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Formation of Epichlorohydrin, a Known Rodent Carcinogen, Following Oral Administration of 1,3-Dichloro-2-propanol in Rats

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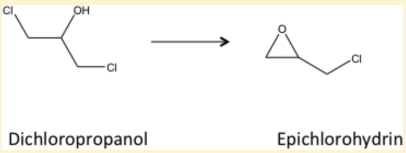
ABSTRACT: The observed toxicity and carcinogenicity of 1,3-dichloro-2-propanol (DCP) in rodents is thought to be due to the formation of reactive metabolites, epichlorohydrin (ECH) and dichloroacetone (DCA). However, there is no direct evidence for the formation of these metabolites from exposure to DCP in rodents due to the challenges of measuring these reactive intermediates directly *in vivo*. The objective of this work was to investigate the metabolism of DCP to ECH and DCA *in vivo* by first developing a sensitive analytical method in a suitable biological matrix and analyzing samples from rats administered DCP. DCA reacted rapidly *in vitro* in rat blood, plasma, and liver homogenate, precluding its detection. Because ECH rapidly disappeared in liver homogenate, but was relatively long-lived in plasma and blood *in vitro*, blood was selected for analysis of this metabolite. Following a single oral dose of 50 mg/kg DCP in male or female Harlan Sprague−Dawley rats, ECH was detected in blood with a maximum concentration reached at ≤13.7 min. ECH was cleared rapidly with a half-life of ca. 33 and 48 min in males and females, respectively. Following a single oral dose of 25 mg/kg ECH in male and female rats, the elimination half-life of ECH was ca. 34 and 20 min, respectively; the oral bioavailability of ECH was low (males, 5.2%; females, 2.1%), suggesting extensive first pass metabolism of ECH following oral administration. The area under the concentration vs time curve for ECH following oral administration of DCP and intravenous administration of ECH was used to estimate the percent of the DCP dose converted to ECH in rats. On the basis of this analysis, we concluded that in male and female rats following oral administration of 50 mg/kg DCP, ≥1.26% or ≥1.78% of the administered dose was metabolized to ECH, respectively.



INTRODUCTION

1,3-Dichloro-2-propanol (DCP) is a member of the broad chemical class halohydrins. DCP is a semivolatile liquid used in high volume as an intermediate in the production of epichlorohydrin (ECH), 1,3-dichloropropene, and 1,2,3-trichloropropane.[1,2] Workers may be exposed to 1,3-DCP via inhalation, dermal, or oral exposure during the manufacture and use of these chemical agents. Exposure to DCP may also occur from ingestion of food containing hydrochloric acid-hydrolyzed vegetable protein or from drinking water in which ECH polyamine polyelectrolytes are used as flocculants and coagulants for water purification.[3,4] Thermal degradation, metabolism, or hydrolysis of the flame retardant tris(1,3-dichloropropyl) phosphate (Fyrol FR-2) might also be a source of consumer exposure to DCP.[5−8]

In subchronic drinking water or gavage studies (doses up to 100 mg/kg/day) in male and female Sprague−Dawley rats, DCP caused decreased body weights, increased liver and kidney weights, and histopathological changes in the stomach, kidney, liver, and nasal tissue.[9] In chronic drinking water studies (doses up to 240 mg/L), dose-related increases in the combined incidences of hepatocellular adenoma and carcinoma in the liver, squamous cell papilloma and carcinoma in the tongue/oral cavity, follicular cell adenoma and carcinoma in the thyroid, and the combined numbers of renal tubular adenoma and carcinoma in the kidney (males only) have been reported in male and female rats.[10] In numerous bacterial and mammalian test systems *in vitro*, DCP was genotoxic in the presence and absence of metabolic activation.[11−16]

In humans, DCP is known to be moderately toxic via inhalation, ingestion, and skin contact. Oral intake resulted in severe irritation of the throat and stomach.[9] Acute hepatitis was reported in 5 of 12 workers exposed to an unknown concentration, likely via inhalation from the cleaning of a DCP manufacturing tank.[17,18] In these individuals, DCP plasma levels at approximately 48 h after exposure were 200 ng/mL. Two of the five workers died from hepatic failure, and autopsy showed hepatocellular necrosis in one of the individuals.

DCP is listed by the International Agency for Research on Cancer (IARC) as being *possibly* carcinogenic to humans (Group 2B).[19] California EPA listed DCP on the Proposition 65 list of chemicals as known to cause cancer in humans.[20] This assessment included consideration of the prospective metabolism of DCP to carcinogenic metabolites, ECH and glycidol, and toxic metabolites, dichloroacetone (DCA) and 3-monochloropropane-1,2-diol. ECH is carcinogenic in experimental animals following exposure via drinking water (375 to 1500 mg/L), gavage (2 and 10 mg/kg), and inhalation (30 and 100 ppm).[21,22] ECH is listed by IARC as *probably* carcinogenic to humans (Group 2A).[23] DCA is known to be a mutagen based on *in vitro* findings.[24,25]

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To date, there is no direct evidence for the formation of ECH or DCA following exposure to DCP in rodents or humans. Indirect evidence includes common metabolites observed in animals administered DCP and ECH. Following oral administration of 50 mg DCP/kg daily for 5 days in male Sprague–Dawley rats, β-chlorolactate (~5% of the dose), *N*,*N*′-bis(acetyl)-*S*,*S*′-(1,3-bis(cysteinyl))propan-2-ol (1%), and *N*-acetyl-S-(2,3-dihydroxypropyl)cysteine were detected in urine.[26] Similar metabolites were observed following oral administration of ECH and hence ECH was proposed as an intermediate in the metabolism of DCP.[26] In another study, following a single subcutaneous injection of DCP at ~68 mg/kg in male Wistar rats, 1,2-propanediol (0.43% of the dose) and 3-chloro-1,2-propanediol (0.35%) were detected in the 24 h urine.[27] On the basis of these observations, two main metabolic pathways were proposed, as shown in Figure 1, one via the formation of ECH and one via the formation of DCA.[20] Several *in vitro* and *in vivo* studies reported the ability of DCP to deplete GSH and to induce and/or be metabolized by CYPs.[28−36] In Wistar rats, diethyldithiocarbamate provided significant protection against DCP hepatotoxicity and inhibited enzyme markers for CYP2E1 activity; ECH was proposed to be the toxic intermediate.[37] In rat hepatocyte cultures, isoniazid increased the rate and extent of GSH depletion as well as the toxicity, whereas cyanamide did not, confirming the involvement of CYP2E1 in the metabolism of DCP. Pretreatment of cultures with 1-aminobenzotriazole (a CYP inhibitor) prevented the toxicity of DCP, whereas pretreatment with diethyl maleate or buthionine sulfoximine (depletion of GSH or inhibition of GSH synthesis) increased its toxicity.[32,33] The observation that pyridine in the microsomal incubations reduced GSH depletion but did not completely eliminate depletion suggests the existence of a second pathway, which was thought to be DCA as a metabolite of DCP via the action of alcohol dehydrogenase or CYPs.[30]

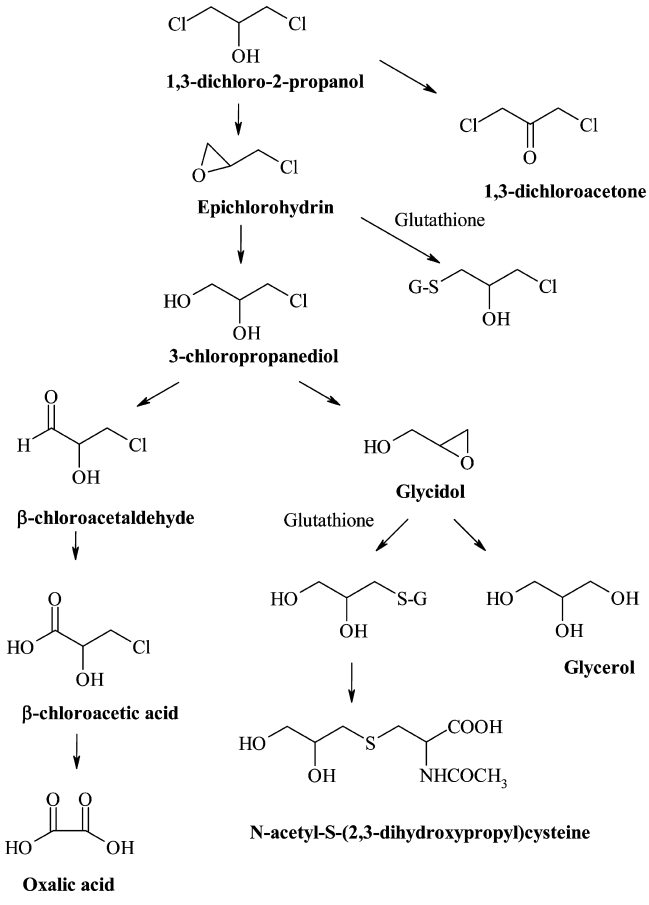

Figure 1. Proposed metabolic pathway of DCP in rodents. Adapted with permission from ref 20. Copyright 2010 Office of Environmental Health Hazard Assessment (OEHHA).

The genotoxic and carcinogenic potency of DCP is thought to arise mainly due to the metabolism of DCP to ECH and/or DCA, although, as pointed out earlier, there is no direct evidence in the literature for the formation of these metabolites. This is likely due to the reactive nature of these electrophilic metabolites that are postulated to be short-lived *in vivo*. DCP was nominated to the National Toxicology Program (NTP) by the National Institute of Environmental Health Sciences for toxicological characterization, including metabolism and disposition, reproductive toxicity, and carcinogenicity studies. Unambiguous identification of ECH and/or DCA from the metabolism of DCP is critical for deciding whether further toxicity and carcinogenicity studies of DCP are needed. Therefore, the purpose of the current work is (1) to develop a sensitive analytical method to detect ECH and DCA in selected biological matrices, (2) to investigate the half-lives of ECH and DCA in selected biological matrices *in vitro* to determine the feasibility of detecting these reactive metabolites *in vivo*, and (3) to investigate the formation of ECH and DCA in adult male and female Harlan Sprague–Dawley rats following oral exposure to DCP. The dose was set at 50 mg/kg based on the rat LD_{50} values as well as the doses used in toxicity studies.[38,39]

MATERIALS AND METHODS

Chemicals and Reagents. DCP was obtained from Acros Organics (Geel, Belgium) with a specified purity of 99.5%. ECH was obtained from Aldrich Chemical Co. (St. Louis, MO) with a vendor purity of 99.9%. Prior to use in studies, the identity of DCP and ECH was confirmed by a combination of GC-MS and ^{1}H and ^{13}C NMR spectroscopy. Purity, estimated by a combination of GC-FID and GC-MS, was 99.4 and 99.9%, respectively, for DCP and ECH. ECH was not detected in DCP. ($^{2}H_5$)(±)-Epichlorohydrin (ECH-d_5) (98.7 atom % D; 98.8% chemical purity) and ($^{2}H_5$)1,3-dichloro-2-propanol (DCP-d_5) (98.8 atom % D; 98.1% chemical purity) was from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). All chemicals and reagents were stored as specified by the vendor. 3A molecular sieves were from Acros Organics (Geel, Belgium). Ethyl acetate and methyl *tert*-butyl ether were obtained from Aldrich Chemical Co. (St. Louis, MO). Heparin was obtained from APP Pharmaceuticals (Schaumburg, IL). Sterile isotonic saline was from Vedco Inc. (St. Joseph, MO) or B. Braun Medical Inc. (Bethlehem, PA).

Half-Life of ECH in Rodent Blood, Plasma, or Liver Homogenate *in Vitro*. Half-lives of ECH in multiple matrices were investigated *in vitro*. Ten microliters of ECH in acetonitrile was added to 90 μL of rat blood, rat plasma, rat liver homogenate (250 mg/mL), or mouse blood at 37 °C or on ice to give a final ECH concentration of 1 or 80 μM. Samples were mixed and incubated for 5, 15, 30, and 60 min. At the end of the reaction, 100 μL of ethyl acetate containing an internal standard mixture (1 μM ECH-d_5) was added, and the samples were vortex mixed and centrifuged. The supernatant was transferred into a microcentrifuge tube containing 10 mg of 3A molecular sieves, vortex mixed, and centrifuged. The supernatant was transferred into a GC vial insert. Standards were prepared containing 0.1, 0.25, 0.5, 1.0, and 2.5 μM ECH, prepared by adding 10 μL of ECH in acetonitrile and 90 μL of internal standard in ethyl acetate. Samples were diluted into the analytical range, as needed. Samples were analyzed by the GC-MS method developed (but not validated) below.

Development and Validation of an Analytical Method for the Quantitation of DCP and ECH in Biological Matrices. A method involving the extraction of blood with ethyl acetate and drying the ethyl acetate extract with 3A molecular sieves followed by gas chromatography−mass spectrometry (GC-MS) in positive ion chemical ionization mode was developed and validated to quantify DCP and ECH as described below. The recovery of DCA from all matrices evaluated was extremely low, likely due to its high reactivity, and hence was not evaluated any further. Solvent calibration curves for DCP and ECH were prepared in ethyl acetate over the range 0.01, 0.025, 0.05, 0.1, 0.2, 0.5, and 1 μM. Matrix calibration curves were prepared using 90 μL of rat blood, plasma, or liver homogenate prepared in water (250 mg/mL) in a microcentrifuge tube, to which 10 μL of a spiking solution of analytes in acetonitrile was added to give final concentrations of 0.01, 0.025, 0.05, 0.1, 0.2, 0.5, and 1 μM in matrix. Calibration curves were also prepared in water similar to that for the matrix calibration curves except that 90 μL of water was used in place of biological matrix. Following the addition of 100 μL of ethyl acetate containing the internal standard mixture (0.25 μM DCP-d_5 and ECH-d_5), samples were vortex-mixed and centrifuged for 5 min. The ethyl acetate layer was dried in a second microfuge tube containing 10 mg of 3A molecular sieves. After vortex mixing, the sample was centrifuged, and the ethyl acetate layer was transferred to a 200 μL silanized insert in a 1.5 mL amber GC vial. All samples were prepared in triplicate.

The method was validated in blood using solvent and matrix standard curves prepared as above. To evaluate the extracted sample storage stability, six sets of quality control (QC) rat blood samples, each with four replicates, were prepared at 0.025 μM (low QC), 0.1 μM (medium QC), and 0.5 μM (high QC) and were processed as described above. One set was analyzed immediately after preparation. Another set was stored in an autosampler tray for 48 h at room temperature. Four sets were stored at −20 °C for 1 week, 3 months, or 6 months prior to analysis. One set of samples was subjected to three freeze−thaw cycles during a 1 week storage period. Study samples were prepared similar to that for the matrix calibration curve samples as described above without the addition of the spiking solution containing DCP and ECH standards.

All samples were analyzed on an Agilent 6890 GC equipped with a programmable temperature vaporization inlet coupled to an Agilent 5973 MSD (Agilent Technologies, Santa Clara, CA) operated in positive chemical ionization mode with selected ion monitoring, with the exception of samples from female rats administered 25 mg/kg ECH by gavage and male and female rats administered 1 mg/kg ECH by intravenous injection (see below). One microliter was injected via pulsed splitless injections onto a DB-5 MS UI column (30 m × 0.25 mm i.d × 0.25 μm, thickness) (Agilent Technologies, Santa Clara, CA). The injector was programmed from 75 °C (0.1 min) to 200 °C (0.5 min) at 500 °C/min with a pulse pressure of 50 psi at the time of injection. The carrier gas was helium at a flow rate of 1.5 mL/min. The GC oven temperature was maintained at an initial temperature of 30 °C (1 min) and then ramped at 5 °C/min to 50 °C. Late eluting compounds were removed by increasing the oven temperature to 200 °C at 80 °C/min. The total run time was ca. 7 min. DCP, DCP-d_5, ECH, and ECH-d_5 were eluted at 6.65, 6.60, 4.02, and 3.99 min, respectively. Mass spectrometer parameters were as follows: ion source temperature, 110 °C; quadrupole temperature, 106 °C; and transfer line temperature, 150 °C. Samples from female rats administered 25 mg/kg ECH by gavage and male and female rats administered 1 mg/kg ECH by intravenous injection and corresponding calibration curves (with extension of the calibration curve to 8 points, including 0.005 μM) were analyzed on an Agilent 7890A GC coupled to an Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA). All GC and mass spectrometer conditions were the same as those given above except for the following: the injector temperature was constant at 150 °C, the ion source temperature was 150 °C, and the total run time was ca. 8 min with DCP, DCP-d_5, ECH, and ECH-d_5 eluting at 4.10, 4.06, 3.15, and 3.12 min, respectively. In all cases, ions monitored were m/z 111 (DCP), 116 (DCP-d_5), 93 (ECH), and 98 (ECH-d_5). Calibrations for both DCP and ECH were performed using seven-point standard curves prepared in rat blood. A $1/x$ weighted linear regression equation was calculated for DCP and ECH, relating the response ratios of the analyte/internal standard to its concentration in matrix standard.

Animals. All studies were conducted at RTI International (RTP, NC) and were approved by the RTI Institutional Animal Care and Use Committee. Animals were housed in a facility that is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Animal procedures were in accordance with the Guide for the Care and Use of Laboratory Animals.[40] Male and female Harlan Sprague−Dawley rats (males, 235−277 g; females, 184−232 g; 8−10 weeks old at dosing) with implanted jugular vein cannulae were obtained from Harlan Laboratories (Indianapolis, IN). The animals were quarantined for up to 6 days before they were used in a study. Animals were provided certified NTP 2000 wafer diet (Zeigler Brothers, Gardners, PA) and city tap water (Durham, NC) *ad libitum*. During the quarantine and study periods, room temperature was maintained at 22 ± 2 °C and relative humidity was maintained within 35 to 65%, with a 12 h light/dark cycle.

Preparation and Analysis of Dose Formulations. Oral dose formulations of DCP were prepared at 10 mg/mL in deionized water on the morning of use and stored on wet ice. Dose formulations were diluted 1:20 with acetonitrile and quantified by GC with a flame ionization detection against a calibration curve from 1−1000 μg/mL. Oral and intravenous dose formulations of ECH were prepared in deionized water at 0.2 and 5 mg/mL and in sterile isotonic saline at 0.5 mg/mL, respectively, on the morning of use and stored on wet ice to prevent any hydrolysis of ECH. ECH dose formulations were analyzed by GC-MS following extraction with chloroform and quantified against a calibration curve from 0.04 to 1000 μg/mL. DCP dose formulations were also analyzed for the presence of ECH using the same procedure as that used for the analysis of ECH dose formulation.

Single Oral or Intravenous Administration of DCP or ECH in Male and Female Harlan Sprague−Dawley Rats. Male or female rats (3 rats per sex per group) were administered a single oral dose of DCP at 50 mg/kg or ECH at 1 and 25 mg/kg. Oral doses were administered via intragastric gavage using a syringe equipped with a ball-tipped 16-gauge stainless steel gavage needle in a dose volume of 5 mL/kg. Male or female rats (3 rats per sex per group) were administered a single intravenous dose of ECH at 1 mg/kg. Doses were administered via the tail vein using a syringe equipped with a 23-guage needle in a dose volume of 2 mL/kg.

Collection and Analysis of Blood Following Oral or Intravenous Administration of DCP or ECH. After dosing, blood was collected into a heparinized syringe via indwelling jugular cannulae from 3 animals per each time point. A blood sample was also collected from each animal prior to dosing, and the cannulae were refilled with heparinized isotonic saline. The target blood sample volume was 100 μL. Blood collection time points were as follows: predose, 5, 15, and 30 min and 1, 2, 4, and 6 h (oral, 50 mg/kg DCP by gavage); predose, 5, 10, 15, 30, 60, and 90 min (25 mg/kg ECH by gavage); predose, 5, 10, 15, 30, 45, 60, and 90 min (1 mg/kg ECH male rats by intravenous); and predose, 5, 10, 15, 20, 25, 30, and 45 min (1 mg/kg ECH female rats by intravenous). Following blood collection, animals were euthanized by exposure to CO_2. Immediately upon collection, blood was weighed in a 1.5 mL plastic centrifuge tube, an equal volume (based on the weight of blood in the tube and the density of ethyl acetate) of internal standard solution (0.25 μM each of DCP-d_5 and ECH-d_5 in ethyl acetate) was added to the tube, and the sample was vortex-mixed. The weight of internal standard solution added was recorded. Tubes were immediately placed on wet ice after internal standard addition and before subsequent extraction steps. The samples were centrifuged, and the ethyl acetate was removed, dried with 3A molecular sieves, and analyzed by GC-MS as described above for DCP and ECH. For each analyte, a triplicate standard curve and QC samples at three concentration levels were run in parallel with each sample set.

Toxicokinetic Analysis. Toxicokinetic analysis was conducted using WinNonlin software, version 5.2 (Pharsight Corporation). Noncompartmental analysis was conducted using a model for

extravascular input.[41] Individual animal data was modeled, with input for the dose of DCP or ECH administered to calculate the dose-normalized area under the curve in blood for DCP or ECH. Parameters estimated were as follows: $t_{1/2}$, half-life of elimination; C_{max}, maximum concentration; C_{max}/D, dose-adjusted maximum concentration; T_{max}, time at which the maximum concentration was achieved; $AUC_{(0-t)}$, area under the blood concentration vs time curve to last time point; $AUC_{(0-t)}/D$, dose-adjusted area under the blood concentration vs time curve to last time point; $AUC_{(0-\infty)}$, area under the blood concentration vs time curve to infinity; $AUC_{(0-\infty)}$, dose-adjusted area under the blood concentration vs time curve to infinity; and V_z/F, volume of distribution adjusted to bioavailability of test chemical.

RESULTS

Development and Validation of the Analytical Method and Evaluation of Half-Life of ECH *in Vitro*. Various approaches including solid-phase microextraction and solvent extraction methods coupled with detection by electron capture or mass spectrometry with electron ionization, negative ion chemical ionization, or positive ion chemical ionization were investigated to detect and quantify ECH and DCA, the postulated reactive metabolites of DCP, in biological matrices. Solvent extraction followed by drying over molecular sieves and analysis by GC-MS in the positive ion chemical ionization mode was found to be the most promising method and was selected as the final method.

Plasma, blood, and liver homogenate were evaluated to determine the most suitable matrix to detect these reactive metabolites *in vivo*. In addition, samples were processed on ice to prevent hydrolysis and/or reaction of ECH and DCA in biological matrices. The recovery of DCA from all biological matrices investigated was very poor. The clearance of ECH from liver homogenate, blood, and plasma was evaluated at 37 °C and on ice at ECH concentrations of 1 or 80 μM. ECH was relatively stable in blood and plasma, but it rapidly cleared in liver homogenate and was not detectable at any of the time points measured. The half-lives estimated for ECH in blood and plasma are shown in Table 1. In rat, ECH cleared more rapidly in blood compared to that in plasma, and at 37 °C, the half-life in rat plasma was between 50 and 53 min, compared with between 20 and 25 min in rat blood. Decreasing the temperature resulted in a substantially longer half-life in both blood and plasma (Table 1). At 37 °C, ECH was cleared in mouse blood more slowly than in rat blood, with a half-life between 43 and 45 min. The initial concentration of ECH had no effect on half-life at 37 °C; however, on ice, the estimated values were longer at higher concentration in both rat blood and plasma (Table 1). Therefore, the final method was optimized for the analysis of DCP and ECH in blood. With sample stability being a concern, the additional manipulation of samples to generate plasma was avoided by choosing blood as the matrix of choice for *in vivo* studies.

Table 1. Half-Life[a] of ECH in Rat and Mouse Blood or Plasma

		half-life (min)	
matrix	concentration (μM)	on ice	37 °C
rat plasma	1	347	53
rat plasma	80	693	50
rat blood	1	129	19.9
rat blood	80	305	24.6
mouse blood	1	ND[b]	44.9
mouse blood	80	ND	42.7

[a]Half-life was determined from semi log plots of ECH concentration vs time curve using the equations $y = a\ e^{-kx}$, and half-life = $0.693/k$. [b]ND, not determined.

To investigate the effect of the sample matrix on extraction efficiency, standard curves were prepared for DCP and ECH in ethyl acetate, water, and blood. The accuracy of response of DCP in blood or water was similar and was 94−102% of the solvent standard. Similar results were observed for ECH, with a response between 91 and 112% over the concentration range examined. The data indicates efficient extraction of both DCP and ECH with ethyl acetate from blood and water and an absence of a matrix effect. The analytical method was validated to quantitate DCP and ECH in blood over the concentration range from 0.005 to 1 μM; the method was linear ($r^2 > 0.997$), accurate (relative error ≤ 15%), and precise (relative standard deviation ≤ 15%) for the quantitation of both DCP and ECH over the concentration range of 0.005 to 1 μM. Stability of analytes in blood extracts was verified using QC samples prepared at low, mid, and high concentrations for up to 48 h at room temperature (105, 98.3, and 102%), 1 week frozen (102, 103, and 106%), and after three freeze−thaw cycles (109, 96.1, and 102%). For DCP, dilution into the concentration range of method was verified for samples above the highest standard. Because two analyses needed to be conducted on each sample, with ECH in the range and DCP above the range, the dilution was verified for DCP only. Additional standards of DCP in ethyl acetate were prepared in the range of 2−400 μM and were diluted with 0.25 μM DCP-d_5 in ethyl acetate to be in the range of 0.1−0.6 μM. The accuracy of the measured concentration was 102.1 ± 7.4%.

Analysis of DCP Dose Formulations for the Presence of ECH. DCP dose formulations were analyzed for the presence of ECH prior to administration in animals. ECH was detected in the 10 mg/mL DCP dose formulation by GC with flame ionization detection, with a peak area corresponding to approximately 0.1% of that of DCP. The peak area ratio did not appear to change when DCP was sampled immediately after being dissolved in water or after 24 h at room temperature. The concentration of ECH in the DCP dose formulations was determined by GC-MS. The DCP and ECH concentrations in the DCP formulation administered in male rats were 9.54 and 0.000424 mg/mL, respectively, and in female rats, 10.13 and 0.00041 mg/mL, respectively. These data indicate that animals received negligible amounts of ECH via administration of DCP.

Single Oral Administration of 50 mg/kg DCP in Male and Female Rats. The doses of DCP administered in male and female rats were 49.8 ± 1.6 mg/kg (0.386 ± 0.014 mmol/kg) and 50.7 ± 1.6 mg/kg (0.393 ± 0.014 mmol/kg), respectively. The estimated doses of ECH administered in male and female rats via the DCP formulation were 2.23 ± 0.08 μg/kg (0.0239 ± 0.0009 μmol/kg) and 1.81 ± 0.07 μg/kg (0.0197 ± 0.0007 μmol/kg), respectively. The dose of DCP administered in male rats was approximately 22 500-fold higher than ECH on a mass basis and 16 140-fold higher on a molar basis. Similarly, the dose of DCP administered in female rats was approximately 27 830-fold higher than ECH on a mass basis and 19 963-fold higher on a molar basis.

ECH was detected in blood following administration of DCP in both male and female rats starting from about 5 min through 120 min. ECH concentrations fell within the calibration range

in blood of animals following administration of DCP; however, for DCP, the concentration for the majority of samples was substantially above the calibration range. Therefore, analyses were conducted with two runs per sample: first, with analysis for ECH followed by dilution of the samples into the range for quantitation of DCP. Blood concentration vs time profiles in male and female rats are shown in Figure 2A and B for DCP and ECH, respectively. Toxicokinetic parameters estimated from noncompartmental analysis are given in Table 2. For DCP, the parameters were similar between male and female rats, with an elimination half-life between 42 and 44 min. The apparent volume of distribution exceeded the reported aqueous body water volume in rats of 0.688 mL/kg,[42] suggesting extensive distribution of DCP into the peripheral compartment. The maximum ECH concentration, C_{max}, was detected at 10 and 13.7 min following administration of DCP in males and females, respectively. ECH was eliminated in male rats (half-life, 33.2 min) slightly faster than that in female rats (half-life, 47.9). As seen with DCP, other toxicokinetic parameters estimated for ECH were very similar between males and females. For both males and females, the apparent volume of distribution was lower than the reported aqueous body water volume in rats, suggesting limited distribution of ECH into the peripheral compartment.

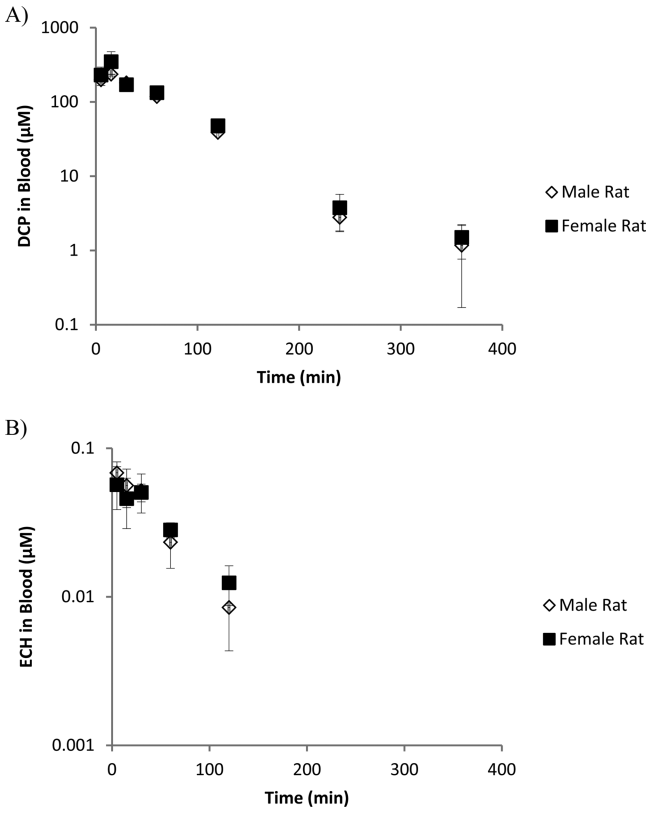

Figure 2. Blood (A) DCP and (B) ECH concentration vs time profiles following a single oral administration of 50 mg/kg DCP in male and female Harlan Sprague–Dawley rats.

Single Oral and Intravenous Administration of ECH in Male and Female Rats. Initially, a group of male rats was administered a single dose of 1 mg/kg by gavage, but no ECH was detectable in blood at time points ranging from 5 min to 6 h. A higher dose of ECH was then used. The oral doses of ECH administered to male and female rats were 26.2 ± 0.3 mg/kg (0.283 ± 0.003 mmol/kg) and 25.3 ± 1.1 mg/kg (0.274 ± 0.012 mmol/kg), respectively. ECH was detected up to 15–90 min following administration in male and female rats. Blood concentration vs time profiles in male and female rats are shown in Figure 3, and toxicokinetic parameters obtained from noncompartmental analysis are given in Table 3. A sex difference in toxicokinetics was observed following administration of ECH in male and female rats. C_{max} and $AUC_{(0-\infty)}$ were about 3-fold lower in females compared with that in males, suggesting that females received less internal dose of ECH than males following oral administration of ECH. ECH was eliminated in females (half-life, 20.1 min) faster than in males (half-life, 33.8 min).

The intravenous doses of ECH administered in male and female rats were 1.02 ± 0.02 mg/kg (0.0111 ± 0.0002 mmol/kg) and 1.03 ± 0.03 mg/kg (0.0111 ± 0.0003 mmol/kg), respectively. Blood concentrations of ECH fell rapidly in male

Table 2. Blood Toxicokinetic Parameters of DCP and ECH Following a Single Oral Administration of 50 mg/kg DCP in Male and Female Harlan Sprague–Dawley Rats[a]

parameter	male rat	female rat
DCP		
$t_{1/2}$ (min)	42.1 ± 1.61	43.8 ± 5.37
C_{max} (μM)	251 ± 90.7	337 ± 133
C_{max}/D (μM kg/mmol[b])	648 ± 242	862 ± 325
T_{max} (min)	12.5 ± 5	12.5 ± 5
$AUC_{(0-last)}$ (μM min)	17 714 ± 4562	19 978 ± 1467
$AUC_{(0-\infty)}$ (μM min)	17 786 ± 4551	20 082 ± 1433
$AUC_{(0-\infty)}/D$ (μM min kg/mmol)	45 873 ± 12 302	51 258 ± 2492
V_z/F (L/kg)	1.39 ± 0.334	1.23 ± 0.187
ECH[b]		
$t_{1/2}$ (min)	33.2 ± 6.97	47.9 ± 12.0
C_{max} (μM)	0.0703 ± 0.0107	0.0623 ± 0.0132
T_{max} (min)	10.0 ± 5.77	13.7 ± 11.8
$AUC_{(0-last)}$ (μM min)	3.50 ± 1.21	3.48 ± 0.81
$AUC_{(0-\infty)}$ (μM min)	4.02 ± 1.15	4.67 ± 0.41
V_z/F (L/kg)	0.302 ± 0.093	0.288 ± 0.060

[a]Average values (±SD) for n = 4 animals are reported. [b]Dose of DCP in mmol/kg was used.

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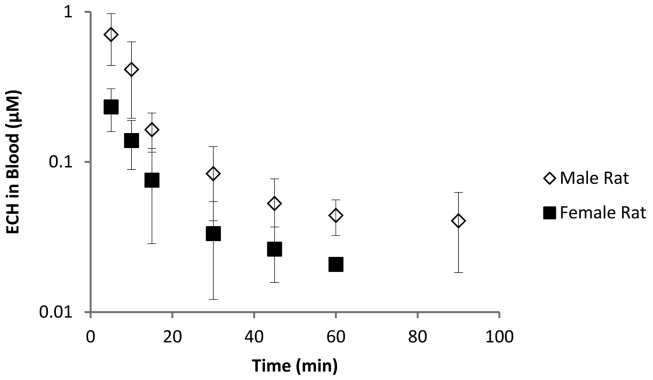

Figure 3. Blood concentration vs time profiles following a single oral administration of 25 mg/kg ECH in male and female Harlan Sprague–Dawley rats.

Table 3. Blood Toxicokinetic Parameters of ECH Following a Single Oral Administration of 25 mg/kg ECH in Male and Female Harlan Sprague–Dawley Rats[a]

parameter	male rat	female rat
$t_{1/2}$ (min)	33.8 ± 22.8	20.1 ± 13.6
C_{max} (μM)	0.760 ± 0.218	0.233 ± 0.074
C_{max}/D (μM kg/mmol)[b]	2.69 ± 0.76	0.857 ± 0.298
T_{max} (min)	7.5 ± 2.9	5 ± 0
$AUC_{(0-last)}$ (μM min)	10.2 ± 1.92	3.28 ± 1.01
$AUC_{(0-\infty)}$ (μM min)	12.1 ± 2.58	3.94 ± 1.06
$AUC_{(0-\infty)}/D$ (μM min kg/mmol)	42.9 ± 9.13	14.4 ± 4.11
V_z/F (L/kg)	1146 ± 828	1956 ± 1267

[a]Average values (±SD) for n = 4 animals are reported. [b]Dose of ECH in mmol/kg was used.

rats following administration and were below the limit of quantitation (LOQ) of the analytical method in some animals at 30 min and in all rats at 45 min. Therefore, in female rats, blood was collected only up to 45 min following administration. In general, concentrations start falling below the lowest standard of 0.005 μM between 10 and 25 min in females. As a result, for one animal, the concentration after 10 min was determined by interpolation below the LOQ in order to have sufficient data points for toxicokinetic analysis. Blood concentration vs time profiles in male and female rats are shown in Figure 4, and toxicokinetic parameters obtained from noncompartmental analysis are given in Table 4. In general,

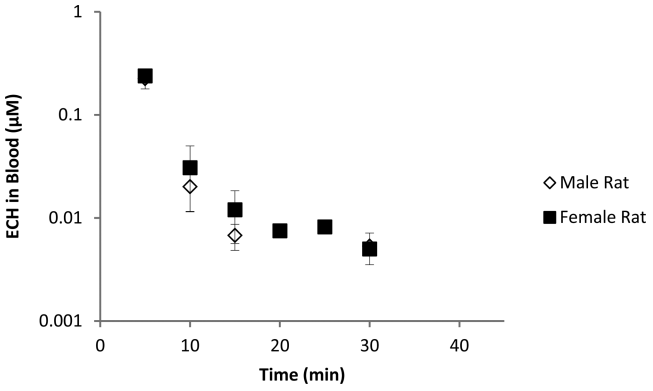

Figure 4. Blood concentration vs time profiles following a single intravenous administration of 1 mg/kg ECH in male and female Harlan Sprague–Dawley rats.

Table 4. Blood Toxicokinetic Parameters of ECH Following a Single Intravenous Administration of 1 mg/kg ECH in Male and Female Harlan Sprague–Dawley Rats[a]

ECH	male rat	female rat
$t_{1/2}$ (min)	6.51 ± 3.2	3.14 ± 0.84
C_0 (μM)	2.96 ± 1.42	2.34 ± 1.17
T_{max} (min)	5 ± 0	5 ± 0
$AUC_{(0-last)}$ (μM min)	8.84 ± 3.43	7.27 ± 2.80
$AUC_{(0-\infty)}$ (μM min)	8.93 ± 3.42	7.30 ± 2.79
$AUC_{(0-\infty)}/D$ (μM min kg/mmol)[b]	827 ± 316	676 ± 259
V_z/F (L/kg)	12.5 ± 8.5	8.53 ± 6.45

[a]Average values (±SD) for n = 4 animals are reported. [b]Dose of ECH in mmol/kg was used.

toxicokinetic parameters between males and females were similar except that, as observed following oral administration, ECH was eliminated in females (half-life, 3.1 min) faster than in males (half-life, 6.51 min).

Oral Bioavailability of ECH. Area under the ECH concentration vs time curve following oral and intravenous administration of ECH, after adjusting for the dose, was used to estimate oral bioavailability of ECH. For male rats, $AUC_{(0-\infty)}$/dose following oral and intravenous administration was 42.9 and 827 μM min kg/mmol, respectively. Therefore, the oral bioavailability was estimated to be 5.2% ((42.9/827) × 100). Similarly for female rats, $AUC_{(0-\infty)}$/dose following oral and intravenous administration was 14.4 and 676 μM min kg/mmol, respectively. Therefore, the oral bioavailability estimated for females was 2.1% ((14.4/676) × 100).

DISCUSSION

Although ECH and DCA were proposed to be reactive species, to the best of our knowledge, there is no direct evidence for the formation of these metabolites from exposure to DCP. The work described here is the first report of the formation of ECH in rodents following exposure to DCP.

In this study, the feasibility of detecting DCA and ECH in DCP-administered animals was investigated by developing a sensitive analytical method to detect these metabolites and by identifying a suitable matrix. The high reactivity of DCA in blood, plasma, and liver homogenate *in vitro* precluded its investigation; DCA was cleared in these matrices with a half-life ≤ 1 min. ECH rapidly disappeared in the liver homogenate. In the presence and absence of diethyl maleate (GSH depleter) or cyclohexene oxide (epoxide hydrolase inhibitor), we confirmed *in vitro* that 80% of the loss of ECH in liver homogenate was due to epoxide hydrolase activity and 18% was due to the reaction of ECH with GSH (data not shown). ECH was relatively long-lived in plasma and blood *in vitro*. Although the estimated half-life of ECH in plasma was longer than that in blood, the time necessary to prepare plasma from blood from animals would result in loss of ECH. Therefore, blood was selected as the preferred matrix for detecting ECH following administration of DCP or ECH in rats. Processing samples on ice slowed the hydrolysis of ECH on blood and plasma *in situ* and hence was incorporated into the final method. An analytical method was developed and validated for successful quantitation of ECH in blood with a limit of quantitation of 0.01 μM, which was subsequently extended to 0.005 μM.

ECH was detected and quantified in blood following oral administration of DCP in male and female rats. To the best of our knowledge, this is first unambiguous detection of ECH

following exposure to DCP. Trace levels of ECH were detected in DCP dose formulations corresponding to 0.0239 and 0.0197 μmol ECH/kg administered in males and females, respectively, suggesting that animals received negligible amounts of ECH compared to DCP via administration of DCP. The initial dose of 1 mg (10.8 μmoles) ECH/kg used for gavage administration in male rats produced no detectable ECH in blood, indicating that the extremely low levels of ECH in the dose of DCP administered would not have caused the ECH in blood from DCP-dosed rats. These data along with the low bioavailability of ECH in male (5.2%) and female rats (2.1%) following oral administration provide evidence for the formation of ECH following oral administration of DCP in rats.

ECH was detected in blood from rats following a single oral dose of 25 mg/kg (half-life of 33.8 and 20.1 min in males and females, respectively) or a single intravenous dose of 1 mg/kg (half-life of 6.51 and 3.14 min in males and females, respectively). In a study by Rossi et al., following a single oral dose of 50 mg/kg ECH in male CD1 Swiss albino mice, levels of ECH were below the limit of quantitation (50 ng/mL) of the analytical method used; however, ECH was detected following an oral dose of 200 mg/kg.[43] The peak blood ECH concentration reported at ∼5 min following administration was 0.49 ± 0.13 μg/mL. The estimated half-life in mice (5.5 min) was much shorter than that observed in male rats (33.8 min) following oral administration of ECH in our study, indicating a clear species difference in the disposition of ECH following oral administration. Following administration of DCP in male and female rats, the maximum ECH concentration was achieved ≤13.7 min. There was no clear sex difference in the disposition of ECH in rats except that the half-life in males (33.2 min) was slightly shorter than that of female rats (47.9 min). This may be partially explained by the reported higher microsomal epoxide hydrolase activity in males compared to that in females.[44] In a pilot study, ECH was also detected and quantified in male B6C3F1/N mice following oral administration of 25 mg/kg DCP (data not shown); ECH was rapidly eliminated in blood with a half-life of 10.1 min in male mice compared to a half-life of 33.2 in rats. In addition, C_{max} and AUC were about 10-fold and ≥40-fold lower in male mice compared with those in male rats, showing a clear species difference in the disposition of ECH following administration of DCP.

The percentage of the dose of DCP converted to ECH in rats following oral administration of DCP in rats was estimated in several ways. An initial evaluation used $AUC_{(0-\infty)}$ values for ECH following oral administration of ECH and following oral administration of DCP. In male rats, for ECH, the dose-adjusted $AUC_{(0-\infty)}$ was 42.9 μM min kg/mmol following oral administration of ECH, and $AUC_{(0-\infty)}$ was 4.02 μM min following oral administration of DCP (Tables 2 and 3). Therefore, the dose of ECH formed in DCP-treated (0.386 mmol/kg DCP) male rats was estimated to be 0.094 mmol/kg (4.02 μM min/42.9 μM min kg/mmol); this is equivalent to ca. 24% (0.094/0.386 × 100) conversion of DCP to ECH in male rats. Similarly, in female rats, for ECH, dose-adjusted $AUC_{(0-\infty)}$ was 14.4 μM min kg/mmol following oral administration of ECH, and $AUC_{(0-\infty)}$ was 4.67 μM min following oral administration of DCP (Tables 2 and 3). Therefore, the dose of ECH formed in ca. 50 mg/kg (or 0.393 mmol/kg) DCP-treated female rats was estimated to be 0.324 mmol/kg (4.67 μM min/14.4 μM min kg/mmol); this is equivalent to ca. 82% (0.324/0.393 × 100) conversion of DCP to ECH in female rats. These estimates may be higher than actual values since ECH may be hydrolyzed in the gut following oral administration of ECH, leading to lower $AUC_{(0-\infty)}$ following oral administration of ECH. This is supported by the estimated low oral bioavailability of 5.1 and 2.1% in males and females, respectively. An alternative approach was then used that avoids the issue of absorption, using the $AUC_{(0-\infty)}$ following intravenous administration of ECH to estimate the percent DCP converted to ECH. Following intravenous administration, the dose-adjusted $AUC_{(0-\infty)}$ values were 827 and 676 μM min kg/mmol in males and females, respectively (Table 4). On the basis of this, the dose of ECH in DCP-treated rats can be calculated as 0.00486 mmol/kg (4.02 μM min/827 μM min kg/mmol) and 0.00696 mmol/kg (4.67 μM min/676 μM min kg/mmol), which is equivalent to 1.26% (0.00486 mmol ECH/0.386 mmol DCP × 100) and 1.78% (0.006961 mmol ECH/0.393 mmol DCP × 100) of DCP converted to ECH in male and female rats, respectively. These values may be slightly underestimated because ECH, once formed in the liver from the metabolism of DCP, can undergo hydrolysis and GSH reaction, thereby decreasing the amount entering systemic circulation. Therefore, we concluded that the percent dose of DCP converted to ECH in male and female rats following a single oral exposure to DCP was ≥1.26% and ≥1.78%, respectively.

The investigation into the formation of DCA from the metabolism of DCP proved to be unsuccessful due to its rapid disappearance. Indirect evidence for the formation of DCA could be provided by the analysis of further metabolites such as glutathione conjugates and protein adducts. However, the major conjugation products of DCP and ECH separately administered to male rats were the same: *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine and *N*,*N*′-bis-acetyl-*S*,*S*′-(bis-cysteinyl)-2-hydroxypropane.[26] In addition, *N*-acetyl-*S*-(3-chloro-2-hydroxypropyl)cysteine, reported to be a metabolite of ECH (Gingell et al.[45]), is also expected to be a metabolite of DCP. This was confirmed by investigation of the reaction of GSH with DCP or ECH (unpublished observations). The formation of adducts from reaction of ECH with globin has been described, and several adducts were identified, including 2,3-dihydroxypropylvaline[46] (which is not specific for ECH but can be formed by reaction with glycidol) and chlorohydroxypropylvaline,[47] which, although formed by ECH, could also be formed by reaction of 3-chloro-1,2-propanediol.[46] Therefore, glutathione conjugates, mercapturic acids, or protein adducts could not be used to distinguish DCP and ECH.

The biological conversion of DCP to ECH has been demonstrated in bacteria and is catalyzed by haloalcohol dehalogenases in Gram-positive and -negative bacteria.[48] Production of ECH from DCP has been reported in a biochemical reactor containing *Escherichia coli* expressing haloalcohol dehalogenase and epoxide hydrolase. Similarities between the active sites of the bacterial haloalcohol dehalogenase and mammalian epoxide hydrolases have been drawn.[49,50] This study has demonstrated the conversion of DCP to ECH in rats. The mechanism by which this conversion occurs and its location within the body should be investigated further to understand the toxicity and carcinogenicity of DCP.

■ CONCLUSIONS

It has been suggested that the observed toxicity and carcinogenicity of DCP in rodents is due to the formation of a reactive metabolite, epichlorohydrin (ECH). To date, there is no direct evidence for the formation of ECH following

exposure of rodents to DCP. The work described here in reports the first unambiguous detection of ECH following oral administration of DCP in rodents. Following a single oral dose of 50 mg/kg DCP, ≥1.26% and ≥1.78% of the administered dose was converted to ECH in male and female rats, respectively. Toxicokinetics of ECH in male and female rats were similar in males and females, eliminating ECH with a half-life of 33.2 and 47.9 min, respectively.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

DCA, 1,3-dichloroacetone; DCP, 1,3-dichloro-2-propanol; DCP-d_5, ($^2H_5$)-1,3-dichloro-2-propanol; ECH, epichlorohydrin; ECH-d_5, ($^2H_5$)-epichlorohydrin; GC-MS, gas chromatography−mass spectrometry; IARC, International Agency for Research on Cancer; LOQ, limit of quantitation; QC, quality control; NTP, National Toxicology Program

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