

BIOAVAILABILITY AND METABOLISM OF HYDROQUINONE AFTER INTRATRACHEAL INSTILLATION IN MALE RATS

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ABSTRACT:

The purpose of this study was to investigate the rate and extent of hydroquinone (HQ) absorption and first pass metabolism in the lungs of male rats *in vivo*. [^{14}C]HQ in physiological saline was administered intratracheally via an indwelling endotracheal tube to simulate inhalation exposure to HQ dust. The bioavailability of HQ was determined by blood sampling simultaneously at arterial and venous sites beginning immediately after administration to conscious rats. Pulmonary absorption and metabolism, and systemic metabolism and elimination were determined by chromatographic analysis of parent compound and metabolites in blood samples after intratracheal administration of [^{14}C]HQ at 0.1, 1.0, and 10 mg/kg. Pulmonary absorption of HQ was found to be very rapid with [^{14}C]HQ detectable in arterial blood, and to a lesser extent in

venous blood, within 5 to 10 s after dose administration. Only [^{14}C]HQ was detected in the initial (5–10 s) arterial blood samples at all dose levels, indicating that pulmonary metabolism of HQ was not extensive. However, later blood samples (45–720 s) indicated rapid metabolism and elimination of the parent compound and metabolites after intratracheal absorption. The elimination half-life from the 0.1 mg/kg dose was allometrically scaled to human proportions and used to estimate the steady-state (maximum) human blood concentrations of HQ resulting from presupposed workplace exposures. The estimates indicated minimal levels of HQ in human blood after respiratory exposures of greater than 1 h at 0.1 or 2.0 mg/m³; these levels were less than background concentrations of HQ detected in human blood in previous studies.

This study investigated the rate and extent of hydroquinone (HQ)¹ absorption and first pass metabolism in the lungs of rats, and subsequent systemic metabolism after intratracheal instillation of a small amount of the chemical in physiological saline. HQ is both a naturally occurring substance (Deisinger et al., 1996) and a large production-volume synthetic chemical (45,000–50,000 tons worldwide estimated for 1994; Krumenacker et al., 1995). HQ is used industrially in photographic, rubber, and chemical processes, and is used commercially as a cosmetic ingredient. HQ is not volatile at ambient temperatures (vapor pressure = 1.8×10^{-5} mm Hg at 25°C), but inhalation exposures to low concentrations of HQ dust can occur occupationally (Pifer et al., 1995).

HQ is known to be biotransformed systemically in the rat to sulfate, glucuronide, and glutathione conjugates (DiVincenzo et al., 1984; Hill et al., 1993). Metabolism is believed to occur predominantly in the liver and, for intragastric exposures, within the intestinal mucosa. However, a

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¹ Abbreviations used are: HQ, hydroquinone; LS, liquid scintillation; AUC, area under the curve; V_{area} , volume of distribution; C_{ss} , steady-state concentration; C_t , concentration at time t ; k_0 , constant rate; k_{elim} , first order elimination rate constant; TWA, time-weighted average.

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number of investigations have addressed the potential for extrahepatic metabolism of phenolic and related compounds, particularly in lung (Dollery et al., 1971; Briant et al., 1973; Cassidy and Houston, 1984; Mistry and Houston, 1985) and in erythrocytes (Eckert, 1988). Although workplace exposure to HQ dust is expected to be <0.1 mg/m³ (Pifer et al., 1995), pulmonary and blood metabolism may reduce the internal, systemic dose of parent compound to levels below what would be assumed in the absence of pulmonary and blood metabolism.

The relatively large amount of chemical required for an inhalation exposure and the potential for radiochemical contamination in the generation of a [^{14}C]labeled dust precluded the execution of an animal inhalation exposure to [^{14}C]HQ. As an alternative, [^{14}C]HQ in a physiological saline solution was administered intratracheally via an indwelling endotracheal tube to conscious rats to simulate exposure by inhalation of HQ dust and its subsequent dissolution in the fluid of the respiratory membrane. Bioavailability of HQ was determined by blood sampling simultaneously at arterial and venous sites at short intervals beginning immediately after intratracheal instillation. The extent of pulmonary metabolism was determined by differences observed in blood concentrations of parent compound and metabolites measured in venous and arterial blood samples after intratracheal administration of HQ over a range of concentrations. The rate of pulmonary absorption and systemic metabolism of HQ was estimated from the HQ concentration in both arterial and venous blood over time and from the decline in parent compound concentration and increase in metabolite concentrations over time.

Materials and Methods

Animals. Male Sprague-Dawley rats [ZML:SD(MBM/VPF)], approximately 8 to 11 weeks old at dosing, were purchased from Zivic-Miller

Laboratories, Inc. (Zelienople, PA). The animals were prepared by the vendor with arterial and venous cannulae and an endotracheal tube. The arterial cannula was placed in the left carotid artery and threaded into the artery so as to position the cannula tip within the aortic arch. This allowed sampling of blood returning from the lung, having passed through the left side of the heart before systemic distribution. The venous cannula was placed in a jugular vein and threaded into the vein so as to position the cannula tip as near to the right atrium as possible; therefore, the blood samples collected from this cannula are returning from systemic circulation. The patency of both cannulae was maintained by daily aspiration and refill of each with a viscous solution of 50% (w/w) polyvinylpyrrolidone (Sigma catalog no. PVP-40; mw 40,000) in sterile saline containing 250 IU/ml sodium heparin (Elkins-Sinn, Inc., Cherry Hill, NJ). An endotracheal tube consisting of 0.012 inches inside diameter \times 0.025 inches outside diameter Micro-Renathane tubing (Braintree Science, Inc., Braintree, MA) was inserted through the wall of the trachea and permanently secured using medical grade cyanoacrylate and suturing to the osmohyoid muscles. The tube was then coupled to 0.020 inches inside diameter \times 0.037 inches outside diameter Micro-Renathane tubing and tunneled s.c. below the nape of the neck. Both cannulae and the endotracheal tube were externalized at the top of the head and mounted in dental acrylic. The use of arterial and venous cannulated rats allowed rapid and accurate serial blood sampling and the presence of the endotracheal tube allowed the dosings to be conducted in conscious animals that were not affected by the respiratory and circulatory depression caused by anesthetics.

Test Chemicals. Unlabeled HQ (CAS 123-31-9) was obtained from Eastman Kodak Company (Rochester, NY) and its structure and purity were confirmed by gas chromatography with mass selective detection (HP 5890/5970, Hewlett-Packard, Wilmington, DE). [UL- ^{14}C]HQ was obtained from Wizard Laboratories (West Sacramento, CA) with an initial specific radioactivity of 20 mCi/mmol. The [^{14}C]HQ was assayed for radiochemical purity by HPLC (Hewlett-Packard 1090 M) with flow-through radiochemical detection (Beckman 171 Radioisotope Detector, Beckman Instruments, Fullerton, CA) using a 170 \times 4.6 mm Supelcosil LC-18, 5 μm particle size reversed phase column (Supelco, Inc., Bellefonte, PA). This analysis indicated a [^{14}C]HQ purity of 99.2% by [^{14}C] peak area.

Dose Administration. The dosing solutions were prepared in degassed saline by combining a suitable amount of [^{14}C]labeled and -unlabeled test substance. Intratracheal administrations were accomplished by attaching a 1-ml syringe to the externalized endotracheal tube and delivering a bolus 0.2 ml/kg (30–70 μl) dose of [^{14}C]HQ in saline pushed by 200 μl of air. Single doses of [^{14}C]HQ were administered at nominal amounts of 0.1, 1.0, or 10 mg/kg body weight, with mean radiochemical doses of 7.2 μCi , 26.0 μCi , and 24.5 μCi per rat, respectively, for each dose group.

Sample Collection and Metabolite Quantitation. Blood samples were obtained by serial sampling from the venous and arterial cannulae at nominal times of 5, 45, 120, 360, and 720 s after dosing. The 350 to 400 μl of blood collected at each time point required approximately 5 s to draw from a fully patent cannula; the interval of each sample collection (start draw time to stop draw time) was accurately recorded. The samples were collected using a 1-cc syringe connected to the cannula port with polyethylene tubing treated with 2 I.U. sodium heparin before blood collection. The blood samples were immediately discharged into ice-cold microcentrifuge tubes and kept on ice. Small aliquots of the whole blood (approximately 10 mg) were added to a one-step digestant/scintillant (Fluorosol; National Diagnostics, Manville NJ) for liquid scintillation (LS) counting using external standard channels ratio quench correction to quantitate total [^{14}C]. The remaining blood sample was transferred to an ice-cold ultrafiltration tube (Centrifree; Amicon, Inc., Beverly, MA) and a protein-free ultrafiltrate was recovered after centrifugation at 3000g for 1 h at 3°C. A small aliquot of the ultrafiltrate (approximately 10 mg) was sampled for LS counting to quantitate recovered [^{14}C] and the balance was transferred to an autosampler vial and held at 3°C until HPLC analysis to separate and quantitate HQ parent compound and metabolites. The difference in [^{14}C] concentration between whole blood and ultrafiltrate was suspected to be due to HQ reversibly bound to protein in the blood. This was investigated by spiking whole blood with [^{14}C]HQ approximating the maximum concentrations of free HQ seen in blood after the 1 mg/kg intratracheal administration. The samples were processed both by ultrafiltration and by ethyl acetate

extraction and the recovered [^{14}C] was assayed by LS counting and by HPLC for quantitation and characterization (see *Results*).

HPLC Radiochemical Analysis for HQ and Metabolites. Injections of the clear ultrafiltrate (100 μl) were analyzed for parent compound and metabolites on an HP 1090 HPLC using a reversed phase column (Supelcosil LC-18, 5 μm , 4.6 \times 170 mm) and an isocratic mobile phase consisting of 50 mM sodium formate buffer (pH 4.5) at 1 ml/min. The column effluent was directed through the UV absorbance detector and then through the radiochemical detector, which was fitted with a 500 μl scintillant-mix flow cell. The 1 ml/min column effluent was mixed with 2 ml/min scintillant (Ultima-Flo M, Packard Instrument Co., Meriden, CT) before passage through the radiochemical detector flow cell. The area percent value for each peak provided by this analysis was multiplied by the radiochemical concentration of the blood ultrafiltrate and then divided by the HQ dose-specific radioactivity to derive a mass quantitation (μg equivalents/g blood ultrafiltrate) for the parent compound and each metabolite.

HQ-sulfate and HQ-glucuronide metabolite standards were obtained from rat urine collected after a 25 mg/kg oral administration of [^{14}C]HQ. Their identities were established previously by negative ion fast atom bombardment mass spectrometry and by specific enzyme hydrolysis. Standards of the mono-, di-, tri-, and tetraglutathione conjugates of HQ were prepared as described previously in the literature (Lau et al., 1988).

Pharmacokinetic Descriptions. A nonlinear least-squares data-fitting program (PKAnalyst, Version 1.0; MicroMath Scientific Software, Salt Lake City, UT) was used to derive pharmacokinetic parameters for total HQ concentrations in blood. A two-compartment model with bolus input and first order output described by the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ was fitted to total HQ concentrations in arterial blood over time, where C_t represents the concentration at time t , A and B represent the y intercepts for the initial and terminal segments of the curve, respectively, and α and β are their respective rate constants. The following parameters were calculated: A , α , B , β , area under the curve (AUC), the half-life of elimination ($T_{1/2}$), and volume of distribution (V_{area}) defined as $V_{\text{area}} = \text{Dose}/\beta \times \text{AUC}$, which is valid for a non-i.v. administration assuming complete absorption and bioavailability (Gibaldi and Perrier, 1982). The data from this experiment demonstrate that no first pass effect occurred in the lung tissue after dose administration, and previous mass balance studies in our laboratory with intratracheal administration of [^{14}C]HQ have indicated $\geq 96\%$ absorption of doses in the range of 5 to 50 mg/kg (H. Lockhart and J. Fox, unpublished data).

Results

The intratracheal instillation of 0.1, 1.0, or 10 mg/kg [^{14}C]HQ to male rats resulted in very rapid absorption and systemic distribution of HQ as indicated by the early detection of [^{14}C] in the blood. Free HQ appeared in the arterial and venous blood within 5 to 10 s after dose administration. Reversibly bound HQ was also present in the earliest samples. The mean concentrations of free plus reversibly bound HQ (total HQ) in the 5- to 10-s arterial blood samples were consistently higher than those in the corresponding venous samples at all dose levels, but showed large interanimal variability (Figs. 1, 2, and 3). By 45 s after administration at all dose levels, the arterial and venous concentrations of total HQ were quite similar and declined beyond this time point at a similar rate in both. Free HQ concentrations were undetectable in both arterial and venous blood by 12 min postdosing in the lowest dose level and were detectable in only 20% of the 12-min blood samples from the 1.0 and 10 mg/kg dose levels. Elimination of parent compound from blood was very rapid and biphasic at all dose levels (Fig. 4). Parameters derived from pharmacokinetic analysis of the total HQ concentrations in arterial blood are presented in Table 1.

The glucuronic acid conjugate of HQ was the major metabolite at all dose levels, detected in both arterial and venous blood samples at comparable concentrations beginning at 45 s postdosing and at increasing concentrations in subsequent samples (Figs. 1, 2, and 3). Only trace amounts of other minor metabolites were detected at the

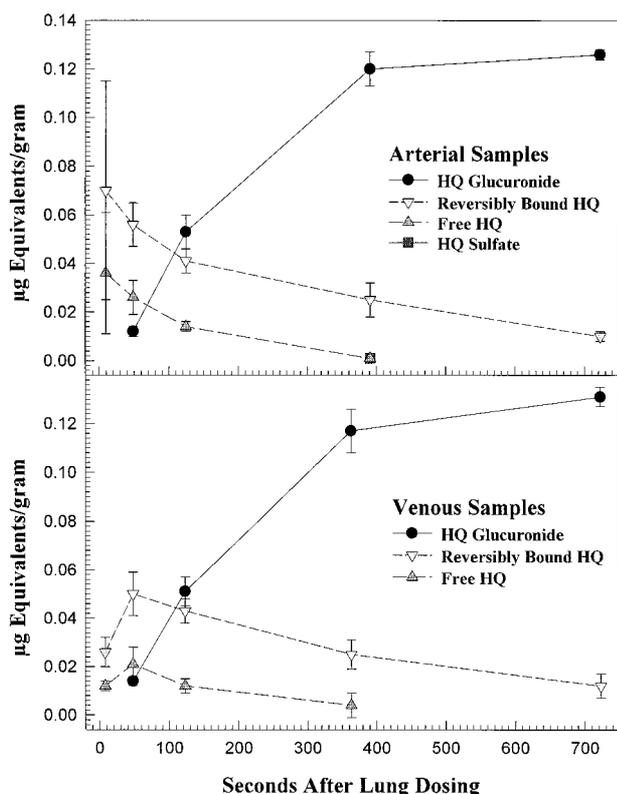


FIG. 1. HQ parent compound and metabolites in blood after a 0.1 mg/kg intratracheal dose of [^{14}C]HQ.

Concentrations of [^{14}C]HQ and [^{14}C] metabolites measured in the blood of male Sprague-Dawley rats after a 0.1 mg/kg intratracheal dose of [^{14}C]HQ. The data points are the mean concentration (S.D.s represented by error bars) of serial samples collected from three animals (two animals at 720 s, arterial time point). Data points that are absent indicate that the chemical was not detected at that time point. Top, data collected from arterial blood samples; bottom, data collected from venous blood samples from the same rat.

0.1 mg/kg dose level, but HQ-sulfate was detected at low concentrations beginning at 45 s postdosing in approximately half the blood samples from the 1.0 mg/kg dose level and consistently, predominantly at the later sampling times, after the 10 mg/kg dose administration. Other minor metabolite peaks appeared inconsistently in the arterial and venous blood samples from the 1.0 and 10 mg/kg dose levels beginning at 45 s postdosing at concentrations of ≤ 0.080 μg equivalents/g (1.0 mg/kg dose level) and ≤ 1.623 μg equivalents/g (10 mg/kg dose level). These minor metabolites appeared sporadically in the blood ultrafiltrate chromatograms and were tentatively identified by coelution with authentic standards. The most common minor metabolite was mono-glutathionyl-HQ, detected at both the 1.0 and 10 mg/kg dose levels, usually in the 45- to 120-s time frame. Also identified by cochromatography in 1.0 and 10 mg/kg dose level samples were peaks corresponding to 2,3-diglutathionyl-HQ or 2,3,5,6,-tetraglutathionyl-HQ and a peak corresponding to 2,3,5-triglutathionyl-HQ. A late eluting peak occasionally seen in trace amounts at all dose levels corresponded in retention time to 2,5-diglutathionyl-HQ or benzoquinone.

The limit of detection for pulmonary metabolites in the initial arterial blood samples depended on the radiochemical content of the dose, the rate of pulmonary absorption, and the rate of blood flow in the pulmonary vein. All three of these parameters were reflected in the [^{14}C] concentration of the initial arterial blood sample; therefore, the limit of detection can be expressed in terms of the blood total [^{14}C] concentration in this sample. At the 0.1 mg/kg dose level, the radio-

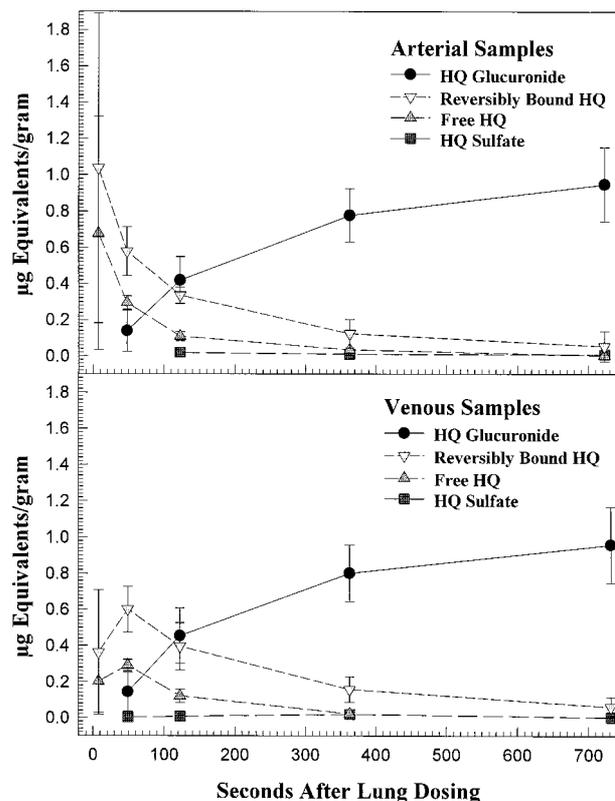


FIG. 2. HQ parent compound and metabolites in blood after a 1.0 mg/kg intratracheal dose of [^{14}C]HQ.

Concentrations of [^{14}C]HQ and [^{14}C] metabolites measured in the blood of male Sprague-Dawley rats after a 1.0 mg/kg intratracheal dose of [^{14}C]HQ. The data points are the mean concentration (S.D.s represented by error bars) of serial samples collected from four animals. Data points that are absent indicate that the chemical was not detected at that time point. Top, data collected from arterial blood samples; bottom, data collected from venous blood samples from the same rat.

chemical concentration of the dose was limited by the specific activity of the stock [^{14}C]HQ and the mean initial arterial blood [^{14}C] concentration was 51,620 dpm/g. The detection limit of the radiochemical flow detector was approximately 3000 dpm/g (as a single peak, 100 μl injection); therefore, a metabolite peak of approximately 5.8% of the whole blood concentration would be detected. For the 1.0 and 10 mg/kg dose levels, the mean initial arterial blood [^{14}C] concentration was 428,500 dpm/g. Factoring in the detection limit from above, a peak of 0.7% of the initial whole blood concentration would be detectable.

The efficiency of [^{14}C] recovery by the blood ultrafiltration procedure was proportional to the sampling time. In the initial blood samples collected 5 to 10 s after dose administration, the concentration ratio of [^{14}C] in blood ultrafiltrate compared to that in whole blood was 0.32 to 0.38 at all dose levels. This ratio steadily increased at later time points to means of 0.92, 0.95, and 0.77 at 12 min for the 0.1, 1.0, and 10 mg/kg dose levels, respectively. To investigate the nature of the [^{14}C] not recovered after ultrafiltration at the early time points, methods experiments were conducted in which whole blood spiked with [^{14}C]HQ in vitro or collected in vivo at 5 to 10 s from rats dosed intratracheally with [^{14}C]HQ was extracted with ethyl acetate. The ethyl acetate extracts contained 98% and 92% of the whole blood [^{14}C] in the in vitro and in vivo experiments, respectively. HPLC analysis of these extracts detected HQ as the only radiolabeled component (Fig. 5). Similarly, direct analysis by HPLC of a small volume of plasma from a 10-min in vitro whole blood incubation with

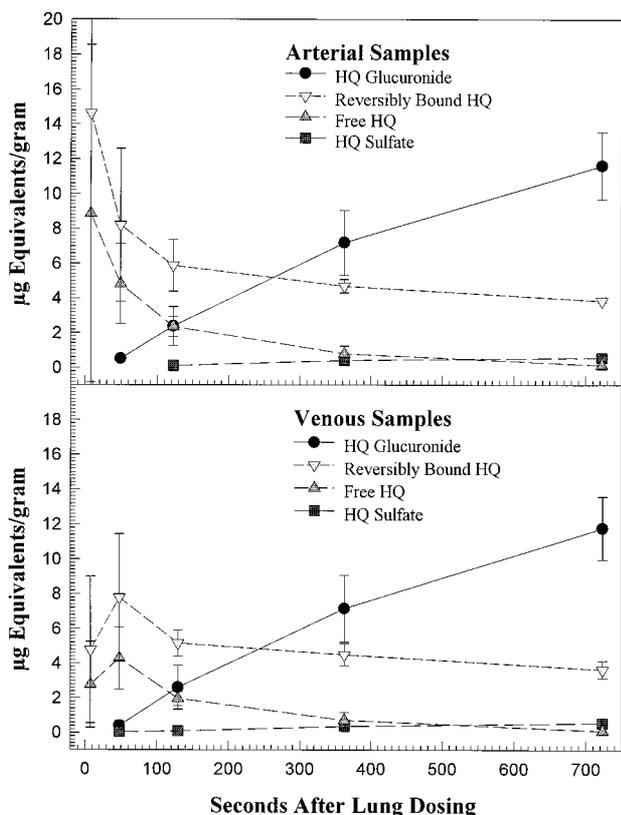


FIG. 3. HQ parent compound and metabolites in blood after a 10 mg/kg intratracheal dose of [^{14}C]HQ.

Concentrations of [^{14}C]HQ and [^{14}C]metabolites measured in the blood of male Sprague-Dawley rats after a 10 mg/kg intratracheal dose of [^{14}C]HQ. The data points are the mean concentration (S.D.s represented by error bars) of serial samples collected from three animals. Data points that are absent indicate that the chemical was not detected at that time point. Top, data collected from arterial blood samples; bottom, data collected from venous blood samples from the same rat.

[^{14}C]HQ resulted in the detection of only [^{14}C]HQ in this fraction of blood.

Discussion

Initial arterial and venous blood samples were collected between 5 and 10 s after intratracheal dosing with [^{14}C]HQ. The very short interval between intratracheal administration and collection of the initial blood samples was designed to detect first pass metabolism in the lung. The arterial cannula was placed to allow sampling of blood returning from the lung before systemic distribution. The initial arterial blood samples were generally higher in [^{14}C] concentration than the corresponding venous samples, indicating pulmonary absorption of the dose had occurred but systemic distribution was incomplete. The initial arterial samples could, therefore, be expected to contain putative pulmonary metabolites, not diluted or masked by hepatic or other systemic metabolism. HPLC characterization of the [^{14}C] in these initial arterial blood samples revealed no metabolic products, with only free and reversibly bound HQ detected at the 0.1, 1.0, and 10 mg/kg dose levels. In corresponding arterial and venous blood samples collected at later time points and containing roughly equal total [^{14}C] concentrations, HQ-glucuronide, the major metabolite, was detected at equal concentrations in both venous and arterial blood (Figs. 1–3), again indicating that no measurable phase two metabolism was occurring in the lung tissue.

The observation that [^{14}C] recovery by the blood ultrafiltration procedure was proportional to the sampling time lead to experiments

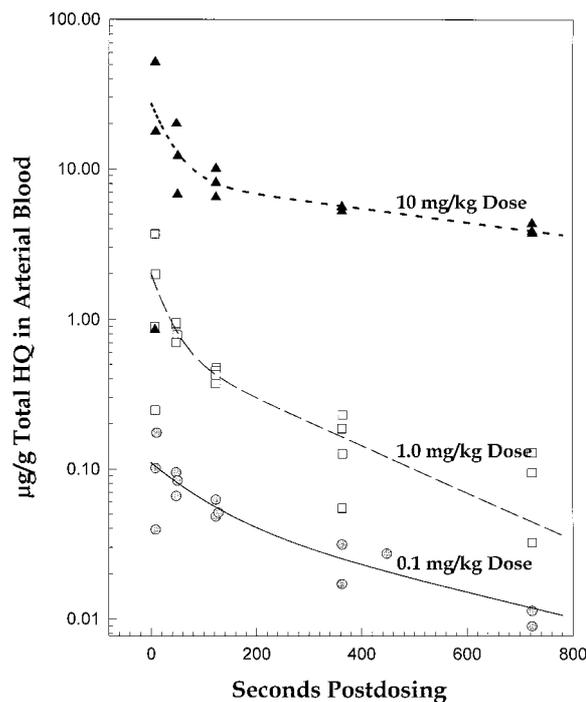


FIG. 4. Pharmacokinetic curve fitting of total HQ concentrations in rat arterial blood.

Least-squares curve fitting of total HQ concentrations versus time in rat arterial blood after a 0.1, 1.0, and 10 mg/kg intratracheal dose of [^{14}C]HQ. The symbols represent individual data points with the least-squares best fit line superimposed. The pharmacokinetic model assumes two compartments with bolus i.v. input and first order output and is described by the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ where A and B represent the y intercepts for the initial and terminal segments of the curve, respectively, and α and β are their respective rate constants.

TABLE 1

Pharmacokinetic analysis of total HQ concentrations in blood after intratracheal dosing

Summary of pharmacokinetic parameter estimates derived from nonlinear least squares fitting (PKAnalyst, MicroMath Scientific Software, Salt Lake City, UT, 1995) of arterial blood total HQ concentration after intratracheal administration of [^{14}C]HQ at 0.1, 1.0, and 10 mg/kg in male Sprague-Dawley rats. The form of the equation is $C_t = Ae^{-\alpha t} + Be^{-\beta t}$

Parameter (dimension)	0.1 mg/kg	1.0 mg/kg	10 mg/kg
A ($\mu\text{g/g}$)	0.0627	1.4098	18.9099
α (s^{-1})	0.0102	0.0301	0.0268
B ($\mu\text{g/g}$)	0.0476	0.6059	8.3537
β (s^{-1})	0.0019	0.0036	0.0011
k_{elim} (s^{-1})	0.0036	0.0094	0.0032
$k_{elim} T_{1/2}$ (s)	194	74	218
AUC ($\mu\text{g/g} \times \text{s}$)	31	215	8580
V_{area} (ml/kg)	1671	1288	1098

to determine the nature of the [^{14}C] not recovered by ultrafiltration in the early samples. These methods experiments established that the [^{14}C] not recovered by ultrafiltration is readily extractable and consists predominantly of [^{14}C]HQ reversibly bound (i.e., hydrogen bonding) to blood proteins in both the plasma and cellular fraction of the blood (Fig. 5). The increased recoveries of [^{14}C] at later sampling times reflect the increasing presence of conjugated metabolites of HQ (Fig. 5), which are not reversibly bound to blood protein. The in vitro experiments also demonstrate the stability of the HQ under the analytical conditions used. Subsequent studies in our laboratories have revealed that HQ in aqueous solution with blood protein is resistant to auto-oxidation, presumably because of hydrogen bonding to protein molecules.

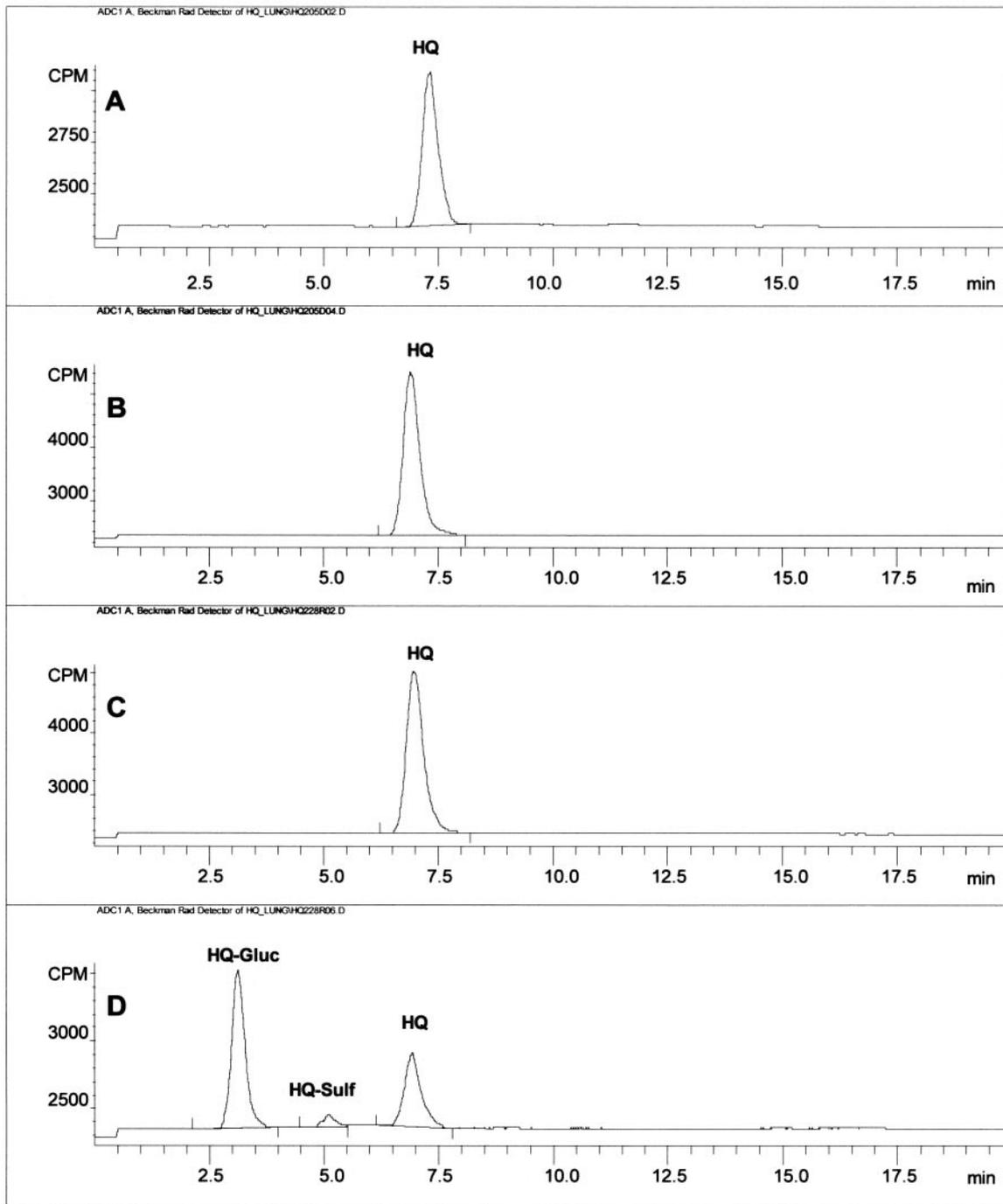


FIG. 5. HPLC chromatograms with radiochemical detection of selected $[^{14}\text{C}]$ HQ blood metabolism samples.

A, whole blood ultrafiltrate and B, ethyl acetate extract of whole blood recovered after a $2\ \mu\text{g/g}$ i.v. dose of $[^{14}\text{C}]$ HQ into whole blood, and whole arterial blood ultrafiltrate samples collected at approximately 9 s (C) and approximately 2 min (D) after a $10\ \text{mg/kg}$ in vivo intratracheal instillation of $[^{14}\text{C}]$ HQ.

Interanimal variability in concentrations of HQ was very large in early arterial blood samples. This is likely related to variable deposition of the doses in the lung, as was seen in methods development dosings using a vital dye. The dye tended to be deposited nonuniformly in several discrete areas of the lung; this may have resulted in variable rates of HQ absorption at the early time points in this study. The rapid pulmonary absorption of HQ, coupled with high blood flow rates in the aorta and very short initial sampling times, may also have contributed to this variability because small variations in sampling

time could result in large variations in resulting $[^{14}\text{C}]$ HQ concentrations.

The appearance of HQ in the arterial blood within 5 to 10 s after intratracheal dosing demonstrates the very rapid pulmonary absorption of this chemical. The rapid absorption of the chemical is followed by rapid elimination of parent compound from the blood, which results in the concentration maxima of total HQ (free plus reversibly bound HQ) in blood occurring in the initial 5- to 10-s arterial blood samples. Pharmacokinetic analysis of the absorption and elimination

of total HQ in arterial blood was best accomplished with a bolus i.v. dose model (Fig. 4). Inhalation exposure to HQ in the workplace would more likely take the form of a continuous exposure to low levels of respirable dust rather than a bolus dose. However, the elimination rate constant observed in the current single-dose study can be applied to the continuous exposure situation (i.e., an i.v. infusion model) to estimate blood HQ concentrations (C) that might be expected at time t , using the relationship

$$C_t = k_0 / (V_{\text{area}} \times k_{\text{elim}}) \times (1 - e^{-k_{\text{elim}} \times t})$$

where k_0 represents the constant input rate, V_{area} the volume of distribution, and k_{elim} the first order elimination rate constant (Clark and Smith, 1981). As exposure time (i.e., t) increases, the value of

$$(1 - e^{-k_{\text{elim}} \times t})$$

approaches 1 and the expression for the steady-state concentration (C_{ss}) of the chemical becomes simply $C_{\text{ss}} = k_0 / (V_{\text{area}} \times k_{\text{elim}})$.

Using the elimination half-life observed for total HQ in rat blood at the lowest (0.1 mg/kg) dose level as the closest approximation to a low-dose respiratory exposure, this value can be allometrically scaled on the basis of body weight to adjust it to a 70-kg human as follows:

Rat 0.1 mg/kg HQ intratracheal dose elimination half-life ($T_{1/2}$) = 194 s (Table 1)

Mean rat body weight for the 0.1 mg/kg doses was 310.07 g.

Allometric scaling formula: $T_{1/2} = a W^b$

where a is the y intercept in seconds, W is body weight in kg, and b is the slope of the log-log plot indicating how the $T_{1/2}$ is changing as a function of weight. It has been shown empirically that this exponent approximates 0.25 in relating biological time to weight (Mordenti and Chappell, 1989).

Therefore, for the rat:

$$194 \text{ s} = a (0.31007 \text{ kg})^{0.25}$$

$$a = 260 \text{ s}$$

Applying this intercept value to a human body weight of 70 kg gives:

$$T_{1/2} \text{ Human} = 260 \text{ s} (70 \text{ kg})^{0.25}$$

$$T_{1/2} \text{ Human} = 752 \text{ s}$$

Substituting a human elimination half-life of 752 s, the resulting rate constant (k_{elim}) would equal 0.000922 s^{-1} based on the relationship $k_{\text{elim}} = \ln 2 / T_{1/2}$.

Using the highest workplace 8-h time-weighted average (TWA) concentration for HQ (0.1 mg/m³) reported by Pifer et al. (1995), and assuming an adult light workload ventilation rate of 0.6 m³/h and a body weight of 70 kg, the constant dose rate (k_0) would be 0.000238 $\mu\text{g/s/kg}$. The V_{area} estimated from the 0.1 mg/kg HQ rat intratracheal dose was 1671 ml/kg, therefore, $C_{\text{ss}} = 0.000238 / (1671 \times 0.000922) = 0.000154 \mu\text{g/ml}$.

A second exposure scenario assumes inhalation of HQ at the current American Conference of Governmental Industrial Hygienists-recommended threshold limit value for HQ expressed as a TWA concentration of 2 mg/m³. As per the above calculations, the k_0 becomes 0.00476 $\mu\text{g/s/kg}$, therefore, $C_{\text{ss}} = 0.00476 / (1671 \times 0.000922) = 0.00309 \mu\text{g/ml}$.

These predicted steady-state blood concentrations of HQ would be 95% attained after 53 min of continuous exposure based on the relationship $t_{95\% \text{ ss}} = (-1/k_{\text{elim}}) \times \ln(1 - 0.95)$.

These calculations of steady state blood concentrations assume that the HQ dust is 100% respirable, i.e., reaches the alveolar region of the deep lung. Characterization of the particle size distribution of photographic grade HQ indicates that the mean particle size is on the order

of 300 μm in length with less than 12% of the particles below 100 μm in length (L.J. Roberson, unpublished report). These relatively large particles are typically removed from the inspired air by inertial impaction in the nasopharyngeal passages, whereas particles in the 5 μm to 0.003 μm size range are more likely to be deposited in the alveolar region of the lung (Witschi and Last, 1996). Apparently only a small fraction, if any, of the airborne particulate HQ is respirable; therefore, correspondingly lower blood concentrations would be expected from inhalation exposures to HQ.

These estimates of the steady-state blood levels of HQ resulting from continuous exposure to the chemical are extremely low because of the very rapid elimination rates for HQ observed in the current study. The steady-state total HQ blood concentration calculated for human exposure to 0.1 mg/m³ of HQ dust is insignificant when compared with the mean plasma concentrations of free HQ (0.023 $\mu\text{g/g}$) reported in humans with no occupational exposure to HQ (Deisinger et al., 1996). Even at the 2 mg/m³ threshold limit value TWA exposure limit for HQ and assuming a 100% respirable dust, the maximum blood levels predicted are 7-fold less than the levels of HQ detected in human blood from subjects with no occupational exposure to HQ and well below those which might be expected from some common natural sources (Deisinger et al., 1996). The maximum blood levels of HQ calculated assuming a 2 mg/m³ \times 8-h exposure and a 100% respirable dust are 300- to 900-fold below the minimum HQ concentration (9 or 25 μM HQ) shown to generate chromosomal aberrations in *in vitro* micronucleus assays using isolated human lymphocytes (Yager et al., 1990; Van Hummelen and Kirsch-Volders, 1992). An *in vivo* study of the genotoxic potential of HQ in mice suggested the existence of a no-effect threshold for the formation of micronuclei in polychromatic erythrocytes at doses between 12.5 and 25 mg/kg i.p. (Grawe et al., 1997). It appears that, in light of the blood elimination kinetics of HQ revealed in this study, inhalation exposures to HQ at or below the current TWA of 2 mg/m³ would appear to be of negligible toxicologic concern and are insignificant in terms of a chromosomal damage endpoint.

The absence of metabolites of HQ in the initial arterial blood samples indicates that pulmonary metabolism of HQ is not extensive. However, systemic metabolism appears to be robust after pulmonary absorption. At the 0.1 and 1.0 mg/kg dose level, HQ-glucuronide accounts for a mean of 93% of the blood [¹⁴C] at 12 min postdosing, whereas at the 10 mg/kg dose level, HQ-glucuronide accounts for a mean of 72% of the blood [¹⁴C] at the corresponding time point, indicating some degree of metabolic saturation. Only a trace of the sulfate conjugate of HQ was detected at the 0.1 mg/kg dose level, but this metabolite represented a mean maximum of 1.5% (6 min) and 3.4% (12 min) of the blood [¹⁴C] for the 1.0 and 10 mg/kg dose levels, respectively, indicating a dose-dependent shift toward this metabolite at higher dose levels. The disproportionately larger AUC for total HQ calculated for the 10 mg/kg dose when compared with the lower dose levels (Table 1), is further indication of the dose-dependent metabolic saturation at the higher dose level.

Comparison of the pharmacokinetic parameters derived from this study to those reported for a 75 mg/kg i.p. dose of HQ in mice (Legathe et al., 1994) indicate much more rapid absorption of HQ after intratracheal administration. The venous time of maximum concentration occurred at approximately 5 min after the i.p. administration compared with less than 1 min for the current intratracheal dosing. However, the venous HQ maximum concentration of 30 $\mu\text{g/ml}$ in the i.p. study is only 3.5 times larger than that in the current study (12 $\mu\text{g/ml}$ total HQ) even though the dose was 7.5 times larger in the i.p. study. This probably resulted from the relatively slower absorption of the i.p. dose with rapid elimination, moderating the

blood levels. The slower absorption of the i.p. dose is also evident in the longer blood $T_{1/2}$ reported for the i.p. dose of HQ (9 ± 2 min compared to <4 min for the 10 mg/kg pulmonary dose), which is probably the result of continuing absorption of HQ from the peritoneum concurrent with elimination from the blood.

In summary, saline solutions of [^{14}C]HQ administered intratracheally to conscious male rats resulted in very rapid absorption of the parent compound into the circulatory system. No evidence was found for the *in vivo* pulmonary metabolism of HQ at any dose level. Systemic metabolism and elimination of HQ after intratracheal administration was very rapid and showed a dose-dependent shift in metabolite pattern at the highest dose level. The rate constants derived from pharmacokinetic analysis of the total HQ blood concentrations in the rat after an intratracheal dose were used to estimate human blood concentrations of HQ resulting from workplace exposures to HQ dusts. The major blood metabolite detected at all dose levels was the glucuronic acid conjugate of HQ. Considering the low levels of respirable HQ present in the workplace and the very rapid elimination of HQ after pulmonary exposure as shown in this study, pulmonary and systemic exposure to HQ from airborne contamination appears to be negligible.

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