ABSTRACT — The present investigation was undertaken to determine the distribution of 1,2-dichloropropane (DCP) in the blood, liver, kidney, lung, and abdominal fat of rats after oral administration. Male rats were orally administered 62 or 125 mg/kg body weight doses of DCP dissolved in corn oil by gavage, and the concentrations in the blood and tissues were measured. The DCP concentration in the abdominal fat was much greater than in the blood and other tissues. Twenty-four-hr after oral administration, DCP could still be detected in the blood and abdominal fat in the 62-mg/kg group, and in the blood, liver, kidney, lung, and abdominal fat in the 125-mg/kg group. Our results are valuable data pertaining to the pharmacokinetics of DCP and to human health risk assessment of oral exposure to DCP.

Key words: 1,2-Dichloropropane, Blood concentration, Tissue concentrations, Oral administration

INTRODUCTION

1,2-Dichloropropane (DCP) was used in the past as a soil fumigant, chemical intermediate, and industrial solvent and was found in paint strippers, varnishes, and furniture finish removers (ATSDR, 1999). Most uses of DCP have now been discontinued; however, DCP is still detected in the ambient air and public water in Japan (Ministry of the Environment, Government of Japan, 2014).

In 1999 the International Agency for Research on Cancer (IARC) evaluated DCP as a Group 3 agent (IARC, 1999): an agent which is not classifiable as to its carcinogenicity in humans. However, since then epidemiological studies have indicated that occupational exposure to paint stripper containing DCP and/or dichloromethane (DCM) was associated with a marked increase in cholangiocarcinomas in an offset printing company in Japan (Kumagai et al., 2013; Kubo et al., 2014). While more recent animal studies demonstrated sufficient evidence of carcinogenicity of DCP, until the studies by Kumagai et al. (2013) and Kubo et al. (2014) were reported, there was no epidemiological data relevant to the carcinogenicity of DCP available. Therefore, as a result of this new data, in 2014 IARC re-evaluated DCP as a Group 1 agent (IARC, 2016): an agent for which there is sufficient evidence of carcinogenicity in humans.

In two-year animal studies, the National Toxicology Program (NTP) reported a dose-related increase of liver tumors in mice of both sexes, but not in rats, administered DCP by oral gavage (NTP, 1986). However, while DCP administration did not result in induction of liver tumors in rats, a dose-related increase in mammary gland adenocarcinomas in female rats was observed (NTP, 1986). Our two-year inhalation carcinogenicity study of 200 ppm (mice) and 500 ppm (rats) DCP vapor showed that inhalation exposure of mice and rats of both sexes to DCP resulted in significantly increased incidences of tumors in the Harderian gland of male mice, the lung of female mice (Matsumoto et al., 2013), and the nasal cavity of male and female rats (Umeda et al., 2010).

To better evaluate the toxicity of chemicals, such as DCP, it is necessary to know the time-course changes of the chemical’s concentration in the blood and tissues consequent to exposure as this is a key factor affecting toxicity. Therefore, understanding the pharmacokinetics of exposure in animal models is one of the fundamental considerations in human risk assessment of exposure to a chemical. Until 2013, only limited information was available about the distribution of DCP in the blood and tissues after inhalation and oral administration.
In 2014, we reported the time-course changes of DCP concentrations in the blood and tissues of rats exposed to 80 or 500 ppm DCP vapor (Take et al., 2014b), concentrations corresponding to the low and high doses used in the two-year inhalation study by Umeda et al. (2010).

In the present study, we investigated DCP concentrations in the blood and tissues of rats after oral administration of DCP at doses of 62 or 125 mg/kg body weight, doses corresponding to the 2 doses used in the two-year oral administration study by the NTP (NTP, 1986).

MATERIALS AND METHODS

Chemical

DCP of analytical grade (greater than 99.5% purity) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals

Seventy-two F344/DuCrI/Crlj (SPF) male rats were obtained at 6-8 weeks of age from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). All animals were quarantined for one week and were then housed in our laboratory until the age of 18 weeks. The body weights of rats at the start of the experiment ranged from 285 to 356 g. Rats were housed in individual cages in a temperature controlled room with a 12-hr light/dark cycle (light: 8:00-20:00). The room temperature and relative humidity were maintained in the ranges of 22 ± 2°C and 55 ± 15%, respectively. All animals had access to food (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum. Animals were cared for in compliance with the “Regulation for Proper Conduct of Animal Experiments in the Japan Bioassay Research Center”, and the present study was approved by the Ethics Committee of Animal Experiments of the Japan Bioassay Research Center.

Experiment design

The rats were divided into 2 groups (62- and 125-mg/kg group), 36 rats in each group. DCP dissolved in corn oil was orally administered by stomach tube at a dose of 62 or 125 mg/kg body weight to rats by a single gavage. The doses correspond to the 2 doses used in the two-year oral administration study by the NTP (NTP, 1986).

Blood and tissue collection and treatment

Blood was collected from the tail vein of each rat. Blood collection was immediately followed by necropsy under isoflurane anesthesia. In each group, blood collection and necropsy were performed at 0, 60, 180, 360, 540, and 1440 min after oral administration of DCP. Six rats were used for each collection. 0.2 mL blood samples were collected into 10-mL headspace sampler (HS)-vials, and 0.2 mL of distilled water was added to each sample (Take et al., 2009, 2010, 2012, 2013, 2014a, 2014b). The lung, liver, kidney, and abdominal fat were removed from each rat, and tissue samples (range from about 0.2 to 0.6 g) were placed into separate 10-mL HS-vials. Then, 5 mL of distilled water was added to the vials, and the vials were immediately sealed with an aluminum crimp cup (Take et al., 2010, 2012, 2013, 2014b).

Measurement of DCP in the blood and tissues

DCP concentrations in the blood and tissue samples were analyzed by HS-Gas Chromatography (GC)/Mass Spectrometer (MS) using Agilent Technologies 7694 (Agilent Technologies, Santa Clara, CA, USA) HS (oven temperature, 100°C; loop temperature, 130°C; 10 min vial equilibration time for blood samples and 30 min for tissue samples) and Agilent Technologies 5973N (Agilent Technologies) GC/MS system (column, J&W DB-1 60 m × 0.25 mm ID × 0.25 μm; oven temperature, 100°C; ion source temperature, 230°C; carrier gas, helium at 1 mL/min; ionization, EI (electron ionization); fragment peak, 63 m/z).

RESULTS AND DISCUSSION

Blood concentrations of DCP

The time-course changes in the blood concentration of DCP are shown in Fig. 1 (Supplemental Table 1). DCP was not measureable in the blood at 0 min. DCP concentration in the blood increased after oral admin-

![Fig. 1. The concentration of DCP in the blood of rats after oral administration of DCP (n = 6 for each collection time point). The concentration of DCP in the blood is expressed as the mean concentration ± S.D.](image-url)
istration, reaching a maximum concentration (Cmax) between the end of oral administration and 60 min after oral administration, and then slowly decreasing in a time-related manner; DCP could still be detected in the blood 1440 min after oral administration in both the 62-mg/kg and 125-mg/kg groups. The peak concentrations of DCP in the blood were similar for both the 62-mg/kg and 125-mg/kg groups; however, in the 62-mg/kg group, DCP remained at its peak concentration in the blood for a limited time while in the 125-mg/kg groups, DCP remained at or near its peak concentration in the blood for several hr.

Timchalk et al. (1991) reported that in rats [14C]DCP could still be detected in the body 24 and 48 hr after oral administration of 1 or 100 mg/kg body weight. In addition, we previously reported that in rats DCP was still detected in the blood 1080 min after the end of a 6-hr inhalation exposure period to 80 or 500 ppm DCP vapor (Take et al., 2014b). Taken together, these data (the present study; Take et al., 2014b; Timchalk et al., 1991) suggest that in rats DCP remains in the blood for a prolonged period of time after administration.

The half-lives (T1/2) of the concentration of DCP in the blood after Cmax and the area-under-the-curve (AUC) values for DCP in the blood from 0 to 1440 min after oral administration in the 2 groups are shown in Table 1. The T1/2 value of the 125-mg/kg group was calculated based on the decrease of DCP in the blood 360 min after administration. The T1/2 of the 125-mg/kg group was longer than that of the 62-mg/kg group. The AUC0-1440 of the 125-mg/kg group was higher than that of the 62-mg/kg group. The ratio of the 125-mg/kg group AUC0-1440 to the 62-mg/kg group AUC0-1440 is 2.8-fold, slightly higher than the ratio of the administered doses (125-/62-mg/kg group = 2).

Timchalk et al. (1991) reported the levels of [14C]DCP levels in the rat 24 and 48 hr after oral administration; however, they did not measure time-course changes in the concentration of DCP in the blood or the T1/2 or AUC of DCP in the blood after administration (Timchalk et al., 1991). Our study is the first report of the time-course changes, T1/2, and AUC of DCP in blood concentration after oral administration and will be valuable in the further development physiologically based pharmacokinetic (PBPK) models of DCP.

### Tissue concentrations of DCP

The time-course changes in the tissue concentrations of DCP in the 62-mg/kg and 125-mg/kg groups are shown in Figs. 2-5 (Supplemental Table 1). DCP was not measurable in any of the tissues at 0 min. The DCP concentration in each of the tissues increased after oral administration, reaching a Cmax between the end of oral administration and 60 min after oral administration, and then slowly decreasing in a time-related manner. In the 62-mg/kg group, DCP was still detectable in the liver, kidney, and lung at 540 min and in the abdominal fat at 1440 min after administration. In the 125-mg/kg group, DCP was still detectable in the liver, kidney, lung, and abdominal fat 1440 min after administration (Figs. 2-5: Supplemental Table 1). The DCP concentration in the abdominal fat was much greater than in the other tissues at each collection time point (Figs. 2-5: Supplemental Table 1).

After oral administration of DCP, a chemical of very high lipid solubility (IARC, 1986), a single large bolus of DCP is absorbed through the gastrointestinal mucosa into the lacteals and blood capillaries of the intestinal villi and then transported to the venous blood returning to the heart by the lymphatic system or to the liver through the portal vein and then to the heart via the vena cava, and after leaving the heart the DCP is distributed to the other tissues in the body. DCP from this bolus is being constantly eliminated from the rat’s body by metabolism and excre-

<table>
<thead>
<tr>
<th>T1/2 and AUCs in the blood and tissues and ratios of tissue AUCs to the ACU of the blood.</th>
<th>62-mg/kg group</th>
<th>125-mg/kg group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood</strong></td>
<td>193&lt;sup&gt;a&lt;/sup&gt;</td>
<td>359&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td>144</td>
<td>2038&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td>144</td>
<td>1034</td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td>114</td>
<td>527</td>
</tr>
<tr>
<td><strong>Abdominal fat</strong></td>
<td>257</td>
<td>17771</td>
</tr>
</tbody>
</table>

<sup>a</sup> min.
<sup>b</sup> μg/mL × min.
<sup>c</sup> mg/mL × min.
<sup>d</sup> AUC<sub>0-1440</sub> value of the liver, kidney, lung, or abdominal fat / AUC<sub>0-1440</sub> value of the blood.
tion. After the Cmax, the higher concentrations of DCP in the liver and kidney could affect elimination of DCP by metabolism (Timchalk et al., 1991; Hutson et al., 1971; Jones and Gibson, 1980; Bartels and Timchalk, 1990; Suzuki et al., 2014) and excretion (Timchalk et al., 1991; Hutson et al., 1971; Jones and Gibson, 1980), and DCP concentration in the lung is expected to decrease due to exhalation (Timchalk et al., 1991; Hutson et al., 1971). After Cmax, elimination of DCP from the rat’s body by metabolism and excretion results in time-course changes of DCP concentration in the tissues (Figs. 2-5: Supplemental Table 1).

Timchalk et al. (1991) reported that [14C]DCP was still detectable in the tissues of rats administered 1 or 100 mg/kg body weight 24- and 48-hr after oral administration, and that [14C]DCP was still detectable in the tissues of rats exposed to 5, 50, or 100 ppm [14C]DCP vapor for a 6-hr period 42 hrs after the end of the exposure period. We previously reported that DCP was still detectable in the tissues of rats exposed to 80 or 500 ppm DCP vapor for a 6-hr period 1080 min after the end of the exposure period (Take et al., 2014b). In our present study, DCP could still be detected in the tissues 1440 min after oral administration. Taken together, similarly to DCP in the blood, these data (the present study; Take et al., 2014b; Timchalk et al., 1991) suggest that DCP remains in the tissues for a prolonged period of time after administration.

Vol. 42 No. 2
Twenty-four hours after oral administration by a single gavage, DCP could still be detected in the blood and abdominal fat in the 62-mg/kg group, and in the blood, liver, kidney, lung, and abdominal fat in the 125-mg/kg group. In 2-week, 13-week, and 2-year animal studies, rats were orally administered from 125 up to 2000 mg/kg DCP daily (2-week study) or from 60 up to 1000 mg/kg DCP 5 days/week (13-week study), or 125 or 250 mg/kg DCP 5 days/week (2-year study, females) or 62 or 125 mg/kg DCP 5 days/week (2-year study, males) (NTP, 1986). As can be seen by the results of our study, even at the lowest doses used, there would be accumulation of DCP in the bodies of the test animals. Therefore, our results suggest that the toxicity seen in these studies was likely associated with repetitive dosage of DCP and its accumulation in the blood and tissues of the exposed rats.

The $T_{1/2}$ values of DCP in the tissues after Cmax are shown in Table 1. In both groups, the $T_{1/2}$ value of the lung was the shortest, and the $T_{1/2}$ value of the abdominal fat was the longest. The $T_{1/2}$ values of the lung, liver, kidney, and abdominal fat of the 125-mg/kg group were longer than those of the 62-mg/kg group. The $T_{1/2}$ values of the abdominal fat were similar to the $T_{1/2}$ values of the blood. The similar $T_{1/2}$ values of DCP in the blood and abdominal fat in the 2 groups coupled with the longer $T_{1/2}$ values of DCP in the lung, liver, and kidney in the 125-mg/kg group compared to the 62-mg/kg group suggest that DCP was transported from the abdominal fat to other tissues by the blood, thereby lengthening the $T_{1/2}$ values in these tissues. We previously reported the $T_{1/2}$ values of DCP of the blood and tissues of rats exposed to DCP by inhalation (Take et al., 2014b). Similarly to the present study, the $T_{1/2}$ values of DCP in the blood and abdominal fat were similar, and the $T_{1/2}$ values of DCP in the blood and abdominal fat were longer than the $T_{1/2}$ values of DCP in the liver, kidney, and lung (Take et al., 2014b). Thus, the tissue retention pattern of DCP after administration by inhalation and oral routes was similar.

The tissue AUC$_{0-1440}$ values and the ratio of the AUC$_{0-1440}$ value for each tissue to the AUC$_{0-1440}$ value for blood are shown in Table 2. As expected, all of the tissue AUC$_{0-1440}$ values were higher in the 125-mg/kg group than in the 62-mg/kg group. Similarly to the tissue concentrations of DCP at individual time points, the ratio of the tissue AUC$_{0-1440}$ values for the 125-mg/kg group to the 62-mg/kg group are 3.2-fold (liver), 3.0-fold (kidney), 3.5-fold (lung), and 2.8-fold (abdominal fat), respectively, and these values were slightly higher than the ratio of the orally administered doses (125-/62-mg/kg group = 2). The ratios of the AUC$_{0-1440}$ values for the liver, kidney, lung, and abdominal fat to the AUC$_{0-1440}$ value for the blood in the 62-mg/kg group are about the same as in the 125-mg/kg group. The $T_{1/2}$ values indicate that the DCP elimination pattern is similar in the two groups.

The elimination rate constants were 0.22/hr (blood),

**Table 2.** Ratios of the blood and tissue partition coefficients and the ratios of the concentrations of DCP in the tissues to the blood.

<table>
<thead>
<tr>
<th>Partition coefficient (Ratio)</th>
<th>Collection time point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td>62-mg/kg group</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>18.7</td>
</tr>
<tr>
<td>Liver</td>
<td>24.8</td>
</tr>
<tr>
<td>Abdominal fat</td>
<td>499</td>
</tr>
<tr>
<td>Kidney</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
</tr>
<tr>
<td>125-mg/kg group</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>18.7</td>
</tr>
<tr>
<td>Liver</td>
<td>24.8</td>
</tr>
<tr>
<td>Abdominal fat</td>
<td>499</td>
</tr>
<tr>
<td>Kidney</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
</tr>
</tbody>
</table>

*Gargas et al., 1989.

b Partition coefficient value of the liver or abdominal fat / partition coefficient value of the blood.

c Mean concentration in the liver, abdominal fat, kidney, or lung (n = 6) / mean concentration in the blood (n = 6).

d Compound not detected.

Table 2. Ratios of the blood and tissue partition coefficients and the ratios of the concentrations of DCP in the tissues to the blood.
0.29/hr (liver), 0.29/hr (kidney), 0.36/hr (lung), and 0.16/hr (abdominal fat) for the 62-mg/kg group, and 0.13/hr (blood), 0.22/hr (liver), 0.22/hr (kidney), 0.25/hr (lung), and 0.13/hr (abdominal fat) for the 125-mg/kg group (The Japanese Society of Toxicology, 2009). The results are useful data pertaining to key toxicokinetic parameters of elimination rate for a one-compartment model of oral administration of DCP.

Timchalk et al. (1991) reported the levels of [14C]DCP levels in the rat 24- and 48-hr after oral administration of 1 or 100 mg/kg body weight; however, they did not measure time-course changes in the concentration of DCP in individual tissues or the T1/2 or AUC of DCP in individual tissues after administration (Timchalk et al., 1991). Our study is the first report of the time-course changes, T1/2, AUC, and toxicokinetic parameters of DCP in individual tissues after oral administration and will be valuable in the further development PBPK models of DCP.

**DCP partition coefficients and tissue concentrations**

The relationship between the partition coefficients and the ratios of the concentrations of DCP in the blood and tissues is shown in Table 2. The partition coefficients of the blood/air, liver/air, and abdominal fat/air are 18.7, 24.8, and 499 (Gargas et al., 1989), respectively, and the ratios of the partition coefficients of the liver and abdominal fat to the blood are 1.33 and 26.68, respectively. We compared our results with ratios of the reported partition coefficients at each collection point.

The ratios of the concentrations of DCP in the liver and in the blood ranged from 2.79 to 6.50 for the 62-mg/kg group and from 0.66 to 9.86 for the 125-mg/kg group. Excluding the 1440 min liver/blood DCP ratio in the 125-mg/kg group, the ratio of the reported partition coefficients of liver/blood (1.33) is lower than 2.79-6.50-fold (62-mg/kg group) and 4.22-9.86-fold (125-mg/kg group). The ratios of the concentrations of DCP in the abdominal fat and in the blood ranged from 30.09 to 80.57 for the 62-mg/kg group and from 41.69 to 80.87 for the 125-mg/kg group. The ratio of the reported partition coefficients of abdominal fat/blood (26.68) is lower than 30.09-80.57-fold (62-mg/kg group) and 41.69-80.87-fold (125-mg/kg group). Thus, the ratios of the concentrations of DCP in the liver and abdominal fat are greater than that of the ratios of the respective partition coefficients. One likely explanation of this is that the clearance rate of DCP from rat's body by metabolism and excretion is slow.

In the kidney and lung, except at 1440 min after administration in 125-mg/kg group, the ratios of the DCP concentrations in the kidney/blood and lung/blood were similar for the 2 groups at each collection time point. The ratios were 44-55% (62-mg/kg group) and 42-66% (125-mg/kg group) lower for the lung compared to the kidney. This result is consistent with the results of our previous study using inhalation route administration (Take et al., 2014b). Gargas et al. (1989) did not report the partition coefficients of kidney/air and lung/air. Therefore, our results are useful for understanding the pharmacokinetics pertaining to the partition coefficients of oral exposure to DCP. Finally, comparing the time-course changes of DCP concentration in each tissue and the reported partition coefficients (Gargas et al., 1989) will be a key factor in understanding the pharmacokinetics of exposure to DCP in animal models.

**DCP equivalent doses of oral administration and inhalation routes**

We previously reported the time-course changes of DCP concentrations in the blood and tissues of rats exposed to 80 or 500 ppm DCP vapor by inhalation (Take et al., 2014b). We found that the DCP concentration in the abdominal fat was much greater than that in the blood and other tissues. Eighteen hrs after the end of inhalation exposure, DCP could still be detected in the abdominal fat in the 80-ppm group, and in the blood, liver, kidney, and abdominal fat in the 500-ppm group.

Similarly to administration by inhalation, in the present study (oral administration route), the DCP concentration in the abdominal fat was much greater than in the blood and other tissues. Twenty-four hours after oral administration, DCP could still be detected in the blood and abdominal fat in the 62-mg/kg group, and in the blood, liver, kidney, lung, and abdominal fat in the 125-mg/kg group. Thus, after administration by both the oral and inhalation routes, the distribution of DCP in the abdominal fat was much greater than that in the blood and other tissues, and this was due to the very high lipid solubility of DCP (IARC, 1986).

The Ministry of the Environment, Government of Japan, has endorsed for the need to interconvert inhalation-dose and oral-dose (Ministry of the Environment, Government of Japan, 2011), the two most common routes of exposure to wide variety of toxic chemicals. We previously reported the estimation of inhalation and oral equivalent doses of chloroform based on the relationship of the AUC inhalation dose curve and the AUC after oral administration (Take et al., 2014a). The total AUC0-1440 of DCP in the blood and tissues in the present study are given in Table 1 and the total AUC0-1440-inhalation of DCP in the blood and tissues is reported in Take et
Effect of 1,2-dichloropropane in rats after oral administration

al. (2014b). The inhalation equivalent dose for the 62- and 125-mg/kg groups were calculated as: inhalation equivalent dose = 80 ppm × total AUC0-1440 of oral dose (Table 1)/total AUC0-1440 of inhalation dose (Take et al., 2014b). Since the age, body weight, and sex of there were the same, this formula does not need to be adjusted. The inhalation equivalent doses for the 62-mg/kg group were 114 ppm (blood), 384 ppm (liver), 261 ppm (kidney), 346 ppm (lung), and 149 ppm (abdominal fat), and for the 125-mg/kg group were 316 ppm (blood), 1211 ppm (liver), 789 ppm (kidney), 1224 ppm (lung), and 416 ppm (abdominal fat). For both the 62-mg/kg and 125-mg/kg groups, the inhalation equivalent doses of the liver and lung were higher than those of blood and other tissues, and inhalation equivalent doses of the blood and abdominal fat were lower than that of the other tissues. In addition, the inhalation equivalent doses of the liver and lung were similar, and those of the blood and abdominal fat were similar. The present results contribute to the comparison of oral and inhalation doses.

In conclusion, in the present study, the distribution of DCP in the blood and tissues after oral administration was investigated. The DCP concentration in the abdominal fat was markedly greater than in the blood and other tissues, and inhalation equivalent doses of the blood and abdominal fat were similar. Present results contribute to the comparison of oral and inhalation doses.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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