Migration of di-(2-ethylhexyl)phthalate (DEHP) and diisononyl phthalate (DINP) from PVC articles

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Zusammenfassung

DEHP und DINP sind Primärweichmacher, die in vielen Weich-PVC-Artikeln wie Beißringen für Kleinkinder eingesetzt werden. Die Ultraschallbad-Extraktion scheint gut geeignet zur Bestimmung der Migration von Phthalaten aus Kunststoff-Gegenständen und liefert Werte, wie sie auch beim Saugen mit Testpersonen erhalten worden waren. Die höheren Migrationsraten, die beim Kauen der Kunststoff-Artikel erhalten wurden, können mit dieser Methode nicht erreicht werden. Dazu ist es notwendig, entsprechende Korrekturfaktoren zu verwenden. Die Ergebnisse der Risikobewertung sind eindeutig bezüglich DEHP. Die Maximalwerte der Saug- und Kauversuche überschreiten den TDI-Wert. Bei zusätzlichen Belastungen mit DEHP liegen auch die Mittelwerte beim Saugen über dem TDI-Wert. Der empfohlene Richtwert wird in allen Fällen um die Faktoren 8 bis 23 überschritten. Bei DINP ist die Situation weniger bedenklich. Unter ungünstigsten Bedingungen beträgt die Aufnahme 56% des TDI-Wertes. Trotzdem sollte diesem Wert in Hinblick auf diverse Unsicherheiten Beachtung geschenkt werden. Der empfohlene Richtwert könnte in manchen Fällen um den Faktor 2 bis 6 überschritten werden.

Summary

DEHP and DINP are primary plasticizers used in many flexible vinyl products such as soft teethers that may be mouthed by young children. The ultrasonic bath extraction seems to be adequate to determine the migration of phthalates from plastic articles and gives values that are comparable with release rates observed in sucking experiments with test persons. However, the higher release rates that occur when articles are chewed are not reflected by the method. Those higher release rates could be taken into account by using appropriate correction factors.
The results of the risk assessment raise clear concern with respect to DEHP. The maximum levels both with respect to sucking and chewing exceed the TDI considerably. If other exposure routes are taken into account additionally then even the average sucking value would lead to intakes above the TDI. In any case the recommended precautionary limit would be exceeded by factors from 8 to 23. Regarding DINP the situation is less dramatic. Under the most aggravating conditions the intake would be about 56% of the TDI. Nevertheless this value should be noticed due to various uncertainties. The recommended precautionary limit could be exceeded in some cases by factors from 2 to 6.

Introduction

Recently, public concern has been raised regarding possible health hazards of di-(2-ethylhexyl)phthalate (DEHP) and diisononyl phthalate (DINP), two chemicals that are used as primary plasticizers in flexible vinyl products. DEHP and DINP are often used in this function in soft vinyl toys such as teethers that may be mouthed by young children.
An enormous number of studies dealing with toxicological aspects of DEHP has been published. Several reviews are also available (1,2,3). Particular attention attracted a phenomenon referred to as "peroxisome proliferation" observed in animal experiments, most notably in mice and rats. This phenomenon which is the strongest in the liver is characterised by an increased number and size of peroxisomes (cell organelles involved in the metabolism of certain fatty acids), by induction of enzyme systems (e.g. peroxisomal β-oxidation, carnitin-

acetyl-transferase, cytochrom P 450 4A) and hepatomegaly (liver enlargement) due to hypertrophy (increased cell size) and hyperplasia (increased cell number). Peroxisomal induction has been associated with the formation of liver tumors although the mechanisms involved have not yet been fully elucidated. Mice and rats are the most sensitive species as far as peroxisome proliferation and tumor induction by peroxisome proliferators is concerned whereas primates seem to be by far less responsive or non responsive. Consequently the relevance of that type of tumor formation to humans has been questioned. DEHP is not considered as genotoxic substance, but appears to be a tumor promotor.

However, peroxisome proliferation induced tumorigenesis is not the only relevant toxicological endpoint. Several studies have shown that DEHP is embryotoxic and teratogenic in rodents. In addition, adverse effects fertility and on the male and female reproductive system including testicular atrophy, reduction in sperm motility and concentration, increase in the number of abnormal sperm as well as histopathological damages have been observed (4,5,6).

Only a limited number of studies concerned with toxicological aspects of DINP exist. An overview is given in (7). Peroxisome proliferation in rat liver seems to be weaker compared to DEHP (8). However, increases in liver and kidney weights as well as histopathological changes in liver and kidneys of male rats have been found without peroxisome induction (9). Hepatocellular carcinoma and adenoma could also be observed in rats and mice. In standard assays DINP has been shown to be nongenotoxic (7).

Data on reproductive and developmental effects are scarce, but in one study (10) it was found that the response to DINP was much weaker in terms of developmental toxicity compared to DEHP.

The purpose of the study was to determine the release of DEHP and DINP from PVC sheets and PVC toys into saliva in practical experiments by using voluntary test persons, to identify a suitable laboratory test method that yields values approximately corresponding to the results of the mimic test and to carry out a risk assessment.

Materials
PVC sheets with a thickness of 0,23 mm containing only DEHP as a plasticizer (made available by the Laboratory of the Government Chemist, Great Britain) were used for the migration tests with the size 5*5 cm.

Teethers "Softy Little Hand and Feet" and "Softy Vinyl Sweets" were obtained from Artsana S.p.A., Como, Italy. Plastic animals and dolls were purchased in local shops in Vienna. Pieces of approximately 10-15 cm² (both surfaces and edges) for the sucking tests and of 20-30 cm² total area (both surfaces and edges) for all other tests were cut out by means of a Stanley-knife.

The saliva simulant BS 6684 according to the British Standard Specification for Safety Harnesses (11) contained the following substances: 4,5g sodium chloride, 0,3g potassium chloride, 0,3g sodium sulfate, 0,4g ammonium chloride, 0,2g urea and 3,0g lactic acid solved in 1000ml distilled water adjusted to pH 4,5 to 5,0 with 5M sodium hydroxide in water. All the substances were analytical grade and purchased from Merck (Germany). DEHP purum and DINP technical mixture of esters with isomeric C_9 alkyl groups were from Fluka (Switzerland); dichloromethane p.a., chloroform p.a., ethanol p.a. and THF p.a. were purchased from Merck (Germany).

As phthalates are ubiquitous environmental contaminants, considerable care was taken to avoid sample contamination. Glassware was used whereever possible, and it was rinsed several times with chloroform or dichloromethane immediately prior to use.

Methods

Migration tests
<u>Migration at static conditions</u>
The PVC sheets (5*5cm) or the cut out pieces of the various PVC articles (20-30cm²) completely covered by the liquid on both sides were extracted without shaking with 100ml of saliva simulant at 37°C for 3 and 6 hours.
<u>Shaking</u>
The PVC sheets (5*5cm) or the cut out pieces of the various PVC articles (20-30cm²) were shaken without mechanical strain covered by the liquid on both sides again with 100ml of saliva simulant at 37°C. The frequency of shaking was always 140/minute.
<u>Ultrasonic Extraction</u>
A glass beaker containing 100ml artificial saliva or de-ionized water, the PVC sheets (5*5cm) or the cut out pieces of the various PVC articles (20-30cm²) were placed in an ultrasonic bath (Bransonic 221,frequency 48 kHz, power output 50 watt, dimension 24x13,5x10 cm) which was adjusted to a temperature of 37°C and the samples were extracted for 1, 3 and 6 hours.
<u>Sucking Experiments</u>
The sample size was smaller than in the other tests. The PVC sheets were 2,5x2,5cm and the cut out pieces of the PVC articles were 2-3 times 2-3 cm with a total area (both sides and edges) of 10-15 cm².
Basically two types of experiments were made. In one test series the instruction was given to suck the item without using teeth, i.e. to keep the sample moving in the mouth without biting it. In the second test series the instruction was given to make use of teeth, i.e. to chew the piece like a chewing gum.
The tests were performed by one of the authors and some students.
The saliva (about 50ml per hour) was collected in Erlenmayer flasks containing 50 or 100 ml dichloromethane or chloroform depending on the duration of the test.

Sample Processing
The (artificial) saliva or water extracts were shaken with 1x50ml and 2x 25ml dichloromethane. The double amount of solvent was used in the 6 hours sucking experiments as the amount of the saliva was about 300ml.
Originally chloroform was used for the determination of the DEHP migration from PVC sheets. It was found that dichloromethane gives the same results. Hence, chloroform was replaced by the less toxic dichloromethane.The combined organic phases were dried with Na_2SO_4 and evaporated to a volume of 1ml (12).

Analytical Determination
High performance thinlayer chromatography (12)
10, 20 or 40µl of each sample (depending on the phthalate concentrations) and 10µl standard solution was applied to HPTLC plates RP 18WF$_{254}$S (10*10cm, 0,2cm layer thickness) from Merck and developed with a mobile phase composed of acetone/water/ethanol (60/20/20 v/v). The quantitative evaluation was carried through with a CAMAG TLC/HPTLC scanner (serie 76510) at 254 nm (deuterium lamp 80V, 0,3A).
This method requires low amounts of solvents, is very fast (10 minutes run time for 10 samples on one plate) and is as sensitive as HPLC because the sample can be concentrated on a very small spot.

High performance liquid chromatography
HPLC was used to verify some of the DC results. A 20cm LiChrosorb RP-18 column (Merck) was used. The mobile phase consisted of 95% acetonitril and 5% water. 20µl were injected (wavelength of detection 254 nm).

Migration of DEHP
The measurements were performed by using a PVC sheet which contained approximately 32% DEHP. Repeated measurements from different locations of the sheet showed a homogenous distribution of the plasticizer (6 determinations, 32,4 +/- 1 % sd).
The samples except for the sucking test were extracted with artificial saliva according to the British standard (pH 5). The values are mean values of 5-7 determinations.

Migration of DINP
Most experiments were made with PVC teethers containing about 36% DINP (5 determinations, 36 +/- 1,8 % sd). Also in this case the plasticizer was evenly distributed. The values are mean values of 3 - 10 determinations.

Results

Fig. 1: DEHP (µg/dm²), summary
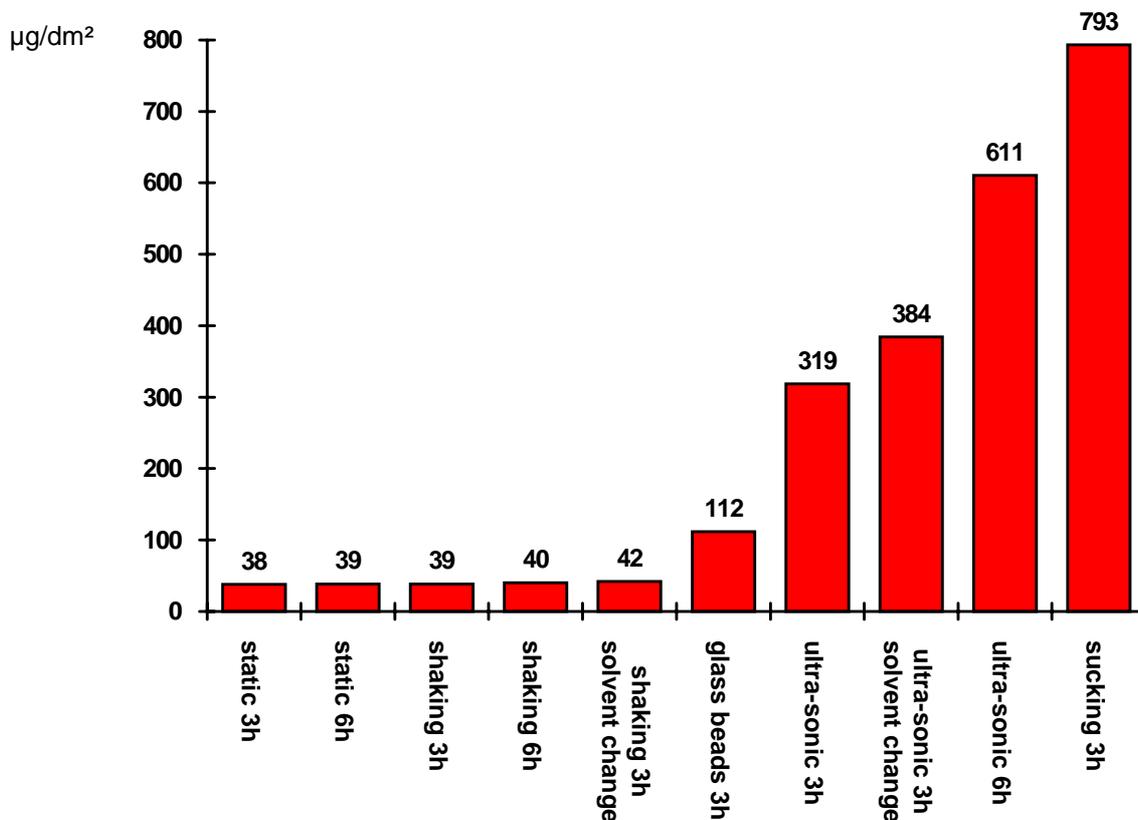
In line with our previous study (12) we could observe a significant difference between the static and the sucking tests (Fig. 1). Whereas the result was 38µg/dm² (3h) for the static test it

was 793 µg/dm² (3h) in the sucking experiment. This corresponds to a factor of about 20. Not in line with our first study was the observation that shaking of the sample did not lead to increased values compared to the static test. The value was 39 µg/dm² (3h) . Neither an extended extraction time of 6 hours nor the replacement of the solvent once per hour led to an increase of the release rates.

Only the use of an ultrasonic bath resulted in values of at least the same order of magnitude as the in vivo tests. An extraction on the ultrasonic bath gave values of 319 µg/dm² (3h) and 611 µg/dm² (6h). It should be noted that the latter value (6h) was still below the result of 3 hours of sucking (793µg/dm²). The replacement of the solvent once per hour did no significantly increase the migration (384 µg/dm² (3h)). From this we concluded that the ultrasonic bath extraction was the most appropriate method to simulate the use by small children.

Fig. 2a: DINP (µg/dm²), yellow teether, summary
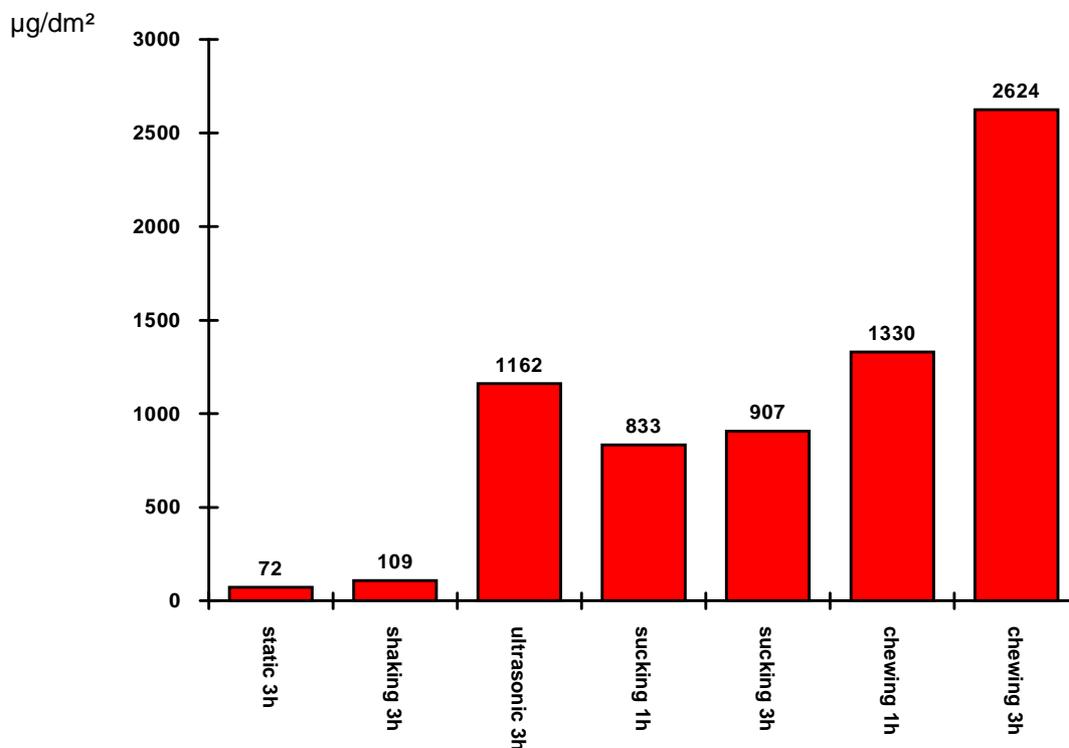
As in the case of DEHP the static values for DINP were significantly lower than the ones measured in the sucking tests (Fig. 2a). There was only a small difference between the static test and shaking of the sample (72 µg/dm² (3h) versus 109 µg/dm² (3h)).

Two types of sucking experiments were carried out. In the first one instructions were given not to use teeth whereas in the second one the test persons were advised to make use of their teeth and to chew the PVC - sample like a chewing gum.

Normal sucking for three hours led to values of 907 µg/dm² (3h). With additional chewing of the specimens values of 2624 µg/dm² (3h) were obtained. Remarkably the result of 1h sucking is not much less (833 µg/dm² (3h)) than the result of 3 hours sucking (907 µg/dm² (3h)), whereas the 3 hours chewing values are twice as high than the one hour chewing values. It should be noted that in this test series the ultrasonic bath extraction gave values (1162 µg/dm² (3h)) that are in quite good agreement with the sucking values (3h).

In the following some results of figure 2a are presented in more detail. Figure 2b shows the variation of the results obtained by using the ultrasonic extraction.

Fig. 2b: DINP, yellow teether, ultrasonic extraction (3h), mean value+/-sd: 1161,8+/-371,6 µg/dm²
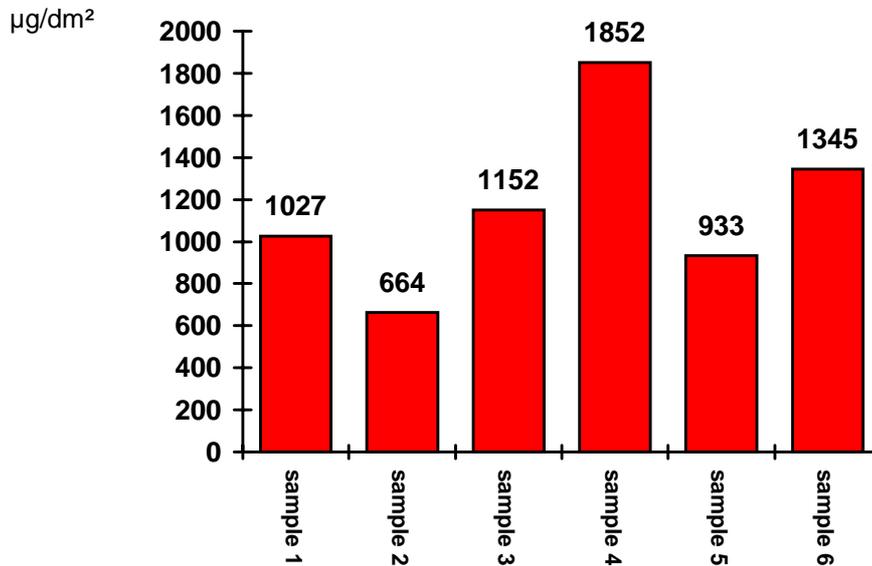
There is a factor of about 3 between the minimum and the maximum value. The variation is not a result of inhomogenous distribution of the plasticizer as explained above.
Figure 2c presents the results of a test series where students sucked a piece of a teether without chewing on it. All students, whose names are indicated, took part in 2 experiments.

Fig. 2c: DINP, yellow teether, sucking experiment by test persons (1h) without chewing, mean value+/-sd: 832+/-397 µg/dm²
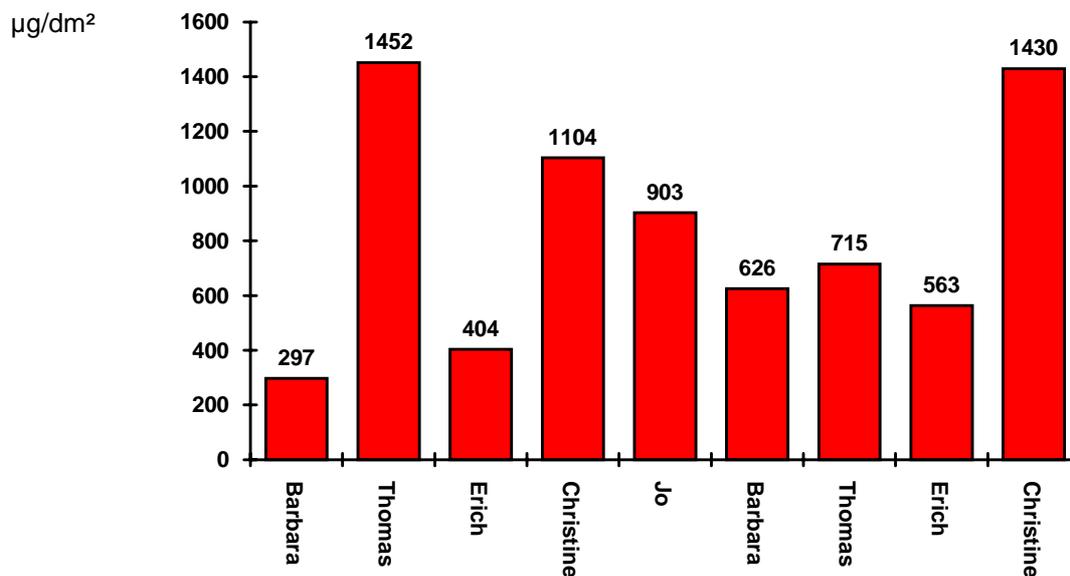
Figure 2d shows the results of a test series where students chewed a piece of a teether and employed their teeth. We excluded the highest value from the calculation of the mean value as it turned out that the student bit off small particles from the sample which were extracted together with the saliva with dichloromethane. Hence, the value does not only reflect the migration of DINP into saliva but also the migration of the plastiziser into the solvent. It cannot be excluded that this happened also in some other tests. Therefore the values presented in figure 2d should be taken with caution.

Fig. 2d: DINP, yellow teether, chewing of test persons (1h), mean value (excluding value5839)+/-sd: 1330 +/-517 µg/dm²
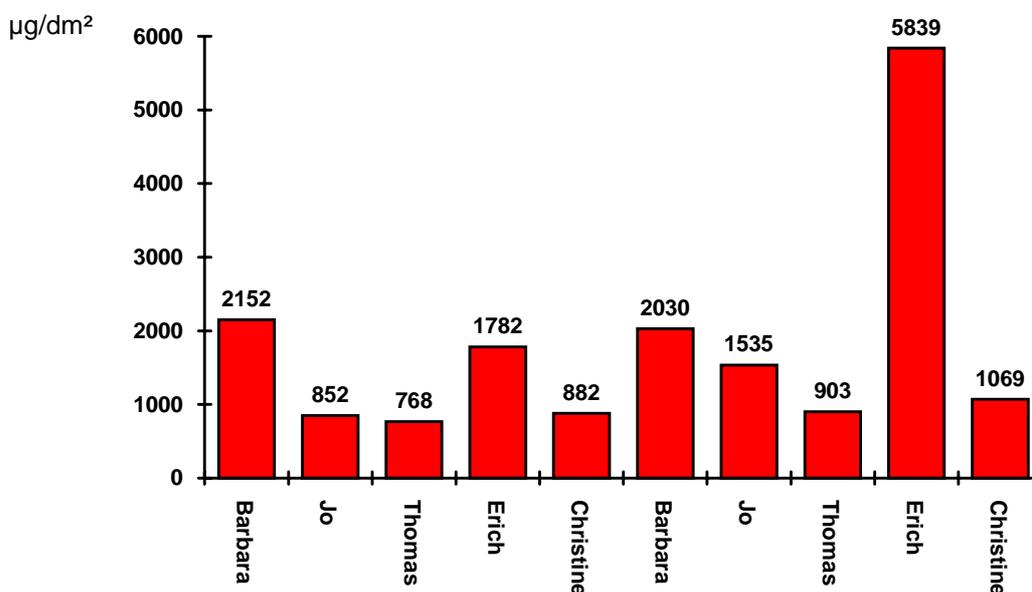
Figures 2b, 2c and 2d also show that the standard deviation for the ultrasonic extraction (32%) is somewhat lower than the ones calculated for sucking without chewing (48%) and for chewing (39%). Any standardized test method should contain a provision to ensure that a sufficient number of samples are tested.

Figure 3a shows the summary of another test series. In this case red teethers were used. The values are mean values of 3 - 10 determinations

Fig. 3a : DINP (µg/dm²), red teether, summary
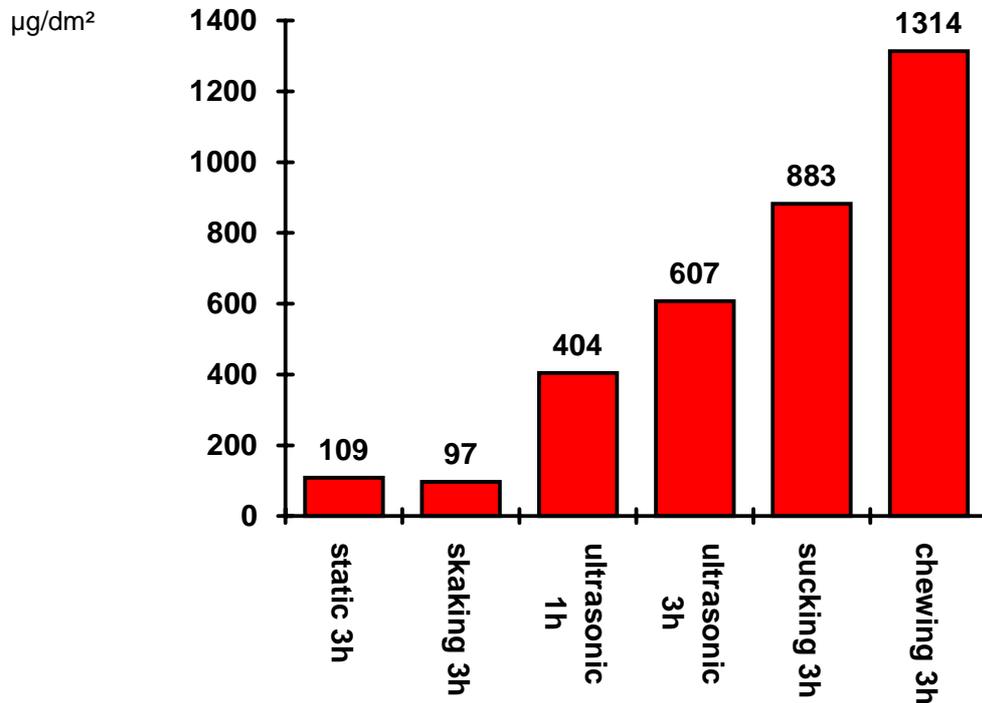
The results of 3h sucking - 883µg/dm² on average - were in good agreement with the results shown in figure 2a (907µg/dm²). Whereas the 3h ultrasonic extraction value in figure 2a was higher than the 3h sucking value in this series the reverse was found.
In figure 3b the influence of the pH value and the composition of the extraction fluid (artificial saliva versus water) is shown. The values are mean values of 3 - 5 determinations.

Fig. 3b: DINP (µg/dm²), red teether, ultrasonic extraction (3h)
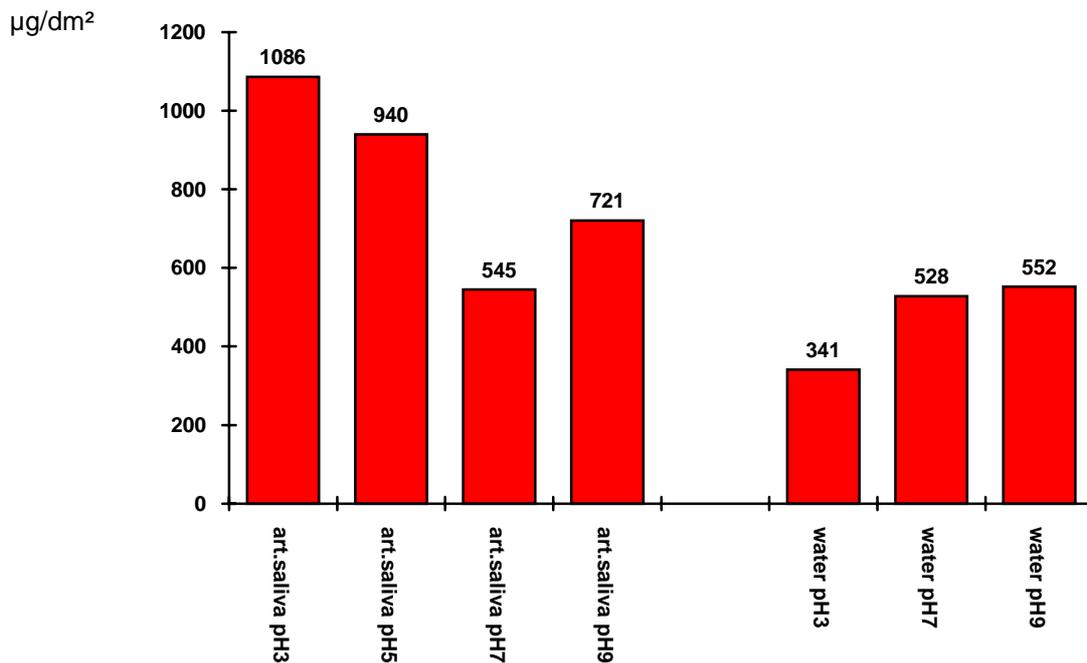
In general, the artificial saliva solutions gave higher release rates than water. There is a factor 2 between pH 7 and pH 3 samples when artificial saliva solutions were used. Consequently the well proven artificial saliva solution according to the British standard with a pH of 5 should continue to be used.

Finally figure 4 gives an overview of ultrasonic extractions (3h) of various plastic articles the average DINP content of which was 34%. The values are mean values of 3 - 6 determinations.

Fig. 4: DINP (µg/dm²), plastic articles, ultrasonic extraction (3h)
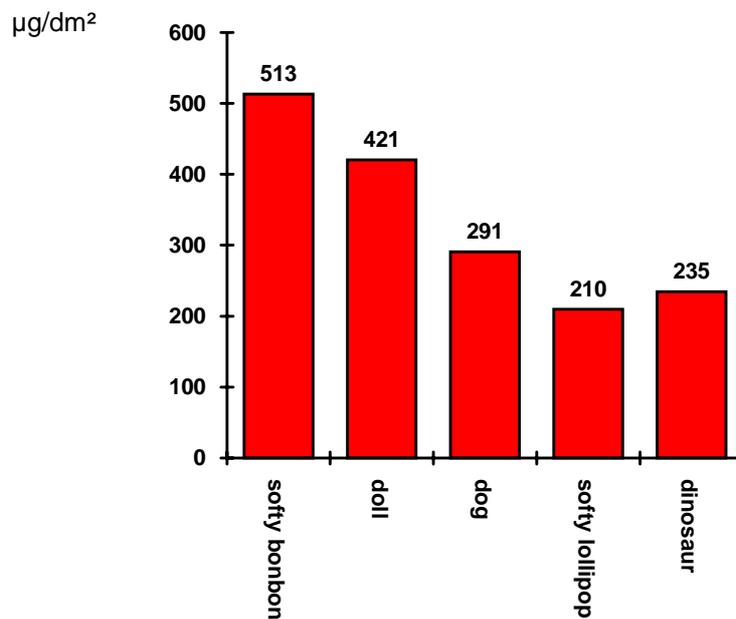
All PVC articles tested had a lower migration into artificial saliva as the yellow and red teethers. However, our data are too limited to draw the conclusion that those articles in general have lower release rates of phthalate plasticizers than teethers.

Discussion

Methodological conclusions

The ultrasonic bath extraction presented in this document seems to be adequate to determine the migration of phthalates from plastic articles and gives values that are comparable with release rates observed in sucking experiments with test persons.

However, the higher release rates that occur when articles are chewed are not reflected by the method. Those higher release rates could be taken into account by using appropriate correction factors.

DINP

Taken together all 3h ultrasonic values presented in figure 2a (yellow teether, 1162 µg/dm²), and figure 3a (red teether, 607 µg/dm²) the total average of 15 measurements is about 830 µg/dm².

In the same way an average figure of about 900 µg/dm² can be calculated from the 14 measurements of the three hour sucking experiments involving both types of teethers.
The values measured after one hour sucking of the yellow teether according to figure 2a was about 830µg/dm². This seems to correspond perfectly with the 3 hours value of the ultrasonic extraction mentioned above (830 µg/dm²).
It shoud be noted that there is only a small difference between one hour and three hours of sucking (830 versus 900 µg/dm²).

DEHP
As far as DEHP is concerned the correspondence between ultrasonic extraction and sucking was less good. Whereas the value for 3h sucking was on average 793 µg/dm² in line with the DINP results, the ultrasonic extractions gave considerably lower values (318 µg/dm²). Even after 6 hours the results of the ultrasonic extraction (610 µg/dm²) were lower than those for the sucking test. A higher extraction time may be needed for DEHP. In any case further data are needed covering other DEHP containing products.

Risk assessment

a) Dose - response relationships

DEHP
The lowest NOAEL (no observed adverse effect level) for peroxisome proliferation in rat (5 mg/kg bodyweight/day) formed the basis for the establishment of a TDI (tolerable daily intake) value of 50 µg/kg bodyweight/day by the European Scientific Committee for Food taking into account a safety factor of 100 (13). The induction of hepatic tumors requires much higher doses of about 300 mg/kg bodyweight/day with reported NOAELS of 50 - 100 mg/kg bodyweight/day (1).
Recent studies suggest, that peroxisome proliferation is not the most sensitive endpoint in rat experiments. Severe damage to the testes of the offspring of female rats exposed to DEHP during and after pregnancy could be observed histologically at levels as low as 3.0 - 3.5 mg/kg bodyweight/day (6). In another study where mild Sertoli cell vacuolation was found a NOAEL of 3.7 mg/kg bodyweight/day was determined (5). The latter was chosen as basis for the risk assessment of the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) regarding phthalate migration from soft PVC toys and child-care articles (14) not just because of the fact that the value is lower than the one previously used but more because of the higher relevance to humans compared to the one based on peroxisome proliferation. The same value was used by ourselves. By applying a safety factor of 100 a TDI value of 37 µg/kg bodyweight/day can be derived.

DINP
The lowest NOAEL with respect to peroxisome proliferation was 18,2 mg/kg bodyweight/day in rodents (7). Increased incidence of hepatocellular carcinoma was observed only at high doses exceeding 600 mg/kg bodyweight/day but not at levels of 300 mg/kg bodyweight/day which one might consider as NOAEL regarding carcinogenesis. The lowest NOAEL regarding the induction of neoplasms in rat was 88 mg/kg bodyweight /day (14).
It has been already outlined above that hepatotoxic effects in rats have been found at dose levels below those necessary for peroxisome induction. Increased organ weights of liver and kidneys as well as histopathological liver changes including spongiosis hepatis, a persinusoidal cell degeneration have been found at a level of 150 mg /kg bodyweight/day but not at the next lower dose of 15 mg/kg bodyweight/day (9). Hence, the latter value is to be considered as the lowest reported NOAEL. It has been used by the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (14,15). Our risk assessment is based on the

same value. The corresponding TDI value by applying a safety factor of 100 is 150 µg/kg bodyweight /day.

b) General exposure assessment

DEHP
The major source of human exposure to DEHP is food as far as the general public is concerned. In the Canadian study (3) the daily intake from food by children aged 0 to 0,5 and 0,5 to 4 years was estimated to be about 8µg/kg bodyweight/day and 18µg/kg bodyweight/day, respectively.
The Ministry of Agriculture, Fisheries and Food in the UK carried out a survey of phthalates in infant formulae (16) on the basis of which average intakes of phthalates by infants up to 6 months of age were estimated. For DEHP values between 6,1 and 35 µg/kg bodyweight/day were found. The latter value is quite close to the TDI of 37 µg/kg bodyweight/day.
Unfortunately there are no published data regarding the concentrations of phthalates in human milk. However, there is mention of a study in the second report by the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (14). In this study the intake of DEHP based on levels in human milk and infant formulae has been estimated. The maximum levels corresponded to 25 µg/kg bodyweight/day which is about 70% of the TDI.
Contact with PVC products containing phthalates may result in skin absorption. Maximum absorption rates for DEHP of 1,06 µg/cm²/h based on in vitro experiments have been reported (17). If the contact area and the contact time are assumed to be 1 dm² and 3 hours the absorbed dose will be about 40 µg/kg bodyweight/day for an 8 kg infant.
Further exposure sources such as contaminated indoor air may also contribute to the total intake. We conclude that the actual exposure to DEHP may constitute a significant proportion of the tolerable daily intake with maximum values almost approaching the TDI even without consideration of exposure to toys.

DINP
No exposure data are available. It appears that DEHP has been replaced to some extent by DINP in the recent past at least as far as toys are concerned. At present DINP accounts roughly for 10 to 15% of the total phthalate production (7) but this may increase in future. It can be assumed that the levels of DINP in the environmental compartments will increase with the prolonged and widespread use of the plasticizer.

Other phthalates
It should be noted that DEHP and DINP are not the only phthalates humans are exposed to. For instance, the average intake of total phthalates from infant formulae was 100 to 130 µg/kg bodyweight/day (16). Apart from DEHP dipropyl-, diisobutyl-, dibutyl-, butylbenzyl-, dihexyl-, dicyclohexyl-, diheptyl-, dioctyl- and dinonylphthalate were identified.

c) Exposure to phthalates from toys and risk characterisation

Following the recommendation of the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (14) it is assumed that the weight of the child is 8 kg, the daily exposure is 3 hours and the mouthed area is 10 cm² . The exposure time has been reduced from 6 hours to 3 hours compared to the first report (15) in the light of the findings of a Dutch investigation (18).
It does not seem to be appropriate to base the risk assessment on values indicating the release of a plasticizer from the same area of 10 cm² in 3 hours. As has been indicated above (figure 2a) there is no big difference between 1 hour and 3 hours sucking. This does not hold true any longer if the realistic assumption is made that the child would change the position of the

article in the mouth. In this case the intake of a plasticizer would of course be much higher as the new area would contain a "full load" of plasticizer. Hence, the assessment is based on the migration value of 10 cm²/hour measured in one hour and this is multiplied by 3 rather than the migration value of 10 cm²/3 hours is used.

In case of DINP the average value for one hour sucking (yellow teether) was 833 µg/dm². Three times this value gives approximately 2500 µg/dm² or 250 µg/10 cm². Given a body weight of 8 kg this means an intake of 31,25 µg/kg bodyweight/day which is about 21% of the TDI value of 150 µg/kg bodyweight/day. The maximum value in the one hour sucking experiment (yellow teether) was 1452 µg/dm². This value corresponds to an intake of 54,5 µg/kg bodyweight/day or about 36% of the TDI.The maximum value in the one hour chewing experiment (yellow teether) was 2252 µg/dm² which is equivalent to an intake of 84,5 µg/kg bodyweight/day or 56,3% of the TDI.

Our data regarding the in vivo release of DEHP are limited. However a comparison between the 3 hours sucking values suggests that the migration of DEHP does not differ from the migration of DINP. Hence, it is assumed that this holds true also with respect to 1 hour sucking and 1 hour chewing. From this follows that the intake values calculated above would be applicable to DEHP as well. The TDI of DEHP is 37 µg/kg bodyweight/day. The average value for one hour sucking would correspond to 83% of the TDI of DEHP, the maximum value of one hour sucking would correspond to 147% of the TDI and the maximum value for one hour chewing would correspond to 228% of the TDI.

The exposure of children to phthalates, however, is not limited to toys. In addition, there is a number of other exposure routes such as air, water, food and articles other than toys as outlined above. In case of DEHP the actual intake under worst case assumptions may already approach the TDI even if exposure to toys is not taken into consideration. Therefore it does not seem to be appropriate to disregard all other sources of exposure. Further, children (and adults) are exposed to a variety of different phthalates. No studies are available dealing with possible additive effects of this group of plasticizers. Very young children may be more sensitive to toxic effects of phthalates. As far as DINP is concerned the data base is rather limited, particularly for reproductive toxicity. The key question is which proportion of the TDI value should be allocated to toys or child care articles in the light of the uncertainties mentioned above.

In a CEN report on safety aspects of child care articles (19) a limit of 10% of the TDI value is suggested which seems to be justified from a precautionary point of view the more so as the exposure to phthalate containing toys and child care articles for small children can be easily avoided. A similar approach was chosen in the European Toys Directive (20) for limits of heavy metals.

Our results raise clear concern with respect to DEHP. The maximum levels both with respect to sucking and chewing exceed the TDI considerably. If other exposure routes are taken into account additionally then even the average sucking value would lead to intakes above the TDI. In any case the precautionary limit would be exceeded by factors from 8 to 23.

Regarding DINP the situation is less dramatic. Under the most onerous conditions the intake would be about 56% of the TDI. Nevertheless this value raises some concern given the uncertainties mentioned above. The precautionary limit would be exceeded by factors from 2 to 6.

In the light of the findings presented above manufacturers are recommended to explore the opportunities to replace the referred substances by less problematic ones. The use of DEHP and DINP should be discontinued in toys and child-care articles intended for small children.

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