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NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT

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Phthalate release from soft PVC baby toys
Report from the Dutch Consensus Group

Könemann, W.H., ed.

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This investigation has been performed at the request of the Ministry of Health, Welfare and Sports

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Samenvatting

Op verzoek van de staatssecretaris van Volksgezondheid, Welzijn en Sport, heeft een werkgroep van vertegenwoordigers van betrokken partijen de afgifte van ftalaten uit zacht PVC babyspeelgoed onderzocht. Omdat di-isononylftalaat (DINP) veruit de meest gebruikte weekmaker in PVC babyspeelgoed is, is alleen dit ftalaat in het verdere onderzoek nader beschouwd. Het volgende experimentele onderzoek is uitgevoerd.

- *een vrijwilligersonderzoek om afgifte van DINP uit PVC-monsters aan speeksel te bepalen (uitgevoerd door TNO Voeding)*

- *een observatie-onderzoek met kinderen om te bepalen hoe lang kinderen via de mond in contact zijn met babyspeelgoed (uitgevoerd door de Landbouwuniversiteit Wageningen)*

- *een nieuwe beoordeling van de blootstelling van baby's aan DINP uit zacht PVC (uitgevoerd door het RIVM)*

- *de ontwikkeling van een routine laboratoriummethode voor de bepaling van de afgifte van DINP uit zacht PVC babyspeelgoed (hoofdzakelijk uitgevoerd door TNO Voeding, met ondersteuning van enkele andere laboratoria)*

Op grond van de resultaten van dit onderzoek en aannemend dat zacht PVC babyspeelgoed de belangrijkste bron van blootstelling van baby's aan DINP is, wordt geconcludeerd dat blootstelling van kinderen die ouder dan 1 jaar zijn, duidelijk onder de maximaal Toelaatbare Dagelijkse Inname (TDI) van 0,15 mg/kg/dag ligt, voor het onderzochte monster. Voor kinderen van 3 - 12 maanden kan de blootstelling in zeldzame gevallen de TDI benaderen of overschrijden.

Er wordt een routine laboratoriummethode voorgesteld voor de bepaling van de afgiftesnelheid van DINP. Tevens wordt een methode voorgesteld om een maximaal toegestane afgiftesnelheid af te leiden.

Summary

At the request of the Secretary of State for Health, Welfare and Sports a working group of representatives from interested parties has investigated the release of phthalates from soft PVC baby toys. Since di-isononylphthalate (DINP) appeared to be the phthalate predominantly used in soft PVC toys, only this phthalate was considered in detail in any further work. The following experimental studies have been carried out

- *a human volunteers study to determine release rates of DINP from PVC samples into saliva (carried out by TNO Nutrition and Food Research Institute).*
- *a child observation study to determine the oral contact time of young children with baby toys (carried out by the Wageningen Agricultural University)*
- *a new assessment of the exposure of babies to DINP from soft PVC toys (carried out by RIVM)*
- *development of a routine laboratory method to determine the release rate of DINP from soft PVC baby toys (carried out mainly by TNO Nutrition and Food Research Institute, with assistance from laboratories of other Consensus Group members)*

Based on the results from these studies and assuming that soft PVC baby toys are the most important source of exposure of babies to DINP, it is concluded that exposure levels of children >12 months were well below the maximal Tolerable Daily Intake (TDI) of 0.15 mg/kg/day, for the sample tested. For children of 3 - 12 months in rare cases the exposure may approximate or exceed the TDI, if the sample tested would be representive for products on the market.

A proposal is given for a routine laboratory method to determine release rates of DINP from toys. Also a method is given to derive a maximum acceptable release rate.

1. Introduction

In 1997 the Netherlands' Health Protection Inspectorate (IGB) determined release rates of phthalates from baby toys. Like in some other countries considerable releases were found.

In a risk assessment carried out by the National Institute of Public Health and the Environment (RIVM)[1] the resulting uptake by young children was estimated to approximate or exceed the tolerable daily intake (TDI). Subsequently the Chief Inspector for Health Protection recommended retailers to voluntary withdraw soft PVC baby toys[2] from the market.

PVC producers, toy industry and retailers questioned the realistic nature of the data used for the risk assessment. In reaction the Secretary of State for Health, Welfare and Sports asked the RIVM to reach consensus with all interested parties (inspectorate, consumer groups, industry, retailers) on a method to assess the risk of the release of phthalates from baby toys, with special emphasis on a method to determine release rates.

2. Overview of activities

A working group of representatives from interested parties, chaired by the RIVM (hereafter called the 'Consensus Group': the list of participants is attached as Annex 1) met and concluded that a laboratory method to determine release rates of phthalates from PVC baby toys should give releases similar to the actual release of phthalates from toys into the mouth of babies. Information on the actual (*in vivo*) release was not available from the literature, however, and the Consensus Group decided that such information should be produced. The Consensus Group also agreed that information on the actual daily of exposure of children to PVC baby toys would be useful, since current estimates were not based on observations of child behaviour or the data were contaminated with data including dummies/pacifiers.

Since di-isononylphthalate (DINP) appeared to be the phthalate predominantly used in soft PVC toys, only this phthalate was considered in detail in any further work.

This has led to the following activities:

- a human volunteers study to determine release rates of DINP from PVC samples into saliva (carried out by TNO Nutrition and Food Research Institute).
- a child observation study to determine the oral contact time of small children with baby toys (carried out by the Wageningen Agricultural University)
- a new assessment of the exposure of babies to DINP from soft PVC specimens as specified in this study (carried out by RIVM)
- development of a routine laboratory method to determine the release rate of DINP from PVC baby toys (carried out mainly by TNO Nutrition and Food Research Institute, with assistance from laboratories of other Consensus Group members)

[1] Phthalates in teething rings/animal figures for infants. (RIVM, 15 January 1998, update of 25 September 1997) P. Janssen, M. van Veen, M. van Apeldoorn, G. Speijers

[2] by this is meant flexible plastisiced PVC products

The studies were all carried out under the responsibility of the Consensus Group. All findings of these studies have been discussed and accepted by the group. Summaries of the reports of these studies can be found as annexes 2 - 5. Full study reports will be made public as soon as possible.

It is not feasible to give here a full account of all discussions held by the Consensus Group. In a complicated issue as the case in question, several implicit or explicit assumptions are made to allow final conclusions. Some indications of the issues discussed will be given, to support the final conclusions of the Consensus Group. Further information on discussions held may be found in the minutes of the Consensus Group meetings (see Annex 1).

2.1 test materials

The Consensus Group decided that standardised test specimens were necessary for its studies to ensure reproducibility of the measurements. The use of reference specimens produced by an industrial research laboratory was preferred, since these specimens could be produced under standardised conditions and with a well defined composition, representative of relevant commercial products (details may be received upon request). From this reference materials, 10 cm^2 disks (total surface) were used (specimen 1). However, since it was argued that PVC toys might be produced under less optimal conditions, also a specimen of PVC toys was obtained, to test a commercial sample. Therefore in the human volunteers study also a 10 cm^2 surface (the tip of a finger of a hand shaped toy) of an intact toy has been used, to ensure that only contact with the outer surface was possible (specimen 2). From these toys also 10 cm^2 disks were punched out and used, to facilitate comparability with the standard disks (specimen 3). The appearance of the inside of this disk differed from the outside. In the development of a routine laboratory method only specimens 1 and 3 have been used.

The composition and appearance of specimens 1, 2 and 3 can be summarised as follows

specimen	*shape*	*appearance*	*total surface*	*thickness*	*DINP content*
# 1	disk	shiny	10 cm^2	3 mm	38 % (w/w)
# 2	fingertip	matt	10 cm^2	ca.. 2.5 mm	ca. 43 % (w/w)
# 3	disk	shiny/matt	10 cm^2	2 - 4 mm	ca. 43 % (w/w)

2.2 volunteers study

It was accepted that it is impossible to carry out studies with young children for practical and ethical reasons. Therefore it was agreed to use adults as a surrogate. Differences in the way of chewing and sucking on toys between children and adults were not considered to introduce a significant error in the exposure estimate. The intra-individual variation in composition of saliva (in particular pH, proteins) between the volunteers did not have influence on the release rate of DINP. Differences in composition of saliva of children and adults might be more significant, but quantitative information was nog available and such differences were therefore ignored.

In the volunteers study the DINP content of saliva was used as the measure for the release rates from PVC. It appeared to be not technically feasible to determine quantitatively the amount adsorbed to the surface of the oral cavity. This could lead to an underestimation of the amount released and absorbed. On the other hand, young children do not swallow all

saliva produced. Because these opposite influences could not be quantified, the consensus group decided to ignore them.

A summary of the results of the volunteers study can be found in Annex 2.

The average levels of release of specimens 1, 2 and 3 (of 1.4 µg/min, 2.4 µg/min and 1.6 µg/min, respectively) correspond well. A possible explanation of the 50% higher releases of specimen 2 is the different shape of this specimen, leading to a different interaction in the mouth during biting and sucking.

Overall, the consensus group found it acceptable to use the release rates as measured in the volunteers experiment as the best estimate of release rates in young children.

2.3 child observation study

A summary of the results of the study can be found in Annex 3.

The major discussion on the child observation study was the definition of the relevant total mouthing (sucking, licking and chewing) time.

The observation time was restricted to the period awake. It was considered unlikely that children could mouth PVC toys during their sleep, because of the shape, size and weight of toys. This in contrast to sucking on pacifiers (which are usually not made from PVC and contain no phthalates), which are specially designed for to keep in the mouth during sleeping time.

In counting the total mouthing time no restriction was made with regard to the nature of the objects on which children were mouthing. Objects varied highly and included toys, fingers and other materials available. One scenario could, however, be the abundant availability of PVC toys, leading to mouthing of PVC toys only. Therefore it was decided to use the total mouthing time per day during the period awake as the relevant parameter for determining exposure of children to PVC toys. The highest mouthing times were found for children of 6 - 12 months.

2.4 exposure assessment

Based on the information of the human volunteers study and of the child observation study a new exposure assessment has been made, for the test materials used in these studies.

For specimens 1, 2 and 3, Annex 4 presents the age dependent exposure levels.

Since the weight of children increases and the mouthing time decreases with increasing age, exposure (expressed as mg/kg/day) was highest for children of 3 - 12 months and is considerably lower (5 - 10x) for children of 36 months.

The release from specimens 1 and 3 differ only slightly. Differences between these specimens and specimen 2 are possibly due to the nature of the surface and the shape of it, as discussed under 'volunteers study'. Since specimen 2 is the most realistic one, these data are used for the updated risk assessment.

2.5 routine laboratory method

A routine laboratory method should consist of several phases:

- sample preparation
- extraction with (artificial) saliva
- transfer from the saliva into a suitable solvent for analytical determination
- quantification with an adequate analytical method

Annex 5 gives an account of the development process for the method.

Problems were encountered with the selection of the artificial saliva and, above all, the degree of mechanical agitation during the extraction phase. Under a variety of conditions it appeared to be possible to obtain releases corresponding with the levels observed in the human volunteers study, but under many conditions it was difficult to achieve an adequate reproducibility. Slightly varying agitation conditions appeared to have large influences on the release. It was possible to reduce this variation within one laboratory, but when comparing the results between laboratories, the influence of differences in equipment appeared to be large. This explains why data from different laboratories in different countries were quite different.

Finally a method has been selected which provides repeatable results and is theoretically the most promising for standardisation between laboratories: the 'head over heels' extraction method.

Under the conditions chosen the average release rates for the specimens 1 and 3 were 3 ± 1 µg/min and of the same order of magnitude as in the human volunteers experiment (1.4 ± 1.2 µg/min and 1.6 ± 1.0 µg/min respectively).

Specimen 2 has not been used in the method development, because the use of whole toys creates many difficulties for the development of a reproducible laboratory extraction procedure. Since specimen 3 is taken from the same toy, however, and since the differences in release rates between specimens 1 and 3 are small, the use of the results of the experiments carried out is considered justified for extrapolation to the *in vivo* situation.

3. safety considerations

The TDI of DINP is 0.15 mg/kg/day[3]. A first estimate for the dietary exposure to phthalates is 0.023 mg/kg/day (source: footnote 1). No specific estimates are known for DINP. No estimate is available for other sources of exposure. It is not up to the Consensus Group to decide which fraction of the TDI should be reserved for exposure to baby toys. As a hypothesis, which should be tested, it is assumed that other sources of exposure are not higher than the exposure to food. This would leave a maximum acceptable uptake of DINP of approximately 0.1 mg/kg/day for maximum acceptable exposure to DINP from toys. This figure is provisionally used for further calculations in this report (the final choice of such a figure is left to policy makers).

[3] Scientific Committee on Toxicology, Ecotoxicology and the Environment, 24 April 1998

If the exposure data of specimen 2 presented in Annex 4 would be representative for all PVC toys, this limit can be exceeded in rare cases for children up to 12 months (so rare that the statistical likelihood cannot be estimated on the basis of the current data). In 99% of the cases the exposure would remain below 0.1 mg/kg/day. In 95% of the cases the exposure would remain under 0.04 mg/kg/day. For children > 12 months the risk is considerably lower and exceeding of the TDI is not observed.

For the interpretation of these data it should be emphasised that exceeding this level does not mean that a negative health impact will occur (because of the safety factors used in deriving the TDI), but only that the safety cannot be guaranteed.

4. policy implications

It is not the job of the Consensus Group to decide on the maximum acceptable release rates for DINP in PVC for baby toys. We will try to give an outline, however, how the above data can be used for policy development based on the measurement of release rates, once the acceptable risk has been set by policy makers.

If the 99 percentile exposure levels are chosen as the reference for acceptable exposure levels, the exposure to DINP from specimen 2 is 75% of the assumed maximum acceptable exposure to DINP from toys. This corresponds with a release rate from specimen 3 (which is punched from the same toy) in the 'head over heels' extraction test of 3 µg/min. The maximum acceptable release rate would therefore be theoretically 3/0.75 µg/min = 4 µg/min (if 0.1 mg/kg/day is used as the maximum acceptable exposure to DINP from toys).

Similar calculations can be made if 95 percentile or maximum exposure levels are chosen as the reference, or if a higher or lower fraction of the TDI is attributed to toys.

For safety purposes it is important to avoid false negatives when testing the release rates from toys. In practice, therefore maximum acceptable release rates should account for limitations of the reproducibility of a routine test method. An interlaboratory comparison study will be essential.

5. limitations

The Consensus Group has focused itself on the development of a method to determine release rates of DINP from soft PVC baby toys. It also developed data to determine the maximum acceptable release rate from PVC toys.

The group did not test other toys than those mentioned above and has therefore no insight in the variation of release rates from PVC toys.

The Consensus Group considered the method developed valid for DINP containing toys, from which 10 cm^2 disks (total surface, thickness 2 - 4 mm) can be punched. For materials with a different size and shape and for other plasticizers, part of the method development will have to be repeated.

Annex 1

THE CONSENSUS GROUP

The Consensus Group consisted of the following experts:

organisation	*name*
National Institute for Public Health and the Environment (RIVM)	dr. W.H. Könemann (chairman) dr. A.K.D. Liem
Health Protection Inspectorate	dr.ir. P.C. Bragt dr. J.C. Wildervanck dr. J.B.H. van Lierop
European Council for Plasticizers and Intermediates (ECPI)	dr. L. van Dijk drs. H.F. van Wijk ir. A. Poppe
PVC Steering Committee	dr. A.H.M. Kayen mr. W. Beerman
Toys Industries Europe (TIE)	ir. M. Twist
Consumers Organisation:	dr. R. Luijk ms.ir. D.C.C. Kok
TNO Nutrition and Food Research Institute	R. Rijk W. Meuling
Laboratory of the Government Chemist (LGC, UK)	dr. J. Braybrook *
Joint Research Centre, Ispra (EU)	ms. dr. C. Simoneau *

* Participants invited after the 4th meeting to provide additional expertise

The Consensus Group has met 10 times. Minutes of the meetings have been published on internet: http://www.minvws.nl/engzoek.asp

Annex 2

HUMAN VOLUNTEERS STUDY (SUMMARY REPORT)

W. Meuling and R. Rijk
TNO Nutrition and Food Research Institute

Twenty volunteers were recruited for a study on the release of plasticizer from PVC baby toys. The purpose of the investigation was to establish the release of plasticizers from PVC under conditions as close as possible to daily use of plasticized materials by babies.
The study has been conducted according to the ICH Guidelines for Good Clinical Practice.

Test specimens

Three plasticized PVC specimens were included in the study

Specimen 1	= Disk (23 * 3 mm, total area 10 cm^2) of a standard PVC sample (prepared under controlled condition, containing 38.5% of diisononylphthalate (DINP)
Specimen 2	= Finger (exposed area 10 cm^2) of a commercial available teething ring
Specimen 3	= Disk (23 * 2-4 mm, total area 10 cm^2) punched from a flat part of the teething ring (specimen 2)
Control specimen	= Disk (23 * 2.5 mm, total area 10 cm^2) of polytetrafluoroethylene (PTFE, Teflon)

Exposure conditions

Volunteers were instructed on biting and sucking procedure and delivery of total amount of saliva produced. According to the protocol all volunteers were invited to suck and bite on the control specimen for 10 or 15 min, in order to obtain blank saliva. After five minutes of rest all volunteers were exposed to test specimen 1 for a period of 15 min. After a rest period of 5 min the exposure procedure was repeated three times with the same specimen. The test specimens were rinsed shortly with water and used in each following exposure periods. In this way a blank and four portions of exposed saliva were collected.
Subsequently the volunteers were randomly divided in two groups of 10 persons. One group was exposed to specimen 2 and the other group to specimen 3 using the same procedure as for specimen 1. All participants completed the study.
Saliva was tested for pH, volume (by weighing), total protein content and amount of DINP. For the determination of DINP in human saliva a method was applied based on extraction of DINP with an organic solvent and quantification by means of HPLC - UV detection at 225 nm. Each collected portion of saliva was analysed in two independent determinations. The method was validated by standard addition of DINP to the blank saliva of each individual volunteer. DINP-values found in the saliva were corrected for recovery percentages.

Results

Overall mean values and standard deviation for each specimen and for the various parameters were calculated.

Table 1. Overview of results from in vivo tests.

Parameter	Specimen 1		Specimen 2		Specimen 3	
	mean	Sd/range	mean	Sd/range	mean	Sd/range
Saliva weight (g)	16.3	9.4	10.9	4.2	16.7	8.5
pH	7.31	0.12	7.26	0.16	7.38	0.11
Protein (mg/l)	497	126	539	189	463	122
DINP release (µg/min)	1.38	0.3-8.3	2.44	0.9-8.9	1.63	0.9-5.7

From the results obtained it appeared that there is no influence from the pH and protein content of the saliva, at the values observed for the volunteers. A significant influence on the total release of DINP in saliva was found from the volume of the saliva produced . However, taking the exposure time as the relevant parameter and expression of DINP release in µg/min, then there appeared to be no influence on the total volume of saliva produced during the exposure time. It was noted that the saliva production during exposure to test specimen 2 was significantly reduced compared to the other specimens.

Release in time appeared to be rather consistent. Below the mean release arranged to exposure period are presented.

Table 2. Release rates over 15 minutes time intervals

Exposure	Specimen 1	Specimen 2	Specimen 3
period	mean (µg/min)	mean (µg/min)	mean (µg/min)
0-15 min	1.50	2.88	1.79
20-35 min	1.16	1.96	1.45
40-45 min	1.29	2.46	1.50
50-65 min	1.57	2.46	1.79

Annex 3

MOUTHING BEHAVIOUR OF YOUNG CHILDREN; AN OBSERVATIONAL STUDY (SUMMARY REPORT)

M.E. Groot, M.C. Lekkerkerk, L.P.A. Steenbekkers
Department of Household and Consumer Studies, Wageningen Agricultural University.

The aim of this study is to quantify mouthing behaviour of young children. By means of an observational study the time is assessed that children lick, suck or bite on objects. Parents of children in the age between 3 and 36 months have observed their child during 10 times one quarter of an hour at two different days. During these quarters they have registered the time during which the child has put something into his/her mouth. In case of a toy they also specified the kind of toy. Data of 42 children are obtained.

The children are divided into 4 age groups, according to developmental period:

- 3 to 6 months (n=5);
- 6 to 12 months (n=14);
- 12 to 18 months (n=12);
- 18 to 36 months (n=11).

Five categories of objects are discerned: dummy/pacifier, teether, fingers, toys and non toys. The parents specified the toys involved. On the basis of this specifications the toys are divided into two groups: toys meant for mouthing and toys not meant for mouthing. This division is made according to the definition producers of toys give. It should be noted that parents make this division in another way.

The total time of mouthing behaviour in the observational period is extrapolated to the time the child is awake and not involved in eating. This will be referred to as the 'awake time per day'. This is the total time of a day that the child has the opportunity to put objects into the mouth. The results presented in figures 1 - 3 all concern these extrapolated times.

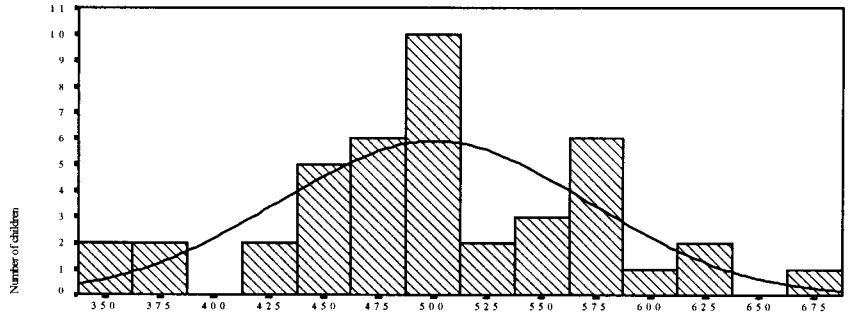

Figure 1. Distribution of the total awake time/day [minutes].

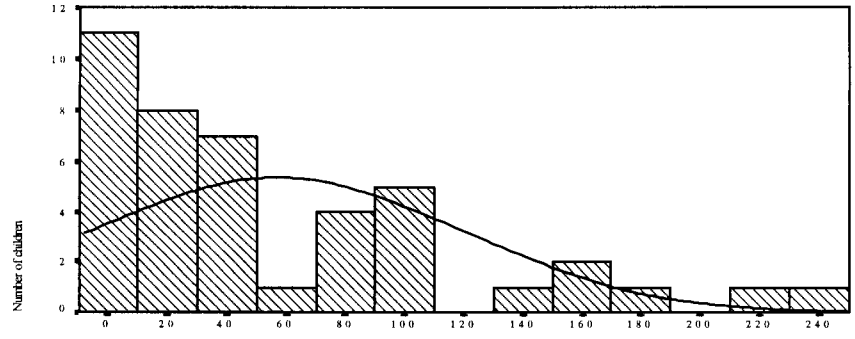

Figure 2. Distribution of the total mouthing time [minutes] during the awake time/day, including dummy.

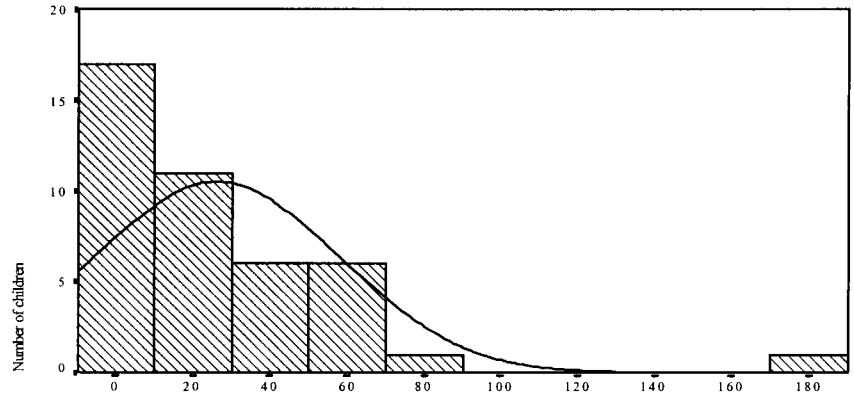

Figure 3. Distribution of the total mouthing time [minutes] during the awake time/day, excluding dummy.

The descriptive statistics of this total mouthing time per age group are given in table 1.

Table 1. Standard deviation, minimum, mean and maximum total mouthing time [minutes], excluding dummy.

	standard deviation	minimum	mean	maximum
3-6 months	19.1	14.5	36.9	67.0
6-12 months	44.7	2.4	44.0	171.5
12-18 months	18.2	0	16.4	53.2
18-36 months	9.8	0	9.3	30.9

Next some boxplots (figures 4 and 5) show the total times per age group (including and excluding dummy).

In the 'box' the median value is given as the black line, the box represents the range between the 25th and 75th percentile. The lines indicate the minimum and maximum value, unless extremes (*) or outliers (o) are identified. 'Extreme' is a value larger than three times the box

height from the upper or lower horizontal lines of the box. An outlier is defined as a value larger than 1.5 times the box height from the upper or lower horizontal lines of the box.

Mean mouthing time [minutes] during the awake time/day, per category of objects and per age group, excluding dummy (Figure 6). It can be concluded that differences in total mouthing time are large, both within and between age groups. The total mouthing time, without dummy, varies in this sample between 0 minutes and approximately 3 hours per day. Mean total time is 26 minutes (standard deviation: 32 minutes) for all age groups taken together. Children in the youngest age group (3 to 6 months) use mostly their fingers to mouth on, whereas children between 6 and 12 months of age spend most of this total time mouthing on toys (not meant for mouthing). In this latter age group largest values for total mouthing time are found.

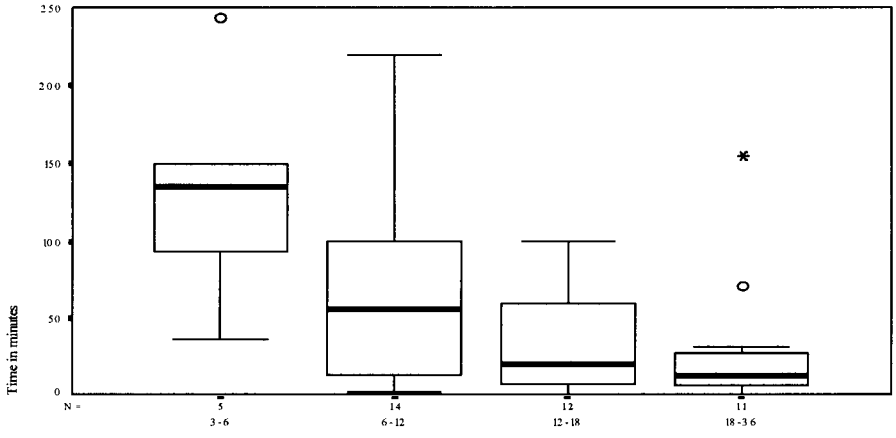

Figure 4. Total mouthing time [minutes] during the awake time/day, per age group, including dummy

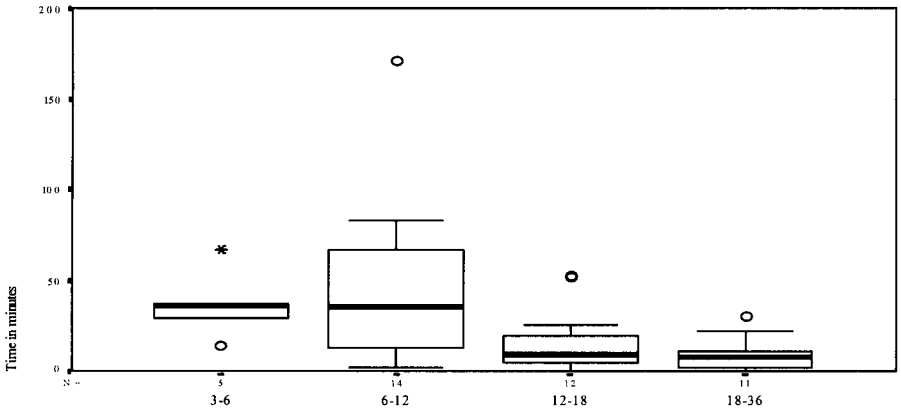

Figure 5. Total mouthing time [minutes] during the awake time/day, per age group, excluding dummy

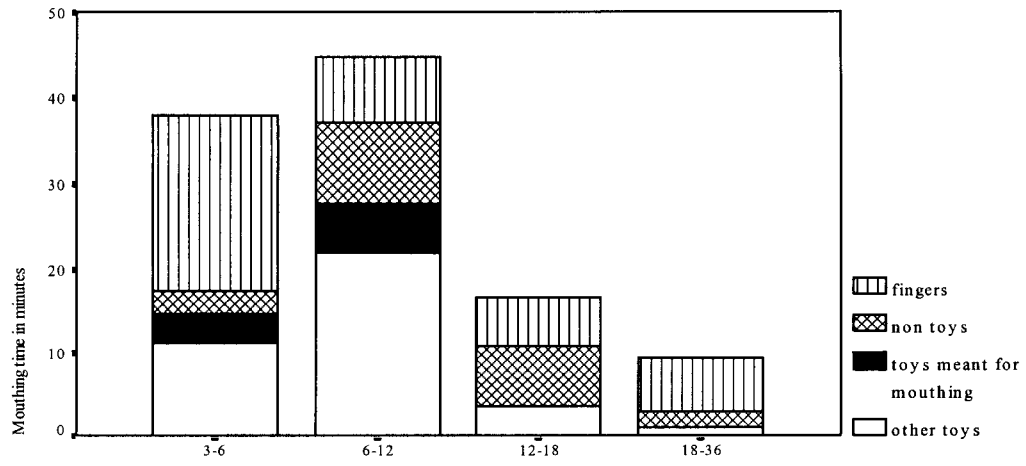

Figure 6. Mean mouthing time [minutes] during the awake time/day, per category of objects and per age group, excluding dummy.

Annex 4

EXPOSURE ASSESSMENT (SUMMARY REPORT)

M.P. van Veen
RIVM/LBO

The assessment of exposure to DINP due to mouthing (licking, sucking and chewing) of soft PVC toys was estimated as follows. The following key parameters were obtained from research or literature.

1. Duration of mouthing. Durations were established by research of Household Studies at the Wageningen Agricultural University (WAU) (Groot et al., in prep). The duration was calculated by summing all mouthing activities except sucking on dummies (meeting of begeleidingscommissie WAU-research, 20/8/98). Durations were expressed as seconds per day.

Four age categories were discerned, 3-6 months (n=5), 6-12 months (n=14), 12-18 months (n=12), and 18-36 months (n=11). Each child was observed on two days. These days are separately taken into account. The distribution of durations includes therefore both inter- and intra-individual variance.

1. Leaching rate. *In vivo* leaching rates were established by research of TNO (Meuling, in prep.). The four samples per test series were all taken into account. Leaching rates were expressed in µg/minute, and for the present analyses recalculated to mg/minute. Leaching rates were available for specimen 1 (n=20), for specimen 2 (n=10), and for specimen 3 (n=10).
2. Body weight. Age specific body weights were obtained from GVO (Groeiboek, p:1-96, GVO Den Haag) and are given in table 1 and 2. Body weights are assumed to have a normal distribution.

The above parameters were brought into the 'product leaching scenario' of CONSEXPO 2.0 (Van Veen, 1997). Additional parameters are product volume and concentration for which a sensitivity analysis was performed. The model is not sensitive at all to these parameters, implicating that the total amount of DINP is not limiting.

The 'product leaching scenario' allows a probabilistic estimation of exposure. Essentially, the key parameters are represented by distributions. Duration and Leaching were expressed as empirical distributions from which subsequent random sampling took place. Body weight was sampled as random normal number (as calculated from the random number generator `ran1`, see Press et al., 1992 for details [Numerical Recipes in C, 2nd press, Cambridge]). A Monte Carlo analysis was performed to establish the final exposure distribution. The measure of exposure is mg phthalate/kg body weight during a day that a child sucks or chews.

Tables 1, 2, and 3 report the estimated dose of phthalates per age category. The dose is calculated for the mean parameter case and at various percentiles of the Monte Carlo exposure distribution. In addition the maximum value of the Monte Carlo analysis is given as 'maximum'. In the mean parameter case, each parameter is not a distribution but is represented by its mean only. The exposure distributions for age category 2 (6-12 months) underlying the tables are depicted in figure 1, 2 and 3. Both distributions are very skewed, the medians (=50 percentile) are much lower than the means, and upper tail percentiles are a factor 5-10 larger than the median value.

Table 1. Dose of DINP from specimen 1.

	dose (mg/kg bw), per age category			
	1	2	3	4
mean parameter case	0.00811	0.00653	0.00194	0.000949
50 percentile	0.00572	0.00362	0.000823	0.000383
95 percentile	0.0207	0.0222	0.00867	0.00333
99 percentile	0.0463	0.0447	0.0185	0.00737
maximum	0.101	0.131	0.0825	0.0205
body weight	6.25 (±0.5) kg	9.25 (±1) kg	11 (±1.25) kg	13.5 (±1.5) kg
age	3-6 month	6-12 months	12-18 months	18-36 months

Table 2. Dose of DINP from specimen 2.

	dose (mg/kg bw), per age category			
	1	2	3	4
mean parameter case	0.0144	0.0116	0.00344	0.00167
50 percentile	0.0103	0.00678	0.00146	0.000748
95 percentile	0.0397	0.0389	0.0160	0.00640
99 percentile	0.0773	0.0753	0.0352	0.0126
maximum	0.112	0.204	0.0894	0.0302
body weight	6.25 (±0.5) kg	9.25 (±1) kg	11 (±1.25) kg	13.5 (±1.5) kg
age	3-6 month	6-12 months	12-18 months	18-36 months

Table 3. Dose of DINP from specimen 3.

	dose (mg/kg bw), per age category			
	1	2	3	4
mean parameter case	0.00966	0.00779	0.00233	0.00113
50 percentile	0.00717	0.00480	0.00106	0.000521
95 percentile	0.0260	0.0255	0.0105	0.00432
99 percentile	0.0398	0.0518	0.0220	0.00783
maximum	0.0707	0.142	0.0511	0.0230
body weight	6.25 (±0.5) kg	9.25 (±1) kg	11 (±1.25) kg	13.5 (±1.5) kg
age	3-6 month	6-12 months	12-18 months	18-36 months

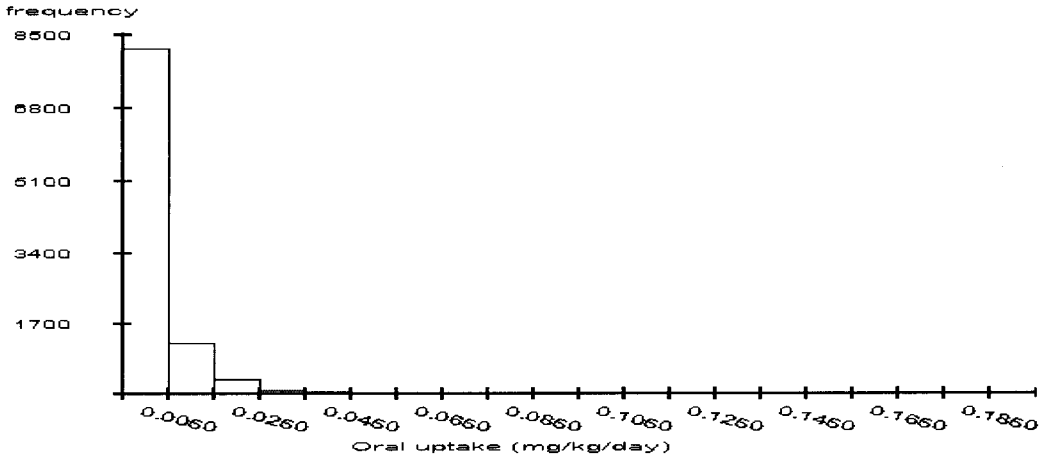

Figure 1. Exposure distribution for the PVC1 case, age category 6-12 months.

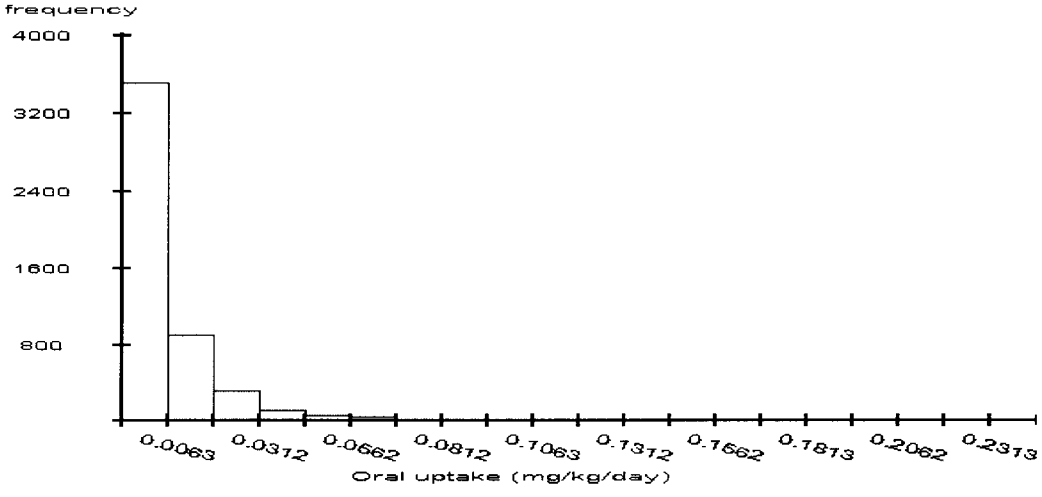

Figure 2. Exposure distribution for specimen 2, age category 6-12 months.

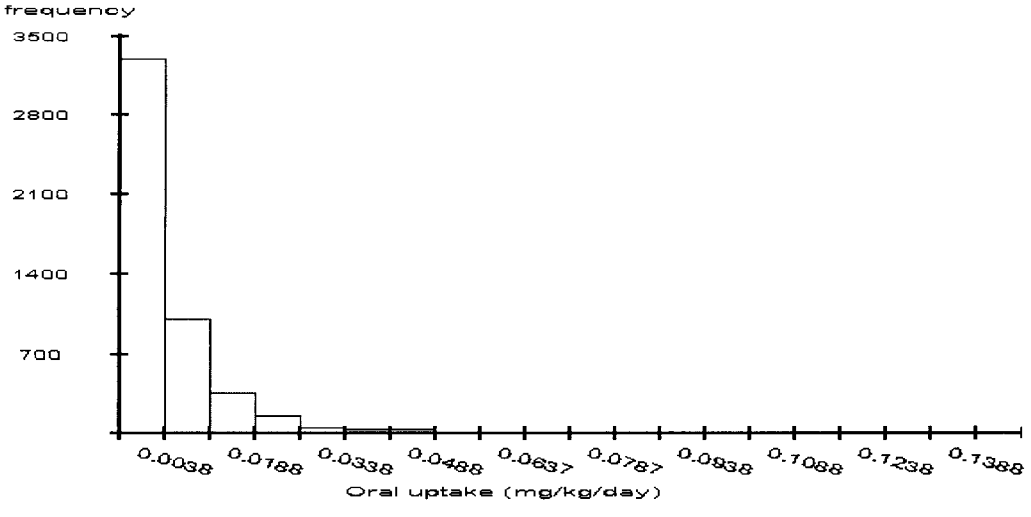

Figure 3. Exposure distribution for specimen 3, age category 6-12 months.

Annex 5

DEVELOPMENT OF A ROUTINE LABORATORY METHOD (SUMMARY REPORT)

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INTRODUCTION

Experiments were carried with the aim to develop an analytical procedure to determine the release of phthalate ester based plasticizers from baby articles intended to be for mouthed. The method should be suitable for routine control of baby toys and leaching values obtained with the laboratory method should harmonise with the in-vivo exposure data.

Within the Dutch Consensus Group saliva simulant, mechanical extraction procedures and analytical methods were discussed taking into account information available from literature. At the start it was decided to select a saliva simulant as close as possible to human saliva, meaning that attention was given to the pH, salt concentration and presence of protein.
The mechanical extraction test should be applicable in any well equipped laboratory. In this respect the use of ultrasonic vibration, shaking machines (orbital and reciprocal), and head over heels rotator were found acceptable whereas the development of special equipment was not encouraged.
The selection of an analytical method to determine the amount of phthalate released to the saliva simulant is not a problem, many acceptable methods are available. Common techniques are GC/MS and HPLC. Using a GC/MS method has the advantage that more information can be obtained on the identity of the components leached, but quantification of complex mixtures as DINP is problematic. Similar problems will occur using reverse phase HPLC.
HPLC has in general the advantage that sample preparation is easier and when using a non-selective method as straight phase HPLC and UV detection a single peak can be obtained for a complex mixture of phthalate isomers. Disadvantage of such a method is that no information is obtained on the identity of the phthalate ester extracted. However information on the plasticizer present in the test article can easily be obtained by investigation of an extract of the test article by e.g. GC/MS. Only in case more than one phthalate ester is present, a selective method of quantification may be useful.
Leaching of plasticizer may depend on the physical properties of the PVC and the homogeneity of the plasticizer in the PVC. Therefore a plasticized PVC sheet was prepared under well controlled conditions ensuring good mixing and with a composition typical for baby toys. In addition a commercial available teething ring in the shape of a hand was selected for testing.

TESTING PROCEDURES

Test specimens

A batch of 3 mm thick PVC pads was prepared in a laboratory using a well defined protocol. The final sheet was composed of PVC (58.8%), Diisononylphthalate (Jayflex® DINP) (38.2%), epoxidized Soybean oil (1.8%) and Ca/Zn Stabilizer (1.2%). From the sheet discs were punched with a diameter of 23 mm. The total area of a disc is approx. 10 cm^2. Further referred to as specimen 1.

One hundred teethers in the shape of a hand, of the same production batch were obtained through the manufacturer. For investigation, disks with a diameter of 23 mm were punched from the hands. Further referred to as specimen 3.

Specimens were left to age for at least 24 hours after punching.

Saliva simulant

Saliva simulant with and without the presence of mucin was used. The salt solution had the following composition:

Compound	Formula	g/l	mmol/l
Magnesium chloride	$MgCl_2$	0.078	0.82
Calcium chloride	$CaCl_2$	0.110	1.0
Dipotassium hydrogen phosphate	K_2HPO_4	0.569	3.3
Potassium carbonate	K_2CO_3	0.530	3.8
Sodium chloride	NaCl	0.330	5.6
Potassium chloride	KCl	0.745	10.0
pH adjusted to 6.8			

Depending on the experiment 1.6 g of mucin was added to 1 l of salt solution.

The choice of the saliva simulant composition is based on natural saliva compositions mentioned in literature. Also the pH is derived from literature data; it is chosen at the low level because the pH of baby saliva is somewhat lower than that of saliva of adults.

Mechanical agitation conditions

For the experiments carried out the following apparatuses were used.
Ultrasonic water bath thermostated at 37°C. 35 kHz,
Shaking water-bath at 37°C, provided with clamps to hold 100 ml conical flasks. Reciprocal at a frequency of 210 cpm (cycles per min) with a stroke size of 24 mm.
Head over heels mixer at approx. 60 rpm at a radius of 150 mm, operating at ambient of 20°C or at 37°C.
Shaking machine, provided with clamps to hold many types of flasks in a horizontal position. Reciprocal at 210 cpm with a stroke size varying from 25 to 54 mm.
Experiments were done using 20 glass beads (diam. 10 mm) in the flask to enhance the mechanical agitation. In other tests 10 stainless steel balls (weight 9g each) were used. Also tests without objects in the extraction flask were carried out. Other laboratories acting as reference laboratories used similar equipment but operating at different conditions.

Extraction periods

Various extraction times combined with different mechanical agitation conditions were performed. Extraction times varied from 15 to 60 min. In all cases one disk with a total surface area of 10 cm^2 was in contact with 25 ml of the simulant. From the simulant a sub-sample was taken and analysed for the content of DINP. In a number of cases the simulant was filtered through a coarse glass frit filter before analysis. In correspondence to in vivo tests, results were expressed in µg DINP/min. Experiments were carried out with replenishment of the simulant after periods of 15 min of mechanical agitation. Also cumulative extractions were performed in which the simulant was sampled every 15 min.

Determination of DINP in simulants

Saliva simulants containing mucin were mixed with ethanol to precipitate the mucin. The mixture was extracted with diethyl ether/hexane (1:1), while water was added to improve separation of the phases. An aliquot of the organic layer was evaporated and re-dissolved in iso-octane.
Simulants containing only salts were extracted with diethyl ether/hexane (1:1) and an aliquot was evaporated and re-dissolved in iso-octane. Initially sub-samples of the saliva simulant was taken for analysis, but in the final stage the total volume of saliva simulant was extracted to avoid adsorption losses.
The iso-octane solutions were analysed by straight phase HPLC using a cyanopropyl column with iso-octane as mobile phase and UV detection at 225 nm. External standard calibration method was applied. Detection limit of the method was found to be 0.04 µg/ml iso-octane. Calibration curves appeared to be linear over the range of 0.2 to 5 µg/ml iso-octane. Reference labs verified the HPLC method and found consistent results.

Results and Discussion

In table 1 the results obtained for the leaching of DINP from standard PVC specimen 1 into saliva simulant with 0.16% of mucin and in the presence of 20 glass beads is listed.
It appeared that leaching using the shaking water bath, rotary evaporator and ultrasonic water bath was close to the detection limit. Leaching, using head over heels rotator and shaking machine is significantly higher but still far below the release found in the in vivo test. Recovery was acceptable using the head over heels rotator and the horizontal shaking machine when applying high stroke sizes. In all other cases recoveries varied from 20 - 55%.

The effect of the mechanical force and the presence of mucin in the saliva simulant was studied for specimens 1 and 3. After a shaking period of 60 min at 210 cpm and a stroke size of 45 mm with 10 stainless steel balls with a total weight 70g in the 100 ml extraction flask the following DINP releases were measured. The values should be compared with the leaching of DINP (0.36 µg/min) when using 20 glass beads (see table 1)

specimen 1	Saliva simulant with 0.16% mucin	1.8 µg/min
	Saliva simulant without mucin	9.3
specimen 3	Saliva simulant with 0.16% mucin	1.5 µg/min
	Saliva simulant without mucin	6.6

Both the presence of severe agitation and the absence of mucin increase the release of DINP. The above values obtained with simulant with mucin are close to the DINP release in vivo. However this method gives such a strong agitation that the glass wall of the flask is attacked, with the consequence that glass particles are present in the simulant, which may cause abrasion of particles.

Using an ultrasonic bath (35 kHz) at 37°C and saliva simulant no significant release of DINP was observed. However a reference lab found a release of 1.0 µg/min when using an ultrasonic bath operating at 40 kHz and 100 watt power.
The influence of pH ranging from 6.5 - 7.7 was examined using saliva simulant with and without mucin combined with the use of glass beads or stainless steel balls. The results are presented in Table 2. There was no significant influence observed from the pH value. However is was noticed that the simulant contained a lot of particles released from the glass wall and the stainless steel balls. After sedimentation of the particles the saliva simulant

without mucin was a clear solution. Upon analysis of the clear solution it was evident that the DINP had settled with the particles.

Table 3 gives an overview of the effects of the stroke size of shaking. With increasing stroke size of the machine release of DINP increases when using the same volume and shape of container. At the fixed stroke size and cycles per min. the DINP release decreases with increasing flask size. If however, glass beads are present in the bottle during the agitation period, the DINP release shows a reverse correlation with the stroke size using a 100 ml flask, whereas in a 250 ml flask the effect is not significant.
Table 3 also shows significant differences between the various analysis under the same conditions (100 ml flask at stroke size 35 mm). No reasons for the differences could be identified and further investigation is required to solve the problem of repeatability.

Specimen 3 was also treated under conditions equal to those of specimen 1 (100 ml flask, stroke size 35 mm) and a release of 4.1 and 5.8 µg/min was found. This value is significantly higher then release from specimen 1 and also higher then the leaching in *in vivo* testing.

The effect of time on DINP leaching was investigated for specimen 1. For that purpose 3 test specimens 1 were shaken at 210 cpm and a stroke size of 37 mm in a 100 ml flask with 25 ml of saliva salt solution at pH 6.8. Each 15 min the saliva was sub-samples and analysed. The release appeared to be linear correlated to extraction time. This means that extraction periods are not a critical parameter as long as the release is expressed in unit of time. The results are presented the table below.

Agitation period	0-15	15-30	30-45	45-60	mean	$Sd_{(n-1)}$
DINP release	0.27	0.35	0.53	0.54	0.42	0.13
(µg/min)	0.53	0.53	0.57	0.72	0.59	0.09
	0.33	0.37	0.35	0.44	0.37	0.05

It was observed that the saliva salt solution, which is completely clear at the start, turns turbid after an agitation period. These turbid solutions appeared stable over a period of at least 24 h. The solutions contain high amount of DINP (far above the solubility properties), but after filtration through a disposable 0.45 µm filter the clear solution does not contain any DINP. This could be caused by the presence of PVC particles or by the over-saturation of the solutions. Therefore saliva salt simulant was spiked with DINP until the solution turned turbid, which occurred at a concentration of 35µg DINP/ml. After filtration of the solution, DINP was not detectable at the level of 0.1 µg/ml. From this experiments it is concluded that simulant can be over-saturated and release of plasticizer may not depend on solubility properties but mainly on mechanical forces to make "suspension like" solutions.

The suitability of a head over heels rotator (approx. 60 rpm and a radius of 150 mm, temperature 20 °C) and influence of flask size was further investigated. Results are presented in Table 4. In addition the reliability of taking sub-samples of the simulant compared to extraction and washing of the extraction flask was investigated. Both test specimens 1 and 3 were included in this part of the study.
The DINP release from specimen 1 using flasks of 250 ml and higher is rather consistent. The spreading of the release values for specimen 3 is rather high. It is remarkable that DINP release using specimen 3 with a 100 ml flask was significantly higher. No reasons for this could be detected although it is the opposite of what is seen with specimen 1.

It is known the phthalates may adhere to the wall surface of the glass container. As the analytical method is based on the analysis of a sub-sample there was a need to examine the absorption to the wall by analysing the total volume of saliva simulant. For the analysis test nr. 5,6 and 15,16 both a sub-sample was analysed and subsequently the remaining total volume was analysed, including rinsing of glass wall. It appeared that the release values increased by 30 to 50%. This means that it is necessary to extract the total volume of saliva simulant. Further investigation are required to examine the need of filtration to remove adhering particles from the disk.

The above results were very promising and therefore more analyses were carried out to validate the head over heels rotator method. Also the influence of temperature was included in the investigation by carrying out extraction at 20°C and 37°C. The leaching from a disk prior used in an extraction test, which was reconditioned for 24 h at ambient was established. The results available so far are presented in Table 5.
No temperature effect was observed on the release of DINP. The standard deviation over a series of analysis varied from 12 to 30%. The standard deviation for the specimen 3 is higher than for specimen 1. Standard deviation for recovery experiments is close to 10% which demonstrates that the analytical method is acceptable. Repeated extraction of the test specimens has limited or no influence on the release of DINP.

Conclusions

In the course of the investigations much experience is obtained in the mechanism of plasticizer release form PVC baby toys and following conclusions have been drawn:

- Release of DINP exceeds solubility parameters in saliva simulant.
- Application of an adequate mechanical force is a pre-requisite to obtain a stable "solution" that correlates with *in vivo* levels of migration.
- Only detailed standardisation of equipment and apparatus will enable acceptable inter-laboratory results.
  Small variations in agitation strength are of significant influence on the release of DINP from test samples. Ultrasonic devises may give satisfactory results, but there are many variables which can give probems for standardisation.
- Head over heels rotation is the proposed method because:
  - DINP release is close to in vivo results for specimens 1 and 2
  - Method is easy to standardise
  - Method is simple to perform
  - Not sensitive to (limited) temperature changes
  - Equipment commercial available, but also easy to produce a home-made apparatus
- Use of mucin in the saliva simulant results in a decrease of DINP release. To obtain the intended release level too strong mechanical forces are required, which is difficult to standardise and makes analyses problematic. Therefore saliva simulant without mucin is advised.
- Test covering the pH range of adult human saliva from 6.5 to 7.7 revealed no influence on DINP release.

Table 1. Determination of leaching of DINP from standard PVC disks into simulated saliva containing 0.16% of Mucin.

experiment description	time min	release µg/min		mean µg/min	Sd(n-1) µg/min
		1	2		
Head over heels at 20°C	blank	0.05	0.08		
60 rpm, radius 150 mm	0-15	1.17	0.68		
20 glass beads diam. 10 mm	15-30	0.45	0.22		
	30-45	0.11	0.51		
	45-60	0.09	0.15	0.42	0.4
Rotary evaporator at 37°C	blank	0.05	-0.02		
360 rpm, pressure 800 mbar	0-15	0.19	0.16		
20 glass beads diam. 10 mm	15-30	0.10	0.10		
	30-45	0.05	0.07		
	45-60	-0.02	0.09	0.09	0.1
Shaking water bath at 37°C	blank	-0.02	0.08		
210 cpm, stroke size. 13 mm	0-15	0.21	0.07		
20 glass beads diam. 10 mm	15-30	0.08	-		
	30-45	0.11	-		
	45-60	0.04	-	0.10	0.1
Shaking machine at 20°C	blank	0.06	0.07		
210 cpm,, stroke size 27 mm	0-15	0.30	0.14		
before filtration of simulant	15-30	0.22	0.21		
20 glass beads diam. 10 mm	30-45	0.30	0.17		
	45-60	0.18	0.14	0.21	0.1
Shaking machine at 20°C	blank	0.04	-0.02		
210 cpm, stroke size 27 mm	0-15	0.32	0.10		
after filtration of simulant	15-30	0.20	0.19		
20 glass beads diam. 10 mm	30-45	0.22	0.18		
	45-60	0.15	0.14	0.19	0.1
Shaking machine at 20°C	blank	0.09	0.00		
210 cpm, stroke size 52 mm	0-15	0.55	0.54		
before filtration of simulant	15-30	0.28	0.24		
20 glass beads diam. 10 mm	30-45	0.37	0.49		
	45-60	0.23	0.20	0.36	0.1
Ultrasonic bath at 37°C	blank	0.08	0.00		
35 kHz	0-15	0.16	0.11		
	15-30	0.12	0.00		
	30-45	0.20	0.06		
	45-60	0.09	0.00	0.09	0.07

Table 2. Influence of pH of simulant.

Test conditions	pH	Mucin content (%)	DINP release (µg/min) simulant *)	DINP release (µg/min) sedimented **)
Shaking machine; 210 cpm, stroke size 45 mm ; 25 ml simulant, pH ranging from 6.5 - 7.7; with 10 stainless steel balls in 100 ml flask	6.5	0	10.6	0.2
	6.8	0	10.7	-0.05
	7.1	0	12.6	0.22
	7.4	0	9.2	0.2
	7.7	0	9.9	-0.05
Shaking machine; 210 cpm, stroke size 45 mm ; 25 ml simulant, pH ranging from 6.5 - 7.7; with 10 stainless steel balls in 100 ml flask	6.5	0.16	1.86	
	6.8	0.16	0.84	
	7.1	0.16	0.74	
	7.4	0.16	1.03	
	7.7	0.16	1.04	
Shaking machine; 210 cpm, stroke size 45 mm ; 25 ml simulant, pH ranging from 6.5 - 7.7; with 20 glass beads in 100 ml flask	6.5	0	6.3	
	6.8	0	5.2	
	7.1	0	6.6	
	7.4	0	7.8	
	7.7	0	11	

*) simulant was homogenised before taking a sub-sample for analysed
**) a sub-sample of the clear simulant was taken for analysis after sedimentation of particles.

Table 3. Release of DINP from new and used standard PVC disks varying agitation amplitude, contents of flask and use of glass beads.

shaking machine at 20°C, 210 cpm, 25 ml saliva simulant	DINP release from new disk (µg/min)	shaking with glass beads (µg/min)	DINP release from used disk (µg/min)
Stroke size 35 mm, 100 ml flask	0.44		0.46
	0.70		0.35
			0.53
			0.37
	1.72	4.80	
	1.64	5.16	
Stroke size 52 mm, 100 ml flask	13.65	8.32	
	12.06	7.49	
Stroke size 52 mm, 250 ml flask	0.46	2.00	
	1.74	2.30	
Stroke size 44 mm, 500 ml flask	0.53		
	0.35		

Table 4. DINP release using a head over heels mixer (introductory experiments).

Test nr	Head over heels mixer Radius 150 mm, 20°C, 64 rpm	DINP release from new disk	
		sub-sample (µg/min)	total saliva volume (µg/min)
1	specimen 1 in 100 ml flask	0.60	
2		0.61	
3	specimen 1 in 250 ml flask	1.46	
4		1.79	
5		1.09	1.53
6		0.81	1.18
7	specimen 1 in 500 ml flask	1.66	
8		2.30	
9		1.42	
10		1.35	
11	specimen 3 in 100 ml flask	6.94	
12		5.51	
13	specimen 3 in 250 ml flask	0.73	
14		3.52	
15		2.02	2.95
16		0.71	1.63
17	specimen 3 in 500 ml flask	3.38	
18		1.10	

Specimen 1 = standard PVC material
Specimen 3 = disk from commercial teether

Table 5. Validation of head over heels method.

Conditions: Head over heels rotator at 64 rpm, radius 150 mm, 25 ml of saliva simulant salt solution, one disk with an area of 10 cm^2. Rotation time 30 min.

Test nr	test specimen	DINP release at 19°C		DINP release at 37°C	
		1e extraction µg/min	2e extraction µg/min	1e extraction µg/min	2e extraction µg/min
1	specimen 1	1.9	in progress	2.5	3.7
2		2.2		2.6	3.5
3		2.1		2.8	5.1
4		2.2		2.6	4.1
5		4.3		2.0	4.2
6		2.5		2.8	3.6
7		2.4		3.1	3.8
8		2.3		3.6	3.4
9		2.5		3.1	4.0
10		2.3		3.3	3.7
	Mean	2.5		2.8	3.9
	$Sd_{(n-1)}$	0.7		0.5	0.5
11	specimen 3	5.9		2.8	3.3
12		4.0		2.6	2.4
13		5.3		2.7	3.3
14		3.4		3.1	3.4
15		6.0		3.1	4.2
16		4.2		3.4	3.6
17		2.7		3.4	3.2
18		3.2		3.1	1.6
19		3.2		4.2	2.0
20		2.8		2.5	2.2
	mean	4.1		3.1	2.9
	$Sd_{(n-1)}$	1.3		0.5	0.8

All extractions were run in series of five. All sample were analysed over a number of days to establish both repeatability and reproducibility. With each series blank simulants and recoveries at the level of 1.5µg/ml (=1.25 µg/min) were taken through the full procedure and subsequently analysed.
In the blanks no interference at the retention time of DINP was detectable.
Average recovery at 19°C was 96.0 ± 9.2% and at 37° 88.3 ± 8.9%.

Annex 6

Mailing list

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