Comparison of Diisononyl Phthalate Migration from Polyvinyl Chloride Products into Human Saliva in Vivo and into Saliva Simulant in Vitro

Tatsuhiro Niino,* a Tohru Ishibashi, a Takeshi Itoh, a Senzo Sakai,a Hajimu Ishiwata,b Takashi Yamada,b and Sukeo Onodera c

a Tokyo Kenbikyo-in Foundation, Center of Food & Environmental Sciences, 44–1 Nihonbashi Hakozaki-cho, Chuo-ku, Tokyo 103–0015, Japan, b National Institute of Health Sciences, 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan, and c Faculty of Pharmaceutical Sciences, Tokyo University of Science, 12 Ichigaya-Funagawara-machi, Shinjuku-ku, Tokyo 162–0826, Japan

(Received December 20, 2001; Accepted February 28, 2002)

Human volunteers chewed polyvinyl chloride (PVC) products under controlled conditions for 60 min (four consecutive periods of 15 min), and then the amount of diisononyl phthalate (DINP) migration into the saliva was determined by HPLC. The PVC products consisted of a molded plate containing added DINP (500 mg/g) and five types of commercial toy containing 160–583 mg/g DINP (mean value 372 mg/g). The DINP migration rates ranged from 3.8 to 32.6 µg/cm²/hr (mean value 16.4 µg/cm²/hr). The DINP contents in the toy products did not correlate with the amount of in vivo migration. The DINP migration rates during the last 15-min period (45–60 min) decreased by 38–75% compared with the first session (0–15 min). In addition, in vitro DINP migration rates from the PVC products into saliva simulant during 15 min of rotary shaking were about 3.6–4.1-fold higher than the rates in vivo over 60 min, and remained essentially fixed for each sample.

Key words —— diisononyl phthalate, in vivo migration, human saliva, in vitro migration, saliva simulant, polyvinyl chloride product

INTRODUCTION

Diisononyl phthalate (DINP) is a mixture of about 100 primarily branched dialkyl chain isomers (Fig. 1.) 1,2 and many structurally dissimilar compounds known as peroxisome proliferators that induce tumors in the rodent liver. 3 In addition to DINP, other dialkyl phthalate esters are frequently used as plasticizers to impart softness and flexibility to normally rigid polyvinyl chloride (PVC) products, such as toys, construction materials, and general consumer products. DINP increases liver weight and changes liver cell histopathology in rodents exposed to chronic high doses. 4 The rodent kidney is also a target for prolonged high-level exposure to DINP. 5

Toys represent a unique source of childhood exposure to DINP since it is the major plasticizer used in these products. 6–8 The rate of DINP migration from PVC toys chewed by children has been measured using two general migration tests. An in vivo test in which adult volunteers chew PVC toys under controlled conditions was developed by Meuling and Rijk in the Netherlands 9 and by Chen in the U.S.A. (US Consumer Product Safety Commission [USCPSC]). 10 The effect of shaking or impacting PVC toys in simulated saliva in vitro has been also tested, but the release rates did not correlate well with the inherent total DINP content. 10,11

At present, there are no standard in vitro methods with good reproducibility for measuring DINP migration. In addition, little data can be obtained from in vivo migration tests from the viewpoints of time and ethics. Therefore a rapid, simple, and reproducible in vitro migration test is required. We present more detailed findings on DINP migration in vivo from PVC products and in vitro after three types of mechanical agitation. These findings provide background information for the development of a standard method with which to mimic exposure during chewing.

MATERIALS AND METHODS

Reagents —— We purchased DINP from Kanto Chemical Co., Inc. (Tokyo, Japan; over 97.9% pure) and from Wako Pure Chemical Ind. (Osaka, Japan; 98.0% pure). All other solvents and reagents were of analytical grade, and confirmed to be free of phthalate esters. The composition of the simulated saliva corresponded to the British Standard Specification for Safety Harnesses. 12 Glassware was heated...
at temperatures over 230°C for at least 5 hr before use.

**HPLC Analysis** — The HPLC conditions were as follows: apparatus, LC-10A (Shimadzu, Kyoto, Japan); column, Inertsil C8-3 (0.46 mm × 250 mm); column oven temperature, 40°C; mobile phase, (A) acetonitrile, (B) water; gradient programming, A% 70–100 (15 min, linear gradient); detector, UV (254 nm); injection volume, 10 µl. The quantity of DINP was determined using an absolute calibration curve after the total area of 6 isomer peaks was summed.

**DINP Migration Test from PVC Plate and Toys** — The PVC plate was molded at Dainippon Resin Laboratory Co. (Yokohama, Japan) as follows. A mixture of PVC 50 g, dibutyl tin mercuprate 2 g, calcium stearate 0.5 g, and DINP 50 g was heated at 180°C, and then the compounds were molded into 1.0-mm-thick plates containing DINP 462 mg/g. The PVC toys included a pacifier, a teething ring (teether), a rattle, a ball, and a soft doll that contained 538, 389, 380, 255, and 160 mg/g DINP, respectively. The plate and toys were punched over a 2.5 × 3.0 cm area (total surface area was approximately 15 cm²).

**Migration Test in Vivo**: One female and three male volunteers gently chewed each of the six samples and phthalate ester-free polypropylene disks as a negative control for 15 min. They performed this task on each sample four times for 15 min each (four sessions), with 5-min breaks. During the experimental periods, saliva was collected in labeled 50-ml glass centrifuge tubes and the total volume and pH were recorded. All saliva samples were diluted to 10 ml with distilled water, mixed with 10 ml of acetonitrile, and then sedimented by centrifugation at 3000 rpm for 10 min. The *in vivo* migration of the pacifier, teether, and rattle was tested by three of the four volunteers, and the amount of DINP which migrated from the samples was quantified by HPLC.

**RESULTS AND DISCUSSION**

**In Vivo Migration of DINP**

Table 1 shows the DINP release rates into human saliva (*in vivo*) from the PVC plate and from five toy samples when volunteers mouthed and gently chewed (referred to below as “chewing”) them for 1 hr (15 min × 4 sessions). The rates of *in vivo* migration from the PVC plates ranged from 3.8 ± 0.9 to 32.6 ± 2.6 µg/cm²/hr, and the coefficients of variation ranged from 8% to 37%. The *in vivo* migration rates of DINP from PVC commercial products (toy ducks) has been tested by volunteers in the Netherlands and in the U.S.A. The Dutch consensus study indicated a mean DINP release rate of 10.8 µg/cm²/hr from samples containing DINP 430 mg/g, which was considerably less than the USCPSC values of 21.9 µg/cm²/hr. The DINP migration rates from the pacifier and rattle were almost identical to those found in the US study, and that from the teether was similar to that found in the Dutch consensus study.

The Product Safety Laboratory of Health Canada found no correlation between the DINP content of PVC products and the *in vitro* release rate. We found similar results with respect to the *in vivo* release rates into human saliva during chewing. In addition, the *in vivo* migration rate from the plate molded in the laboratory was significantly faster than that from the five toys, which are commercial products with a complex surface structure. Furthermore, the coefficient of variation was minimal on the plate. The *in vivo* migration rate might be more related to the surface design of PVC products.

The rates of saliva produced by four volunteers chewing the six samples ranged from 37.6 ± 7.7 to 53.6 ± 5.1 ml/hr (Table 1). The pH of the saliva
The normal salivation rate in humans is 60–120 ml/hr when gum is chewed, and the fluctuation range is wide. The rate of saliva production induced by chewing the samples seemed low and was affected by each sample. However, the total release of DINP into the saliva was not altered by the salivation rate and pH of the saliva.

Figure 2 shows the effect of chewing time on the DINP release and rate of saliva produced by four volunteers chewing the six samples. The migration rate of DINP was the most rapid during the first 15 min and thereafter decreased with repetition of the task. The migration rates at the fourth session (45–60 min of chewing time) from the plate, pacifier, teether, rattle, ball, and soft doll were 71, 75, 75, 69, 47, and 38% respectively, of those at the first session (0–15 min). This indicates that DINP gradually migrated from the surface of PVC polymer, because the rate decreased over time. Our findings showed that DINP initially migrated into the saliva through chewing, indicating that the molecular forces that bound DINP and PVC polymer in the samples are weak. The rates of saliva produced during each session were almost identical, and the quantity did not appear to affect DINP migration significantly.

Comparison of in Vitro Migration of DINP with in Vivo Migration

Table 2 shows the in vitro migration rates of DINP into saliva simulant agitated on three mechanical shakers for 15 min. The DINP release rates from the PVC products ranged from 22.4 to 148.5 µg/cm²/hr. The amount of DINP migration from the rattle after rotation, vertical, shaking and in vivo chewing were equivalent to the mean values of each of the four products, but the amount that migrated after horizontal shaking was half of these values. The USCPSC reported that the DINP release rate obtained by impaction using an air-driven piston ranged from 0.1 to 4.4 µg/cm²/hr. The release rates obtained in vivo are higher than those obtained in vitro. Considerably more DINP seems to have migrated due to mechanical shaking than either to

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**Table 1. DINP Contents in PVC Samples and in Vivo Migration Rates into Human Saliva after Mouthing and Gentle Chewing**

<table>
<thead>
<tr>
<th>PVC sample</th>
<th>Contents(a) (mg/g)</th>
<th>In vivo migration(b) µg/cm²/h</th>
<th>Saliva produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate</td>
<td>462</td>
<td>32.6 ± 2.6</td>
<td>49.2 ± 5.4</td>
</tr>
<tr>
<td>Toys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacifier</td>
<td>583</td>
<td>20.0 ± 6.0</td>
<td>37.6 ± 7.7</td>
</tr>
<tr>
<td>Teether</td>
<td>389</td>
<td>12.5 ± 1.9</td>
<td>41.0 ± 3.7</td>
</tr>
<tr>
<td>Rattle</td>
<td>380</td>
<td>21.9 ± 2.6</td>
<td>40.1 ± 4.0</td>
</tr>
<tr>
<td>Ball</td>
<td>255</td>
<td>7.8 ± 2.9</td>
<td>45.2 ± 3.7</td>
</tr>
<tr>
<td>Soft doll</td>
<td>160</td>
<td>3.8 ± 0.9</td>
<td>53.6 ± 5.1</td>
</tr>
<tr>
<td>Mean value</td>
<td>372</td>
<td>16.4 ± 2.8</td>
<td>— — — — —</td>
</tr>
</tbody>
</table>

(a) DINP contents in PVC samples were measured by extraction with acetone after rotary shaking at 300 rpm for 3 hr. (b) Release rates obtained from in vivo migration tests after four volunteers chewed PVC samples for 60 min (15 min × 4 sessions). Values are means ± S.D. (pacifier, teether, and rattle, n = 3; plate, ball, and soft doll, n = 4).
the type of impaction used at the USCPSC or from chewing by adult volunteers.

The in vitro migration rates from samples agitated by rotary, vertical, and horizontal shaking for 15 min were 3.6–4.1, 4.6–7.4, and 1.1–3.4-fold higher, respectively, than those of in vivo migration for 60 min. The in vitro release rates obtained by rotary shaking compared with the in vivo migration rates were almost fixed for each sample. The regression lines of the migration rates in vitro to those in vivo showed linearity, and the correlation coefficients (r) were ranged from 0.7841 to 0.9962 (Table 2). We found that there was a highly positive correlation between the migration rates after rotation shaking in vitro and chewing in vivo. In addition, the coefficient of variation in the migration rates caused by rotary shaking was smaller than that caused by either vertical or horizontal shaking.

On the bases of comprehensive reviews of data relating to the toxicology of DNP and its rate of migration from PVC toys during the mouthing activities of children, Wilkinson and Lamb concluded that DNP in PVC toys does not present a significant risk to the health of children. The present study found that the in vivo migration test results were equivalent to those obtained in the U.S.A. and in the Netherlands. In addition, the migration test using a rotary shaker may be useful as an in vitro standard method of mimicking exposure while chewing. Both the in vivo and in vitro migration rates into saliva are used as partial means of estimating exposure to DNP derived from toys in Japan.

Acknowledgements This study was partly supported by Health Science Research Grants (1998 to 1999) from the Ministry of Health and Welfare of Japan.

REFERENCES

Product Safety Bureau, Environmental Health Directorate, Health Protection Branch, Ottawa, Ontario.


