg / (kg bw x day)

Total internal exposure: 9.8 µg / (kg bw x day)

## 4. Risk assessment

For DINCH a complete toxicological dataset was available, including a chronic rat bioassay and a 2-generation reproduction study in rats. For DEHT a similarly elaborate dataset was available. Both compounds showed no activity for the

reproductive toxicity endpoints studied. For both compounds overall-NOAELs were derivable that can be used as point of departure in the risk assessment. For TXIB the data set was much more limited. No chronic toxicity study was done and for reproductive toxicity only two screening assays were available. In one of these studies the compound showed reproductive toxicity at a high dose level and questionable changes in sperm parameters at lower dose levels. Despite the limitations in the data set we derived an overall-NOAEL for TXIB. Given the fact that liver toxicity and reproductive effects were observed only at considerably higher dose levels than this NOAEL, we consider it not necessary to apply an extra factor to compensate for data base limitations.

Based on the low potential for induction of skin irritation and sensitzation as found in the relevant studies with the three plasticizers we judge the risk for local skin reactions due to contact with the toy items to be low.

Based on the available toxicity data the following overall-NOAELs were selected for the three plasticizers.

<u>Overall NOAELs</u>

| | |
|---|---|
| TXIB | 30 mg/kg bw/day |
| DEHT | 79 mg/kg bw/day |
| DINCH | 100 mg/kg bw/day |

For calculating the margin of safety these NOAELs must be converted to the corresponding internal dose levels using the oral absorption factors of 80, 60 and 50% for TXIB, DEHT and DINCH respectively. This is done in the table below (fourth column). The table also presents the calculated margins of safety (fifth column).

*Table 2: Margins of safety, scenario 1; exposure due to mouthing*

| | Internal exposure [mg/kg bw/day] | External NOAEL [mg/kg bw/day] | Internal NOAEL[mg/kg bw/day] | MoS |
|---|---|---|---|---|
| TXIB | 0.041 | 30 | 24 | 580 |
| DEHT | 0.0066 | 79 | 48 | 7300 |
| DINCH | 0.0095 | 100 | 50 | 5300 |

*Table 3: Margins of safety, scenario 2; exposure due to dermal contact*

| | Internal exposure [mg/kg bw/day] | NOAEL [mg/kg bw/day] | Internal NOAEL[mg/kg bw/day] | MoS |
|---|---|---|---|---|
| TXIB | 0.14 | 30 | 24 | 170 |
| DEHT | 0.0040 | 79 | 48 | 12000 |
| DINCH | 0.0098 | 100 | 50 | 5100 |

The calculated margins of safety for DEHT and DINCH are very high, leading to the conclusion that these compounds are not expected to pose any health risk for toy-users at the migrated levels. For TXIB the margin of safety was considerably lower but still above 100, which is the margin usually taken into account in deriving safe levels for humans based on animal NOAELs. The margin of safety for TXIB, however, should also be judged against the limitations in the toxicological dataset for the compound. The conclusion for TXIB is that the presently available information indicates absence of risk but further relevant experimental evidence for this compound would strengthen the risk assessment for its use in toys.

## 5. References


Bremmer, HJ, WM Blom, PH van Hoeven-Arentzen, LCH Prud'homme de Lodder, MTM van Raaij, EHFM Straetmans, MP van Veen, JGM van Engelen (2006) Pest Control Products Fact Sheet. To assess the risks for the consumer. Updated version for ConsExpo 4. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 320005002.

Cain, WS Wijk RA de, Jalowayski AA, Pilla Caminha, G, Schmidt, R. (2005) *Indoor Air* 15, 445-457.

CSTEE (1998) EU Scientific Committee on Toxicity, Ecotoxicity and the Environment. Phthalate migration from soft PVC toys and child-care articles. Opinion expressed at the CSTEE third plenary meeting Brussels, 24 April 1998.

CSTEE (1998a) EU Scientific Committee on Toxicity, Ecotoxicity and the Environment. Opinion on Phthalate migration from soft PVC toys and child-care articles - Data made available since the 16th of June 1998, opinion expressed at the 6th CSTEE plenary meeting, Brussels, 26/27 November 1998.

EFSA (2006) Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 12th list of substances for food contact materials Question N° EFSA-Q-2004-073, EFSA-Q-2006-024, EFSA-Q-2005-238a, EFSA-Q-2005-238b, EFSA-Q-2005-238c, EFSA-Q-2006-012, EFSA-Q-2006-027 Adopted on 26 & 27 September 2006. The EFSA Journal (2006) **395-401**, 1-21.

EFSA (2008) Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to the 18th list of substances for food contact materials Question N° EFSA-Q-2007-167, EFSA-Q-2006-177, EFSA-Q-2005-152, EFSA-Q-2007-022, EFSA-Q-2007-004, EFSA-Q-2007-024, Adopted on 31 January 2008. The EFSA Journal (2008) 628-633, 1-19

EU-Risk assessment report (2008) Bis(2-ethylhexyl)phthalate (DEHP), EINECS No204-211-0, European Chemicals Bureau.

IARC (1999) Species differences in thyroid, kidney and urinary bladder carcinogenesis. IARC scientific publications no. 147. Eds. Capen, CC, Dybing, E, Rice, JM, Wilbourn, JD. International Agency for Research on Cancer, WHO, Lyon, France.

Rosell L (1990) Indoor Air '90, The 5th International Conference on Indoor Air Quality and Climate, Toronto, July 29 August 3, 1990, Volume 3, pages 707-712, 4 references, 1990. (Abstract only). http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~x9MBtu:3 (Retrieved on 24-06-2009)

Schakel, DJ (2005) Determination of the migration of diisinonylphthate in saliva simulant with straight phase HPLC-UV detection [in Dutch]. Food and Consumer Product Safety Authority, April 2005.

Schuur, G., B.J. Baars (2004) Advice concerning the exposure of Dutch children to phthalates via scoubidou strings [in Dutch]. RIVM/SIR 10796AOO

Simoneau, C., Rijk R., (2001) *Standard Operation Procedure for the Determination of the Release of Di-isononylphthalate (DINP) in Saliva Simulant from Toys and Childcare Articles using a Head over Heels Dynamic Agitation Device*, EUR Report EUR 19899 EN 200. As cited in VWA (2009).

VWA (2008) Plasticizers in toys and childcare articles [Weekmakers in speelgoed- en kinderverzorgingsartikelen [In Dutch]. Ir MYP van den Vondel. VWA report no. ND072201, dated 7 March 2008.

VWA (2009) The Application of alternative plasticizers in toys and health care articles [Toepassing alternatieve weekmakers in speelgoed- en kinderverzorgingartikelen. Inventarisatie en migratie-onderzoek] [In Dutch]. Ir. M.P.Y. van Vondel, Voedsel en Waren Autoriteit Regio Noord. (Deel)projectnummer: ND082211. Report dated October 2009.

## Appendix 1

# Toxicity profile 1,2-Cyclohexanedicarboxylic acid, diisononylester (DINCH)

1,2-Cyclohexanedicarboxylic acid, diisononylester (DINCH, CAS-number 166412-78-8) is a clear colourless liquid. It is used as a plasticiser in PVC in concentrations up to 40%. The compound is lipophilic with a calculated $LogK_{ow}$ of 10.0. Its water solubility is low, as is its vapour pressure.

DINCH toxicity was evaluated by EFSA (2006) for application in food contact materials. This use was specified as plasticizer in PVC cling films for fresh meat packaging (10%), for aqueous food and fruits and vegetables (35%), artificial corks (35%), sealing gaskets for beverage containers (35%), flexible tubes for beverages, alcoholic and non-alcoholic (40%), conveyor belts for fatty foods (12%) and other foods (12%) and as polystyrene impact modifier (3%) (EFSA 2006). In view of possible use in medical devices the compound was reviewed by the EU-SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks) in 2008. A further review was performed within the scope of the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2008). The latter provides the most complete description of results of the various studies.

A comprehensive toxicological database for DINCH is available including data on toxicokinetics in rats, acute and subacute toxicity in rats, skin/eye irritation and dermal sensitisation studies, semichronic toxicity in rats, chronic toxicity and carcinogenicity in rats, genotoxicity, reproduction toxicity and developmental toxicity. In addition liver phase I and II enzyme induction was examined in rats after oral administration for 2 weeks. Also in rats S-phase cell proliferation in liver, kidneys and thyroid glands was determined after oral administration for 1, 4 and 13 weeks. The mechanism of the effect on thyroid was studied using the perchlorate discharge assay in rats.

**Toxicokinetics**

Absorption, distribution, elimination and toxicokinetics were studied *in vivo* in rats after single gavage dosing at several levels of $^{14}C$-labelled DINCH in 0.5% aqueous carboxymethylcellulose and 1% Cremophor EL. In a separate group of rats the labelled compound was given intravenously. At a high dose of 1000 mg/kg bw 68-92% of the radioactive dose was excreted in faeces and about 5% in urine. At a low dose of 20 mg/kg bw this was 59-63% and about 30% respectively. This pattern indicates saturation of gastro-intestinal absorption to occur. After intravenous administration of 3 mg/kg bw about 45% was excreted in both urine and faeces. At the low oral dose 6 to 12% of 14C was excreted via bile (at the high dose this was

about 0.5%). In urine the major metabolite was cyclohexanedicarboxylic acid (50-75% of 14C in urine). In addition two to five minor metabolites were found in urine. Urinary metabolite pattern was similar after oral and intravenous administration. In faeces 84-100% of the radioactivity present was unchanged parent compound (unabsorbed). In bile the most prominent metabolite was identified as the glucuronic acid conjugate of the monoisononyl ester, which represented about 3.7-7.6 % of of the radioactive dose. This is about half of the radioactivity present in bile. Other minor metabolites in bile were the monoisononyl ester itself and degradation products thereof (with or without further conjugation) and other derivatives/conjugates that may lack both isononyl groups. Oral bioavailability was calculated to be ~5-6% (high dose) and ~40-49% (low dose), based on the total excreted radioactivity. After intravenous administration metabolism to polar metabolites was efficient and complete: no unmetabolised diisononyl ester of cyclohexane dicarboxylic acid was detected in urine or faeces (NICNAS 2008).

**Acute and short-term toxicity**

In toxicity studies DINCH exhibited only low acute oral toxicity (LD50 rat > 5000 mg/kg bw) and low irritation potential for skin and eye when tested undilutedly. Available studies indicate absence of sensitising potential upon dermal contact. In a 28-days oral diet study in rats indications of mild renal function impairment were found in males at the highest test concentration of 15000 ppm (epithelial cells in urine, elevated serum Na+/K+). Females at this concentration showed increased γ-glutamyltransferase and reduced serum bilirubin. This may be associated with hepatic microsomal enzyme induction due to stimulation of phase II reactions. The NOAEL in this study was 3000 ppm (about 330 mg/kg bw/day). In a subsequent 90-days study in rats effects were as follows: increased γ-glutamyltransferase in females (15000 ppm), increased TSH (15000 ppm), presence of epithelial cells in urine in males (4500 and 15000 ppm), accumulation of α2μ-microglobulin in renal tubuli and increased kidney weights in males (4500 and 15000 ppm), increased kidney weight in females (without histopathological correlate), increased incidence of minimal to slight hypertrophy/hyperplasia of thyroid gland follicular epithelia (all dose levels). The authors explain the effect on thyroid as arising from liver enzyme induction. Liver enzyme induction results in increased catabolism of thyroxine, which leads to increased TSH levels through a physiological feedback mechanism. Increased TSH levels result in thyroid follicular hypertrophy. The NOAEL in this study was <1500 ppm (107 and 128 mg/kg bw/day in males and females respectively) (NICNAS 2008, EFSA 2006).

**Chronic toxicity and carcinogenicity**

In a combined chronic/carcinogenicity study in rats in which DINCH was given via the diet at nominal dose levels of 0, 40, 200 and 1000 mg/kg bw/day, similar thyroid effects were observed as in the 90-days study. Increased incidences of thyroid

hyplasia and adenomas and increased thyroid weight were observed in both sexes at the high dose, and at medium dose in males. Other effects were: increased platelet counts in females (high dose), increased γ-glutamyltransferase and decreased bilirubin in females (high dose), increased amount of degenerated transitional epithelial cells in urinary sediments in males (high dose) and granular and/or epithelial cell casts in urine of males (medium and high dose). Because the latter effect was present after 3 months only and was not accompanied by histological renal changes it was considered adaptive. In this study, the NOAEL for thyroid effects was 40 mg/kg bw/day. The NOAEL for other adverse effects was 200 mg/kg bw/day (based on increased platelet counts in females at 1000 mg/kg bw/day) (NICNAS 2008, EFSA 2006).

**Genotoxicity**

DINCH was tested in three *in vitro* mutagenicity assays (reversion in bacteria, forward mutation and chromosomal aberration tests in mammalian cells) and in the micronucleus test in mouse bone marrow (i.p. injection). Based on the negative results obtained, it is concluded that DINCH is not genotoxic (EFSA 2006).

**Reproductive and developmental toxicity**

Developmental toxicity was studied in rats and rabbits. In rats dose levels up to 1200 mg/kg bw/dag given from day 6 through 19 of gestation. No effects were found. In rabbits dosages of up to 1000 mg/kg bw/day were administered from day 6 through day 29 of gestation. Again no effects were observed. In a further rat developmental study pregnant rats were given 0, 750 or 1000 mg/kg bw/day by oral gavage from day 6 of gestation through day 20 post partum. No maternal effects were seen. Reproductive parameters also were unaffected. Examination for sexual maturation (testes descending, day of vaginal opening/balanopreputial separation) of all male pups and up to 3 female pups per litter that had been raised until days 100 to 105 post partum (with no additional exposure) showed no effect. Clinical examinations, sexual maturation, organ weights, gross and histopathological findings (including gonads) and sperm motility in these animals showed no indications of substance-related adverse effects. The NOAEL in this study was 1000 mg/kg bw/day (NICNAS 2008, SCENIHR 2008).

In a 2-generation reproduction study rats received DINCH in the diet at nominal dose levels of 0, 100, 300 and 1000 mg/kg bw/day. No effect on reproduction parameters was seen. Gross and histopathological findings did not indicate that the test substance adversely affected reproductive performance or fertility in the parental or first filial generation rats for all dose groups (no histpothology done in F2-animals). In F0 parent animals γ-glutamyltransferase was increased and serum bilirubin decreased in females (medium and high dose) and weights of liver and kidneys were increased in males and females (medium and high dose). The same effects were seen in F1 parent

animals. In addition in the F1 generation vacuolization of kidney tubular epithelia in males (medium and high dose) was found and thyroid hyperplasia in females (medium and high dose). The NOAEL in this study was 100 mg/kg bw/day (EFSA 2006, NICNAS 2008).

**Special studies**

In a 2-week study in rats a number parameters indicative of liver enzyme induction were measured after administration of 15000 ppm in the diet (cytochrome P450 concentration, phase I enzyme activities ethoxyresorufin O-deethylase, pentoxyresorufin O-depentylase, benzoxyresorufin O-debenzylase, 4-methylumbelliferone glucuronyltransferase, 4-hydroxybiphenyl glucuronyltransferase). The result showed marked increases in all parameters (NICNAS 2008). Also in rats cell proliferation (S-phase response) was assessed in liver, kidneys and thyroid glands using staining with 5'-bromo-2-deoxyuridine after adminstering DINCH in the diet for 1, 4 or 13 weeks (dose levels 0, 40, 200 and 1000 mg/kg bw/day). Induction of cell proliferation was found in all three organs. The effect was greatest after 1 week of treatment, decreasing somewhat after 4 weeks while approaching control levels after 13 weeks of treatment. The liver and kidney cell proliferation was found after 1 and 4 weeks in medium and high dose males (effect absent after 13 weeks). Thyroid cell proliferation was found at all dose levels in males and females after 1 and 4 weeks and again was absent after 13 weeks of dosing (NICNAS 2008). In male rats thyroid function was studied using the perchlorate discharge as diagnostic test for evaluating thyroid uptake of iodine. DINCH was given via the diet at a concentration of 15000 ppm for 4 weeks. Phenobarbital was used as positive control for indirect disturbance of thyroid function (via liver enzyme induction) and propylthiouracil was positive control for direct inhibition of iodine uptake by the thyroid. DINCH produced a similar reponse as phenobarbital in this study (insignificant changes in T3, T4 and TSH levels in blood, increased thyroid weight, increased iodine uptake with and without perchlorate injection) (NICNAS 2008).

**TDI derived by EFSA (2006)**

EFSA (2006) concluded that given the absence of genotoxic properties, the induction of follicular cell hyperplasia and adenomas in rat thyroid as seen in the chronic rat study can be attributed to a non-genotoxic, indirect mechanism. Because known high sensitivity of rodents to chemical disturbance of thyroid function (IARC, 1999) compared to humans, the thyroid effects as observed in 90 days and chronic toxicity/carcinogenicity studies were considered inappropriate as basis for a TDI. Instead, the renal toxicity as observed in the rat subchronic toxicity study and in the 2-generation rat study were taken as the pivotal effect, for which a NOAEL of 100 mg/kg bw/day was identified. Using a default uncertainty factor of 100 a TDI of 1 mg/kg bw was derived for DINCH (EFSA 2006).

## References

EFSA (2006) Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 12th list of substances for food contact materials Question N° EFSA-Q-2004-073, EFSA-Q-2006-024, EFSA-Q-2005-238a, EFSA-Q-2005-238b, EFSA-Q-2005-238c, EFSA-Q-2006-012, EFSA-Q-2006-027 Adopted on 26 & 27 September 2006. The EFSA Journal (2006) **395-401**, 1-21.

IARC (1999) Species differences in thyroid, kidney and urinary bladder carcinogenesis. IARC scientific publications no. 147. Eds. Capen, C.C., Dybing, E., Rice, J.M., Wilbourn, J.D. International Agency for Research on Cancer, WHO, Lyon, France.

NICNAS (2008) National Industrial Chemicals Notification And Assessment Scheme - Full Public Report 1,2-Cyclohexanedicarboxylic Acid, 1,2-Diisononyl Ester ('Hexamoll Dinch'). File No: STD/1259 August 2008.

SCENIHR (2008) Scientific Committee on Emerging and Newly-Identified Health Risks. Opinion on the safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk. Adopted after public consultation by the SCENIHR during the 22nd Plenary of 6 February 2008

## Appendix 2

# Toxicity profile di-(2-ethylhexyl)-terephthalate (DEHT)

Di-(2-ethylhexyl)-terephthalate (DEHT, CAS-number 6422-86-2) is a colourless liquid. It is used as a plasticiser in PVC in concentrations up to 30%. The compound is lipophilic with a $LogK_{ow}$ of 8.39. Its water solubility is low, as is its vapour pressure.

DEHT toxicity was evaluated by EFSA (2008) for application in food contact materials. This use was specified as plasticizer up to approximately 30% in PVC-materials, coming into contact with all kinds of foodstuffs under all conditions both for single and repeated use. Typical products could be wraps, tubing, conveyor belts and sealing gaskets (EFSA 2008). In view of possible use in medical devices the compound was reviewed by the EU-SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks) in 2008. An earlier review was within the scope of the OECD SIDS programme (SIDS 2004). For the present report Eastman Chemical Company provided a summary of toxicological information (ECC 2007).

A comprehensive toxicological database for DEHT is available including data on toxicokinetics in rats, acute and subacute toxicity in rats, skin/eye irritation and dermal sensitisation studies, semichronic toxicity in rats, chronic toxicity and carcinogenicity in rats, genotoxicity, reproduction toxicity and developmental toxicity. Further its potency for induction of peroxisome proliferation in rats was determined as was its *in vitro* estrogenicity.

**Toxicokinetics**

The metabolic hydrolysis rate of DEHT was studied *in vitro* using a rat intestinal homogenate. The degree of formation of free ethylhexanol indicated virtually complete hydrolysis to occur. The half-life for disappearance of parent compound was 53 minutes. Diethylhexylphthalate showed more rapid disappearance (half-life 12.6 minutes) and lower conversion to free ethylhexanol, indicating that for this compound the mono-ester remains intact. Toxicokinetics was also studied *in vivo* in rats with single gavage dosing of 100 mg/kg bw of $^{14}C$-labelled DEHT in corn oil. The mean amount of unchanged parent compound in the faeces was 36.6% of the dose. A percentage of 50.5% of the dose was recovered in the urine as unlabeled terephthalic acid. A small part of 3.6% of the $^{14}C$ was exhaled as $CO_2$. Only minor amounts of mono(2-ethylhexyl)terephthalate were found. Based on the recovered dose as unchanged compound, the free acid or as $CO_2$ the amount of mono-ester and its metabolites amount to a maximum of 9.3% of the orally administered dose (ECC 2007).

Percutaneous absorption of DEHT was examined in human skin *in vitro*. An excess of [carboxyl-$^{14}$C]-labelled DEHT was applied to sections of human skin contained in glass diffusion cells. Solubility of the test substance in receptor solution was determined not to be a rate-limiting step in skin absorption. The total recovery of the test substance was measured by determining the percentage $^{14}$C-labeled test substance remaining in test system components for each test substance cell. The total mean (± SD) $^{14}$C-labeled test substance recovery was 104% ± 6%. The majority of $^{14}$C was recovered from the donor cell and only 0.056% ± 0.032% was associated with the skin. The measured absorption rate (mean ± SD) was 0.103 ± 0.052 µg/cm$^2$/hr, and the permeability constant was (8.39 ± 2.17) x $10^{-8}$ cm/hr. Based on this study dermal absorption through human skin is classified as extremely low (ECC 2007).

**Acute and short-term toxicity**

In toxicity studies DEHT exhibited only low acute oral toxicity (LD50 rat > 5000 mg/kg bw, mouse >3200 mg/kg bw) and low irritation potential for skin and eye when tested undilutedly. Available studies indicate absence of sensitising potential upon dermal contact. In a 21-days oral diet study in rats reduced feed consumption and growth retardation were observed at the highest test concentration of 2.5%. Increased absolute weights of kidney and testis were also seen at this level. Increased liver weights were observed at ≥1.2%. At 2.5% only blood serum triglycerides and cholesterol were increased and liver electron microscopy showed signs indicating peroxisome proliferation in the liver. Significant increases in some hepatic enzyme activities associated with peroxisome proliferation were also seen at this level. The peroxisome proliferation may be related to the decreased feed consumption, the study-authors point out. The NOAEL in this study was 1.0% (1000 mg/kg bw/day) (ECC 2007; SIDS 2004). In a 90-days oral diet study in rats carried out subsequently the only change was an increased liver weight at the highest test concentration of 1.0% without any signs of peroxisome proliferation or histopathological changes. The toxicological significance of this effect is questionable. The NOAEL in this study was 0.5% (309 mg/kg bw/day) (ECC 2007, SIDS 2004).

**Chronic toxicity and carcinogenicity**

A chronic toxicity/carcinogenicity study was carried in rats with dosing via the diet at concentrations of 0, 0.15, 0.6 and 1.2%. Toxic responses were confined to slightly decreased growth and food conversion efficiency in males and females at 0.6 and 1.2% and increased incidence lense opacity in females 0.6 and 1.2%. No increase in tumour incidence was noted in this study. The NOAEL was in the study was 0.15% (equivalent to 79 mg/kg/day in males and 102 mg/kg/day in females) (ECC 2007, Deyo 2008).

**Genotoxicity**

Genotoxicity of DEHT was studied *in vitro* in three test systems, i.e. Ames-test, chromosome aberrations in mamalian cells and HGPRT mutation assay in mammalian cells. All three test were negative, thus indicating that DEHT lacks genotoxic potential.

The impurity terephthalic acid, (2-ethylhexyl) methyl ester showed lack of mutagenic activity in bacteria and mammalian cells *in vitro*.

**Reproductive and developmental toxicity**

In a 2-generation reproduction study in rats in which DEHT was administered via the diet at test concentrations of 0, 0.3, 0.6 or 1.0% (approx. 0,150-200, 300-400, 500-700 mg/kg/day for males and 0, 250-300, 500-600, 800-1000 mg/kg/day for females) no effect on reproduction or sperm production was found. Parental body weights were lower at 1.0% and offspring body weights were lower at 0.6 and 1.0%. The NOAEL for toxicity in this study was 0.3% (150 mg/kg). The same test concentrations were used in a standard rat developmental study. Decreased maternal weight gain at the highest test concentration was the only effect seen in this study (no effect of fetuses). In a further developmental study in rats dams were allowed to give birth and pups were examined extensively for reproductive organ development. Male mating behaviour was also evaluated. In this study single dose levels of both DEHT and DEHP were tested using daily application via gavage from gestation day 14 until postnatal day 3. DEHT at 750 mg/kg bw/day produced no effect whereas DEHP produced shortened anogenital distance, reduced testis weight and testis abnormalities (ECC 2007, Gray et al. 2000). Developmental toxicity was also studied in mice using dietary test concentrations of 0, 0.1, 0.3 and 0.7% (0, 197, 592, and 1,382 mg/kg bw/day), administered from gestation day 0 through 18. No effect was found on fetuses. Changes in maternal liver weights (unspecified) were found at 0.3 and 0.7%. The NOAEL for maternal toxicity was 0.1% (197 mg/kg) and for developmental toxicity 0.7% (1,382 mg/kg) (ECC 2007).

Oral estrogenicity of DEHT was studied in the rat uterotrophic assay. Immature female rats were given gavage dosages of 20, 200, 2000 mg/kg bw/day once daily on postnatal days 19 to 21. No effect on uterine weight was noted at sacrifice on postnatal day 22. The positive control compound ethinyl-estradiol induced a 3-4 fold increase in uterine weight in this study (ECC 2007).

**TDI derived by EFSA (2008)**

Based on the NOAEL of 79 mg/kg bw/day from the chronic toxicity/carcinogenicity study, a TDI of 1 mg/kg bw/day for DEHT was derived. EFSA noted that previously a TDI of 1 mg/kg bw/day had been derived for the dimethyl ester of terephthalic acid (EFSA 2008).

## References

ECC (2007) Toxicity summary for Eastman® 168 plasticizer. Prepared by Product Safety & Stewardship Eastman Chemical Company Kingsport, Tennessee 37662, USA. May 21, 2007. Data supplied by Eastman Kodak.

EFSA (2008) Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to the 18th list of substances for food contact materials Question N° EFSA-Q-2007-167, EFSA-Q-2006-177, EFSA-Q-2005-152, EFSA-Q-2007-022, EFSA-Q-2007-004, EFSA-Q-2007-024, Adopted on 31 January 2008. The EFSA Journal (2008) 628-633, 1-19

Deyo, JA (2008) Carcinogenicity and chronic toxicity of di-2-ethylhexyl terephthalate (DEHT) following a 2-year dietary exposure in Fischer 344 rats. *Food and Chemical Toxicology* **46** (2008) 990–1005.

Gray, LE et al. (2000) Perinatal Exposure to the Phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, Alters Sexual Differentiation of the Male Rat. *Toxicological Sciences* **58,** 350-365.

SIDS (2004) SIDS Initial Assessment Profile: Di(2-ethylhexyl)terephthalate (DEHT). CAS 6422-86-2 Summary conclusions of the SIAR. SIAM 19, 19-22 October 2004.

## Appendix 3

# Toxicity profile 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)

2,2,-Trimethyl-1,3-pentanediol diisobutyrate (TXIB, CAS-number 6846-50-0) is a colourless liquid. It is used as a plasticiser in PVC in concentrations up to 20%. The compound is lipophilic with a $LogK_{ow}$ of >4.1. Its water solubility is low, as is its vapour pressure (0.088 Pa at 20 °C) (SIDS 1995).

TXIB toxicity was evaluated by EFSA (2006) for application in food contact materials. This specified use was in plasticised PVC single use gloves for contact with food. The gloves will come in contact with all kinds of food for a period of maximally 30 min and at a temperature not exceeding 40°C (EFSA 2006). Earlier reviews are those by RIVM (2001) as constituent in drinking-water materials and by OECD within the SIDS programme (SIDS 1995). For the present report Eastman Chemical Company provided a summary of toxicological information (ECC 2007).

The toxicological database for TXIB includes limited data on toxicokinetics in rats, acute and subacute toxicity in rats, skin/eye irritation and dermal sensitisation studies, semichronic toxicity in rats and dogs, genotoxicity, reproduction toxicity and developmental toxicity.

**Toxicokinetics**
Available information dates back to 1966. After a single oral dose of $^{14}$C-TXIB (dose levels 236-895 mg/kg bw) given to rats, 47-72% of the dose was present in urine within 5 days and 14-31% in faeces within 7 days. No $^{14}CO_2$ was detected. In total, excretions accounted for 95-99% of the dose. Identification of metabolites showed the feces to contain both 2,2,4-trimethyl pentanediol (TMPD) and unchanged TXIB indicating partial esterase cleavage of the two isobutyrates. A small portion of the absorbed material in the urine was unchanged TXIB while the majority consisted of metabolites consistent with complete cleavage to the glycol (TMPD) parent molecule. Much of the urinary metabolites however was unidentified (ECC 2007).

**Acute and short-term toxicity**
In toxicity studies TXIB exhibited only low acute oral toxicity (LD50 rat > 3200 mg/kg bw, mouse >6400 mg/kg bw) and low irritation potential for skin and eye when tested undilutedly. Available studies indicate absence of sensitising potential upon dermal contact.

An early semichronic study in rats (from 1963) showed liver as the target organ with increased weight and increased SGOT at test concentrations in the diet of 0.1 and 1.0% (79 and 815 mg/kg bw/day respectively). In a more recent rat study, carried out according to FDA guidelines, TXIB was given via diet at dosage levels of 0, 30, 150 and 750 mg/kg bw/day. This study included a functional observational battery assessment for detecting neurological effects. Compound-related effects consisted of increases in serum cholesterol and bilirubin at 750 mg/kg and, in males only, at the same dose level increased kidney weight and hyaline droplet due to accumulation of alpha-2μ-globulin accompanied by an increased incidence rate of chronic progressive nephropathy. The severity of the nephropathy was comparable to that in the control group. No effect on gonad histopathology was found. The NOAEL in this study was 150 mg/kg bw/day (ECC 2007, EFSA 2006). Semichronic toxicity was also studied in dogs (study from 1966). After giving TXIB in the diet at 0.1, 0.35 or 1.0% (equivalent to 25, 90 and 250 mg/kg bw/day respectively) increased weights of liver and pituitary were the only effects observed. No histological changes were observed in the tissues examined (including gonads) (ECC 2007).

**Chronic toxicity and carcinogenicity**
No data.

**Genotoxicity**
Genotoxicity of TXIB was studied *in vitro* in three test systems, i.e. Ames-test, chromosome aberrations in mamalian cells and HGPRT mutation assay in mammalian cells. All three test were negative, thus indicating that TXIB lacks genotoxic potential (ECC 2007, EFSA 2006).

**Reproductive and developmental toxicity**
In a combined repeated-dose and reproductive/developmental toxicity screening test (OECD 422), male and female rats were given TXIB at 0, 30, 150 and 750 mg/kg bw/day by gavage for 28 days (2 weeks before mating and 2 weeks during mating) followed by 10 additional days in males and throughout gestation and until day 3 of lactation in females. No effect on parameters of reproductive performance was observed. No external abnormalities were found among pups. The mean estrous cycle of the high dose (4.1 days) was significantly shorter than the control value (4.6 days). This change was still within historical controls (4.0 days). Toxic effects in parent animals in this study included increased serum creatinine and bilirubin (150 and 750 mg/kg), increased blood protein (750 mg/kg), brown coloured livers (750 mg/kg), basophilic changes in renal tubular epithelium and hyaline droplet degeneration (males 150 and 750 mg/kg), necrosis and fibrosis of proximal renal tubuli, dilatation of distal renal tubuli (males 750 mg/kg), decreased fatty changes and swelling of the liver cells (males 750 mg/kg). The NOAEL for toxic effects in this study was 30 mg/kg bw/day (SIDS, 1995; ECC 2007). In a further combined

reproductive/developmental toxicity screening study in rats (OECD 421), TXIB was given in the diet from pre-mating (14 days), mating (1 to 8), gestation (21-23 days), through early lactation (4 to 5 days). Test concentrations were 0.15, 0.45, or 1.5 % (daily doses of 91, 276, 905 mg/kg in males and 120, 359, 1135 mg/kg in females). No effect on weights of testes, epididymis and ovaries occurred. Neither was there an effect on histopathology in these organs. No effect on sperm motility was observed. Total count of epididymal spermatozoa, however, was reduced at all dose levels. Other changes of significance noted in the high dose group consisted of a reduction in the numbers of sperm/tissue and weight-adjusted testicular spermatids heads. The latter effect was also present in the low dose males but not in the medium-dose group. There were no neonatal observations of significance and none of the pups showed any external abnormalities. Statistically significant reproductive effects observed in the high dose group included reduced numbers of implantation sites and corpora lutea, decreased mean litter weights on days 0 and 4, and the number of live pups on day 4. There were no abnormalities in any other parameter: reproductive performance fertility index, fecundity index, precoital interval, gestation duration, pup survival, post-implantation loss, number of implants, live and dead pups, sex ratio, and pup body weight and body weight change. The relevance of the effect on the sperm count parameters was evaluated in a separate report. There it is concluded that the effect probably is not compound-related because primary determinants of sperm counts in epididymis and testis occur prior to the period TXIB exposure as used in this study. Sperm motility values also suggest there was no functional problem with the sperm (ECC 2008). The full report of this study was obtained from the study sponsor and evaluated. Based on this the observed effects in this study are evaluated as follows. The reductions in implantation sites and corpora lutea as observed in the highest dose group clearly indicate an early effect on reproduction. The biological significance of the observed changes on sperm count parameters, however, is doubtful. The variability in these parameters is known to be high and the observed changes lacked a dose related pattern. Histopathology in testes and epididymus, which can be regarded as a more stable measure of male reproductive toxicity, showed no effect. In conclusion this study does not provide clear evidence for a specific effect on sperm production. Based on the reductions in implantations and corpora lutea, the NOAEL for reproductive effects from this study is 276 mg/kg/day in males and 359 mg/kg bw/day in females.

**TDI derived by EFSA (2006)**

No TDI was derived for TXIB (not needed within the Food Contact evaluation system) (EFSA 2006).

## References

ECC (2007) Toxicity Summary for Eastman® TXIB. Prepared by: Product Safety & Health Eastman Chemical Company Kingsport, Tennessee 37660. November 28, 2007.

EFSA (2006) Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 12th list of substances for food contact materials Question N° EFSA-Q-2004-073, EFSA-Q-2006-024, EFSA-Q-2005-238a, EFSA-Q-2005-238b, EFSA-Q-2005-238c, EFSA-Q-2006-012, EFSA-Q-2006-027. Adopted on 26 & 27 September 2006. The EFSA Journal (2006) 395 to 401, 1-21

Gearhart, S, David, RM (2001) 2,2,4-Trimethyl-1,3-Pentanediol Diisobutyrate – Reproduction/developmental toxicity screening test in the rat. Toxicological Sciences Laboratory Health and Environment Laboratories Eastman Kodak Company Rochester, New York 14652-6272 USA. Final report. Supplied by Eastman Chemicals upon request by RIVM.

RIVM (2001) Drinking Water Material Constituent Summary Safety Data Sheet: 2,2,4-Trimethyl-1,3-Pentanediol Diisobutyrate, trade name: TXIB, applicant: Plasticon, application date: July 2001.

SIDS (1995) 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate. SIDS Initial Assessment Report for SIAM 3, Williamsburg, Virginia, 13-15 February 1995.