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Kinetics of Monochloroacetic Acid in Adult Male Rats after Intravenous Injection of a Subtoxic and a Toxic Dose

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ABSTRACT

Distribution, metabolism, and excretion of monochloroacetic acid (MCA) were examined in adult male rats at a subtoxic (10 mg/kg) and a toxic (75 mg/kg) dose. Rats were injected i.v. with [^{14}C]MCA and housed individually. Urine and feces were collected. Animals were euthanized at different time intervals after dosing and tissues procured. Radioactivity in aliquots showed very rapid distribution of MCA to tissues. Concentrations of MCA in plasma, liver, heart, lungs, and brown fat paralleled each other, whereas those in brain and thymus did not. There was no dose proportionality in tissue concentrations. Elimination of MCA from plasma required modeling by two compartments. Most of the radioactivity found in plasma was parent MCA. Elimination rate constant (K_{10}) and distribution rate constant (K_{12}) were greatly reduced at the toxic dose. Elimination of the toxic dose was further retarded due to increased retention of MCA in the peripheral compartment as indicated by increased mean residence times in most tissues. A very large fraction of dose was found in the gastrointestinal tract, almost all of which was reabsorbed. Attempts to reduce toxicity by blocking the enterohepatic circulation with activated charcoal or cholestyramine failed. Radioactivity found in bile was associated with one metabolite more polar than the parent compound. A very large fraction of dose (73 and 59%) was found in urine, 55 to 68% of which was parent MCA. The rate-determining step in the toxicity of MCA was identified as its detoxification by the liver. A therapeutic approach in MCA intoxications is suggested.

Monochloroacetic acid (MCA) is a chlorinated analog of acetic acid that is used as a postemergence contact herbicide and defoliant, detergent, disinfectant, and drying agent for canning processes. It is also a chemical intermediate for a number of synthetic products such as caffeine, vitamin A, 2,4-D, 2,4,5-T, and dyes (Woodard et al., 1941; Chenoweth, 1949; Webb, 1966; Sittig, 1985; U.S. Environmental Protection Agency, 1988; Budavari, 1989; National Toxicology Program, 1992). Total worldwide annual production of MCA was reported at about 400,000 tons (Kulling et al., 1992). MCA is one of the most commonly detected disinfection by-products in the drinking water supply of the United States (Christman et al., 1983; Norwood et al., 1983; Uden and Miller, 1983; Krasner et al., 1989). MCA is also produced as one of the metabolites of other widely used chemicals such as vinyl chloride, vinylidene chloride, 1,1,2-trichloroethane, and 1,2-dichloroethane (Yllner, 1971b; Bartsch et al., 1976; Hathway, 1977).

MCA is rapidly and efficiently absorbed through the skin. It is not only highly corrosive to tissues topically but also can cause death systemically after various routes, including exposure through the skin (Mann, 1969; Quick et al., 1983; Kusch et al., 1990; Kulling et al., 1992). It has been recommended to hospitalize all individuals who had exposure to as little as 1% of the skin (Kusch et al., 1990; Kulling et al., 1992). In addition to exposure via drinking water, thousands of workers are exposed to MCA occupationally (Kaphalia et al., 1992). Several cases of accidental poisoning have been reported in the literature, mostly fatal, to humans and animals (Zeldenrust, 1951; Christiansen and Dalgaard-Mikkelsen, 1961; Mann, 1969; Quick et al., 1983; Kusch et al., 1990; Kulling et al., 1992). The toxic mechanism of MCA is not completely understood. However, MCA is known to cause severe damage to energy-rich tissues (Hayes et al., 1973; Kulling et al., 1992) by interfering with the metabolism of fuel molecules of the tricarboxylic acid (TCA) cycle and gluconeogenesis. It enters the TCA cycle as a two-carbon chloroacetate and forms chlorocitrate, inhibiting further acetate oxidation in the TCA cycle. It also inhibits pyruvate carboxylase in the gluconeogenesis pathway (Fuhrman et al., 1955; Doedens and Ashmore, 1972; Hayes et al., 1973; Gosselin et al., 1984), which further aggravates energy depletion. MCA also reduces sulfhydryl content in rat liver and kidneys

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ABBREVIATIONS: MCA, monochloroacetic acid; TCA, tricarboxylic acid; GI, gastrointestinal; HPLC, high performance liquid chromatography; AUC, area under the curve; MRT, mean residence time.

(Fuhrman et al., 1955; Hayes et al., 1973; Gosselin et al., 1984). These effects result in severe tissue damage to organs such as liver, heart, central nervous system, kidney, and skeletal muscles (Hayes et al., 1973; Kulling et al., 1992). MCA in mice is mainly conjugated with glutathione to form an *S*-carboxymethyl derivative, which is converted to *S*-carboxymethylcysteine and eventually to thiodiacetic acid. These metabolites along with parent MCA are primarily excreted into urine (Yllner, 1971a). A minor pathway is the metabolism of MCA to glycolic acid, probably by enzymatic hydrolysis of the carbon-chlorine bond, which is further oxidized mainly to carbon dioxide and exhaled (Yllner, 1971a).

There are many studies describing the acute, subchronic, and chronic toxicity of MCA (Hayes et al., 1973; Davis and Bernt, 1987; Bhat et al., 1991; Bryant et al., 1992; National Toxicology Program, 1992), including its lack of mutagenic (Rannug et al., 1976) and carcinogenic potency (Innes et al., 1969; van Duuren et al., 1974; National Toxicology Program, 1992). Information on its kinetics, however, is incomplete. One study looked at MCA distribution to tissues by whole-body autoradiography (Bhat et al., 1990). This is very informative, however, it cannot be used for quantitative kinetic analysis. This study was designed, therefore, to determine a complete kinetic profile of MCA in adult male rats at a subtoxic dose of 10 mg/kg and to compare this with a highly toxic ($\sim LD_{50}$) dose of 75 mg/kg. Another goal of this study was to understand the kinetics of MCA in sufficient detail to guide the healthcare professional toward a more meaningful therapeutic intervention in patients accidentally exposed to potentially lethal doses of MCA.

Materials and Methods

Chemicals. Uniformly radiolabeled [^{14}C]MCA (mol. wt. 94.5, specific activity 4.5 mCi/mmol, >95% radiochemical purity) was purchased from Sigma Chemical Co. (St. Louis, MO). The chemical purity of [^{14}C]MCA was greater than 95%. It was used without further purification. Nonradiolabeled MCA (purity >95%) was added to adjust the concentration of the dosing solutions. Rats received uniformly 12.5 μCi/kg radioactivity. Dosing solutions were prepared in 0.9% saline and kept in the dark at 4°C. The pH of the dosing solutions was adjusted to 7 to 8 with NaOH. Dosing solutions were brought to room temperature before administration to animals.

Animals (Care and Dosing). Adult male Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN) and allowed to acclimatize to the animal facility for 1 week before their use in experimentation. Animals were housed in polycarbonate cages (three per cage) with corncob bedding and stainless steel wire tops under standard conditions (22 ± 1°C, 55% relative humidity, 12-h light/dark cycle). Rats were provided with Teclad 7001 Rodent Chow (Harlan, Madison, WI) and water ad libitum. After acclimatization, animals (160–190 g) were randomly selected for experimentation. Rats were given a single bolus i.v. dose into the tail vein at a volume of 1 ml/kg.

To select an acutely highly toxic dose of MCA for the kinetic experiments, groups of six to seven rats were treated i.v. with nonradiolabeled MCA at doses ranging from 50 to 125 mg/kg. The onset of toxicity (coma and mortality) was closely monitored for up to 72 h after injection.

On the basis of the acute toxicity results, rats were dosed with [^{14}C]MCA at an acutely subtoxic dose of 10 mg/kg or an acutely highly toxic ($\sim LD_{50}$) dose of 75 mg/kg for the kinetic studies. After completion of injections, animals were transferred to individual metabolism cages and provided with ground feed and water.

Sample Collection and Analysis. Five animals were scheduled to be euthanized by decapitation at various time intervals (5, 15, and 45 min; 2, 4, 8, and 16 h) after dosing. Some rats died at the last four time points (2–16 h) of the 75-mg/kg dose, reducing the number to three to four animals per group (n = 3 at 2, 8, and 16 h; n = 4 at 4 h). Blood was collected from cervical stumps in tubes containing EDTA and separated into plasma and red blood cells by centrifugation. Area around the injection site (~1 square inch) was excised to monitor the accuracy of dosing. Urinary bladders were manually emptied and the collected liquids added to the appropriate urine samples. Various organs [liver, kidney, heart, brain, thymus, fat, brown fat, lung, testis, spleen, muscle, skin (ear), and intestine] were removed from each animal by gross dissection. The gastrointestinal tract (GI) was separated into stomach (esophagus and stomach), small intestine, and large intestine. Contents were procured from each GI segment. After flushing with saline these and other tissues were stored. Tissues were rinsed gently but thoroughly with water to remove remaining traces of blood before storage. Dissecting instruments were also washed between tissue procurements to avoid cross-contamination. The same tissues as described above were also collected from rats that died before their scheduled sacrifice. All samples were stored at −10°C immediately after harvesting until further analysis. MCA is stable at this temperature for months without any significant degradation (I. Schultz and R. Bull, personal communication).

Total radioactivity in the collected samples was quantitated in duplicate (except for thyroid and brown fat). Weighed tissue aliquots (~0.2 g) were digested overnight in 1 to 2 ml of Soluene-350 tissue solubilizer (Packard, Meriden, CT) at 50°C. Colored samples were decolorized by adding a maximum of 0.2 ml of 30% H_2O_2. Scintillation cocktail (Hyonic Fluor; Packard, Meriden, CT) was then added and radioactivity determined in a Packard Tri-Carb 1900 TR scintillation spectrophotometer (Packard Instrument Co., Downers Grove, IL). Feces and intestinal contents were thoroughly homogenized before taking aliquots.

The scintillation counter was calibrated to correct for quenching by establishing a curve using quench standards. Rate of chemiluminescence was also monitored and samples were counted until chemiluminescence completely decayed. The counting efficiency of the scintillation counter was >95%. Total administered doses were corrected for radioactivity remaining at the injection site. Background radioactivity obtained from a rat not given [^{14}C]MCA was subtracted from each sample before calculating concentrations.

Metabolites. Radioactivity found in plasma, urine, and bile was analyzed for parent MCA. The time course of parent MCA in plasma (mixed with 1 volume of water) and urine (without dilution) was determined by HPLC. Due to low specific activity of MCA in these samples (ratio of [^{14}C]MCA versus unlabeled MCA was 1:38 and 1:286 for the 10- and 75-mg/kg dosage groups, respectively), fractions containing parent compound were collected and counted by liquid scintillation for more accurate measurements. The collection-time window was determined by multiple injections of [^{14}C]MCA standard of higher specific activity and monitoring the hill and valley of their peaks. The ratio of the total radioactivity in the sample and that of parent MCA was used to calculate the concentration of parent compound in each sample.

For the analysis of bile, additional rats were treated with 10 mg/kg MCA. Animals were anesthetized with pentobarbital (35 mg/kg i.p.) 30 min after administration of MCA. The bile duct was cannulated and bile collected for a period of 1 h. The bile collected was mixed with 4 volumes of water and analyzed for metabolite(s).

The HPLC system consisted of Shimadzu LC-6A pumps (Shimadzu Corp., Tokyo, Japan), a C18 column (5 μm, 250 × 4.6 mm i.d.; Alltech, Deerfield, IL) with a Beckman 171 radioisotope detector and an isocratic mobile phase (15 mM sodium phosphate buffer, pH 2.4, with 5% acetonitrile) at a flow rate of 1 ml/min. A C18 guard column (20 × 4.0 mm i.d.) was used routinely to protect the analytical column.

Kinetic Analysis. Average plasma concentration-time profiles of total radioactivity and parent MCA measured after i.v. injection of

the subtoxic dose of 10 mg/kg and the highly toxic dose of 75 mg/kg were analyzed by a compartmental modeling method using a nonlinear least-squares regression program (WinNonlin; Pharsight Corp., Cary, NC). The best fit of the data was obtained by using a two-compartment model rather than a one- or three-compartment model with $1/(\hat{Y})^2$ iterative weighting scheme, where $\hat{Y}$ is the concentration of MCA or total radioactivity in plasma predicted by the model. The WinNonlin program calculated model predictions by using standard equations (Gibaldi and Perrier, 1982). Renal clearance (Cl_{renal}) was calculated as $Cl_{renal} = X_{u\ 0\to16}/AUC_{0\to16}$ with $X_{u\ 0\to16}$ and $AUC_{0\to16}$ being the amount of total radioactivity or MCA in urine and the plasma AUC, respectively. The apparent volume of tissue compartment (V_t) was calculated as $V_t = V_c \cdot K_{12}/K_{21}$ with V_c being the volume of the central compartment and K_{12} and K_{21} the interdepartmental rate constants to and from the second compartment in the model. A noncompartmental modeling method was applied to determine $AUC_{0\to\infty}$ by extrapolating the terminal portion of the curve to infinity.

The time course of total [^{14}C]MCA-associated radioactivity in selected tissues was also analyzed to determine $AUC_{0\to16}$ and $AUC_{0\to\infty}$, as well as mean residence time (MRT_∞) using noncompartmental modeling methods. The $AUC_{0\to\infty}$ and MRT_∞ of total radioactivity in each tissue were predicted after extrapolating the terminal portion of the curves to infinity.

Antidotal Treatment. After finding very high biliary excretion of radioactivity in the kinetic experiments, attempts were made to trap presumed MCA excreted with bile to block its recirculation. A total of 30 rats was gavaged with two different agents (activated charcoal, 0.3 g/rat in an aqueous suspension, or cholestyramine, 0.5 g/rat in water). At 2, 3, 4, and 5 h after administration of these agents, rats were administered 100 mg/kg MCA (the highest dose in the bracket causing 100% coma and 50% mortality) through the tail vein, as described before. The dosing schedule for the antidotes was chosen to ascertain the presence of binding agent in the duodenum during the time period of maximal biliary excretion of radioactivity (up to 4 h after dosing). Twelve rats were treated with MCA alone at the same concentration for comparison. Mortality was recorded for up to 72 h following treatment in each group.

Statistics. Significant differences between treatments with different doses were assessed by Student's t test. Data in text, tables, and figures are given as means ± S.E.

Results

Table 1 shows the acute toxicity of MCA following single i.v. injections of doses ranging from 10 to 125 mg/kg. The onset of toxicity was very abrupt: no apparent signs of toxicity were observed up to 50 mg/kg, whereas 43% of the rats died at 60 mg/kg and >50% at 70 mg/kg. Doses between 70 and 100 mg/kg induced almost the same mortality (50–67%) without any apparent dose response and were not significantly different from each other (p = 0.43–1.0). Doses ≥80 mg/kg induced 100% incidence of coma. The incidence of mortality increased to 100% at doses ≥110 mg/kg (Table 1). The onset of coma was usually accompanied by clonic and tonic convulsions. In some instances animals died within minutes after going into coma, whereas in other instances, they remained in deep stupor for extended periods of time and either died or recovered. The onset of coma took place at 70 ± 5 min (n = 53) and death occurred within 75 ± 6 min (n = 43). Most of the animals that went into coma and did not die within 90 min of dosing regained consciousness suddenly and recovered. Estimation of a median lethal dose (LD_{50}) of MCA after single i.v. injections was not possible due to a range of doses (70–100 mg/kg) causing the same mortality. However, on the basis of this acute toxicity information, a dose of 75 mg/kg was chosen to study the high (toxic)-dose kinetics of MCA and to compare it with low (subtoxic)-dose kinetics of 10 mg/kg.

TABLE 1
Acute toxicity of monochloroacetic acid in adult male Sprague-Dawley rats after single intravenous injections

Treatment	Manifestation of Toxicity		n
	Percentage of Coma[a]	Percentage of Death[b,c]	
mg/kg			
10	0	0	7
50	0	0	6
60	57.2	42.9	7
70	83.3	66.7	6
75	71.4	57.2	7
80	100	66.7	6
90	100	66.7	6
100	100	50.0	6
110	100	100	7
120	100	100	7
125	100	100	7

[a] Mean time to coma was 70 ± 5 min (n = 53).
[b] Mean time to death was 75 ± 6 min (n = 43).
[c] Mortality among the doses ranging from 60 to 100 mg/kg was not significantly different from each other (p = 0.43–1.0).

Tissue Distribution, Metabolism, and Excretion. Tables 2 and 3 depict the time course of distribution and excretion of MCA at doses of 10 and 75 mg/kg, respectively. Table 4 compares AUCs and MRTs of selected tissues and excreta of the two doses. MCA was rapidly distributed to tissues; after 5 min only 0.6 and 1.0% of dose/ml remained in the systemic circulation at doses of 10 and 75 mg/kg, respectively. Within 8 h, concentration of radioactivity in plasma dropped to ≤0.1% of dose/ml. Most of the radioactivity associated with plasma was parent MCA (Tables 2 and 3; Fig. 1). Binding of radioactivity to red blood cells was negligible being <0.08% of the dose/g at the early time points when plasma concentrations were at highest (data not shown). Distribution of MCA to tissues and its elimination from them appeared to be diminished/saturated at the toxic dose; the AUC of total and parent MCA in plasma was 22 to 23 times higher at a dose of 75 mg/kg than at a dose of 10 mg/kg instead of the expected 7- to 8-fold quotient (representing the ratio of doses), reflecting the slower distribution and/or clearance at the higher dose (Table 4).

A higher percent of radioactivity was found in liver and kidney at the subtoxic dose (10 mg/kg), compared with the toxic (75 mg/kg) dose. Distribution of the 10-mg/kg dose to liver was rapid and paralleled the plasma concentration profile, whereas after the 75-mg/kg dose, liver concentrations peaked at 15 min post injection (Tables 2 and 3). The slower initial distribution of the toxic dose to the liver was also apparent from a smaller (2- to 3-fold) concentration ratio than expected based on dose proportionality (7- to 8-fold) between the subtoxic and toxic doses up to 15 min. Concentration in kidney peaked at 45 min (2.2% dose/g) after the subtoxic dose, whereas it peaked at 4 h (1.6% dose/g) after the toxic dose (Tables 2 and 3). The AUCs for liver and kidney were about 11 times higher after 75 mg/kg of MCA than after 10 mg/kg.

Distribution of radioactivity to heart, lungs, muscle, and skin was rapid and followed the pattern of plasma concentrations: highest concentrations occurred at 5 min and declined with time at both doses (Tables 2 and 3). The MRT in

TABLE 2
Concentration of radioactivity in different rat tissues after administration of a single i.v. dose of 10 mg/kg [^{14}C]monochloroacetic acid

Tissue	Percentage of Dose per Gram of Tissue or Total in Excreta and GI Tract						
	5 min	15 min	45 min	2 h	4 h	8 h	16 h
Plasma$_{(\text{total } [^{14}\text{C}]\text{MCA})}$	0.64 ± 0.03[a]	0.17 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.05 ± 0.004	0.02 ± 0.001	0.01 ± 0.001
Plasma$_{(\text{parent MCA})}$	0.32 ± 0.01	0.10 ± 0.03	0.11 ± 0.01	0.09 ± 0.01	0.04 ± 0.003	0.01 ± 0.002	0.004 ± 0.001
Liver	2.95 ± 0.11	2.74 ± 0.05	0.72 ± 0.03	0.58 ± 0.03	0.33 ± 0.03	0.13 ± 0.01	0.06 ± 0.01
Kidney	0.90 ± 0.02	1.39 ± 0.03	2.24 ± 0.09	1.69 ± 0.07	0.54 ± 0.03	0.16 ± 0.01	0.06 ± 0.003
Heart	0.61 ± 0.03	0.40 ± 0.01	0.34 ± 0.02	0.18 ± 0.01	0.05 ± 0.002	0.02 ± 0.001	0.02 ± 0.001
Brain	0.39 ± 0.01	0.29 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.18 ± 0.01
Thymus	0.41 ± 0.01	0.19 ± 0.01	0.42 ± 0.01	1.20 ± 0.03	1.11 ± 0.02	0.95 ± 0.04	0.56 ± 0.02
Thyroid	0.67 ± 0.07	0.20 ± 0.01	0.17 ± 0.04	0.11 ± 0.03	0.04 ± 0.02	B.D.	B.D.
Brown fat	0.99 ± 0.06	0.93 ± 0.05	0.98 ± 0.13	0.62 ± 0.09	0.18 ± 0.02	0.05 ± 0.01	0.03 ± 0.003
White fat	0.31 ± 0.02	0.24 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.08 ± 0.01	0.04 ± 0.003	0.02 ± 0.002
Lungs	0.45 ± 0.02	0.24 ± 0.01	0.18 ± 0.01	0.14 ± 0.01	0.09 ± 0.003	0.05 ± 0.002	0.02 ± 0.001
Testis	0.21 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.39 ± 0.01	0.22 ± 0.01	0.08 ± 0.003	0.04 ± 0.002
Spleen	0.35 ± 0.01	0.16 ± 0.01	0.24 ± 0.01	0.44 ± 0.01	0.30 ± 0.004	0.11 ± 0.01	0.03 ± 0.002
Muscle	0.30 ± 0.01	0.10 ± 0.01	0.08 ± 0.001	0.09 ± 0.01	0.03 ± 0.001	0.02 ± 0.001	0.01 ± 0.001
Skin (ears)	0.44 ± 0.02	0.21 ± 0.01	0.12 ± 0.01	0.16 ± 0.01	0.08 ± 0.01	0.04 ± 0.002	0.02 ± 0.001
Stomach	0.62 ± 0.04	0.67 ± 0.07	0.57 ± 0.08	0.41 ± 0.01	0.30 ± 0.02	0.13 ± 0.01	0.05 ± 0.002
Stomach[b]	0.02 ± 0.01	0.14 ± 0.06	0.19 ± 0.07	0.02 ± 0.01	0.02 ± 0.004	0.02 ± 0.01	0.03 ± 0.01
Small intestine	4.29 ± 0.29	6.12 ± 0.74	8.73 ± 0.72	6.36 ± 0.67	1.18 ± 0.09	0.31 ± 0.01	0.11 ± 0.01
Small intestine[b]	3.83 ± 0.57	23.69 ± 1.30	36.59 ± 0.68	6.87 ± 0.76	0.52 ± 0.06	0.09 ± 0.01	0.05 ± 0.01
Colon	1.05 ± 0.06	0.71 ± 0.04	0.52 ± 0.02	0.71 ± 0.08	0.60 ± 0.07	0.16 ± 0.01	0.06 ± 0.003
Colon[b]	0.80 ± 0.04	1.14 ± 0.06	0.57 ± 0.04	2.16 ± 0.81	4.27 ± 0.61	0.64 ± 0.06	0.12 ± 0.02
Urine$_{(\text{total } [^{14}\text{C}]\text{MCA})}$	0.03 ± 0.01	0.82 ± 0.03	7.95 ± 0.24	26.65 ± 3.23	55.30 ± 0.97	62.57 ± 1.38	72.80 ± 0.89
Urine$_{(\text{parent MCA})}$	B.D.	B.D.	4.28 ± 0.65	15.66 ± 2.39	23.55 ± 3.46	24.70 ± 2.11	40.22 ± 1.48
Feces	0.10 ± 0.01	0.07 ± 0.02	0.15 ± 0.02	0.04 ± 0.01	0.02 ± 0.003	0.08 ± 0.03	0.56 ± 0.05

B.D., below detection limit.
[a] Mean ± standard error (n = 5).
[b] Contents.

TABLE 3
Concentration of radioactivity in different rat tissues after administration of a single i.v. dose of 75 mg/kg [^{14}C]monochloroacetic acid

Tissue	Percentage of Dose per Gram of Tissue or Total in Excreta and GI Tract							
	5 min	15 min	45 min	2 h	4 h	8 h	16 h	Dead[a]
Plasma$_{(\text{total } [^{14}\text{C}]\text{MCA})}$	1.04 ± 0.01[b]	0.64 ± 0.01	0.56 ± 0.01	0.39 ± 0.01	0.13 ± 0.01	0.04 ± 0.004	0.02 ± 0.003	0.59 ± 0.06
Plasma$_{(\text{parent MCA})}$	0.83 ± 0.02	0.47 ± 0.02	0.45 ± 0.02	0.25 ± 0.04	0.09 ± 0.01	0.03 ± 0.002	0.02 ± 0.004	N.D.
Liver	0.90 ± 0.01	0.97 ± 0.03	0.87 ± 0.03	0.64 ± 0.04	0.43 ± 0.03	0.33 ± 0.04	0.17 ± 0.04	0.58 ± 0.02
Kidney	0.76 ± 0.02	0.79 ± 0.02	1.03 ± 0.03	1.29 ± 0.09	1.58 ± 0.12	0.41 ± 0.05	0.16 ± 0.05	0.70 ± 0.02
Heart	0.66 ± 0.02	0.52 ± 0.01	0.51 ± 0.01	0.35 ± 0.02	0.21 ± 0.01	0.08 ± 0.01	0.04 ± 0.001	0.42 ± 0.02
Brain	0.40 ± 0.01	0.38 ± 0.01	0.43 ± 0.01	0.36 ± 0.02	0.28 ± 0.01	0.26 ± 0.01	0.26 ± 0.03	0.42 ± 0.02
Thymus	0.50 ± 0.01	0.40 ± 0.01	0.45 ± 0.01	0.67 ± 0.01	1.01 ± 0.03	1.02 ± 0.04	0.52 ± 0.02	0.35 ± 0.01
Brown fat	0.65 ± 0.01	0.52 ± 0.01	0.62 ± 0.05	0.75 ± 0.09	0.57 ± 0.04	0.22 ± 0.04	0.07 ± 0.004	0.34 ± 0.02
White fat	0.21 ± 0.01	0.17 ± 0.02	0.18 ± 0.01	0.20 ± 0.02	0.22 ± 0.01	0.13 ± 0.02	0.03 ± 0.002	0.27 ± 0.02
Lungs	0.52 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.31 ± 0.01	0.18 ± 0.004	0.11 ± 0.01	0.06 ± 0.01	0.35 ± 0.01
Testis	0.27 ± 0.01	0.33 ± 0.01	0.43 ± 0.01	0.41 ± 0.02	0.38 ± 0.02	0.30 ± 0.02	0.19 ± 0.03	0.56 ± 0.05
Spleen	0.47 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.39 ± 0.02	0.44 ± 0.01	0.27 ± 0.03	0.08 ± 0.01	0.53 ± 0.02
Muscle	0.43 ± 0.01	0.30 ± 0.01	0.25 ± 0.01	0.17 ± 0.01	0.08 ± 0.003	0.06 ± 0.001	0.05 ± 0.003	0.49 ± 0.02
Skin (ears)	0.76 ± 0.05	0.56 ± 0.02	0.39 ± 0.01	0.27 ± 0.03	0.13 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.38 ± 0.03
Stomach	0.66 ± 0.02	0.59 ± 0.02	0.57 ± 0.02	0.58 ± 0.06	0.41 ± 0.09	0.28 ± 0.04	0.10 ± 0.01	0.75 ± 0.03
Stomach[c]	0.01 ± 0.001	0.09 ± 0.03	0.20 ± 0.03	0.73 ± 0.05	0.66 ± 0.34	0.47 ± 0.25	0.43 ± 0.13	1.41 ± 0.11
Small intestine	3.34 ± 0.16	3.35 ± 0.08	4.98 ± 0.15	5.26 ± 0.49	3.26 ± 0.32	0.86 ± 0.12	0.24 ± 0.02	3.50 ± 0.11
Small intestine[c]	2.39 ± 0.17	6.14 ± 0.24	11.85 ± 0.33	8.98 ± 0.35	5.35 ± 0.53	0.69 ± 0.27	0.16 ± 0.03	3.80 ± 0.23
Colon	1.08 ± 0.03	1.32 ± 0.03	1.38 ± 0.03	1.21 ± 0.07	0.57 ± 0.02	0.27 ± 0.02	0.14 ± 0.01	1.39 ± 0.07
Colon[c]	2.02 ± 0.07	3.33 ± 0.09	3.93 ± 0.20	2.31 ± 0.29	0.91 ± 0.06	1.08 ± 0.36	0.30 ± 0.04	3.25 ± 0.18
Urine$_{(\text{total } [^{14}\text{C}]\text{MCA})}$	0.74 ± 0.46	2.19 ± 0.28	5.92 ± 0.39	11.37 ± 1.27	27.65 ± 3.52	57.30 ± 1.13	58.62 ± 4.24	N.D.
Urine$_{(\text{parent MCA})}$	B.D.	1.81 ± 0.21	2.91 ± 0.38	8.18 ± 1.30	17.96 ± 1.84	39.72 ± 0.85	39.85 ± 4.89	N.D.
Feces	—[d]	0.26 ± 0.06	0.61 ± 0.16	1.53 ± 0.44	1.76 ± 0.68	1.21 ± 0.33	0.86 ± 0.21	N.D.

N.D., not determined; B.D., below detection limit.
[a] Collected from dead rats (all rats died between 50 and 70 min after dosing). Radioactivity was determined in the whole blood of dead rats.
[b] Mean ± standard error (n = 5 from 5–45 min, n = at least 3 from 2–16 h due to mortality, n = 7 for dead rats).
[c] Contents.
[d] —, no feces excreted.

heart was almost identical at both doses (about 5 h), whereas the toxic dose's MRT was much longer in liver, kidney, brown fat, and small intestine (Table 4). Distribution of both of the doses to spleen and testes was slower, peaking at about 2 to 4 h after dosing; however, the difference in distribution of radioactivity was within the expected dose proportionality (Tables 2 and 3).

Radioactivity associated with MCA entered the brain rapidly. It was retained there at almost the same concentration throughout the experimental period. The ratio of accumulation was proportionate to the dose (Tables 2–4). Distribution of radioactivity to thymus occurred with both doses in two distinct steps: an early rapid distribution, when plasma concentrations were very high, followed by a decline within 15

TABLE 4

AUC and MRT of [^{14}C]monochloroacetic acid in excreta and selected tissues of adult male Sprague-Dawley rats after a single intravenous injection of a subtoxic dose of 10 mg/kg and a highly toxic dose of 75 mg/kg

Parameter estimates were calculated from the average concentrations of MCA (total radioactivity) in tissues of three to five rats at each time point using a noncompartmental modeling method. $AUC_{0\to\infty}$ and MRT_{∞} were calculated by extrapolating the terminal slope of the curves to infinity.

Tissues	10 mg/kg			75 mg/kg		
	$AUC_{0\to16}$[a]	$AUC_{0\to\infty}$[a]	MRT_{∞}	$AUC_{0\to16}$[a]	$AUC_{0\to\infty}$[a]	MRT_{∞}
	$\mu g\ h\ g^{-1}$	$\mu g\ h\ g^{-1}$	h	$\mu g\ h\ g^{-1}$	$\mu g\ h\ g^{-1}$	h
Plasma$_{(total\ [^{14}C]MCA)}$	12.29	12.67	3.78	281.4	288.0	3.70
Plasma$_{(parent\ MCA)}$	9.75	10.04	3.65	209.5	216.2	3.62
Liver	85.46	90.18	4.51	808.8	1,077	11.21
Kidney	141.7	144.5	3.53	1,491	1,592	6.01
Heart	20.30	22.31	5.09	338.1	361.6	5.58
Brain	61.62	142.3	28.51	590.1	1,483	31.73
Lung	20.74	23.50	6.86	324.9	381.0	8.30
Muscle	9.96	11.25	7.13	187.9	220.4	8.97
Skin	18.19	20.26	6.51	265.9	293.3	7.07
Brown fat	57.11	58.23	3.33	699.1	745.0	5.69
White fat	18.97	20.51	5.87	280.1	299.9	6.40
Small intestine	1,475	1,494	1.95	12,004	12,502	4.69
Colon	475.7	484.3	4.74	3,433	3,953	7.25
Urine$_{(total\ [^{14}C]MCA)}$	15,074	—[b]	—	88,878	—	—
Urine$_{(parent\ MCA)}$	6,946	—	—	60,569	—	—
Feces	49.57	—	—	2,546	—	—

[a] AUC of the small intestine, colon, urine, and feces is in μg h representing total radioactivity or parent MCA.
[b] Could not be calculated due to continuously rising values with time.

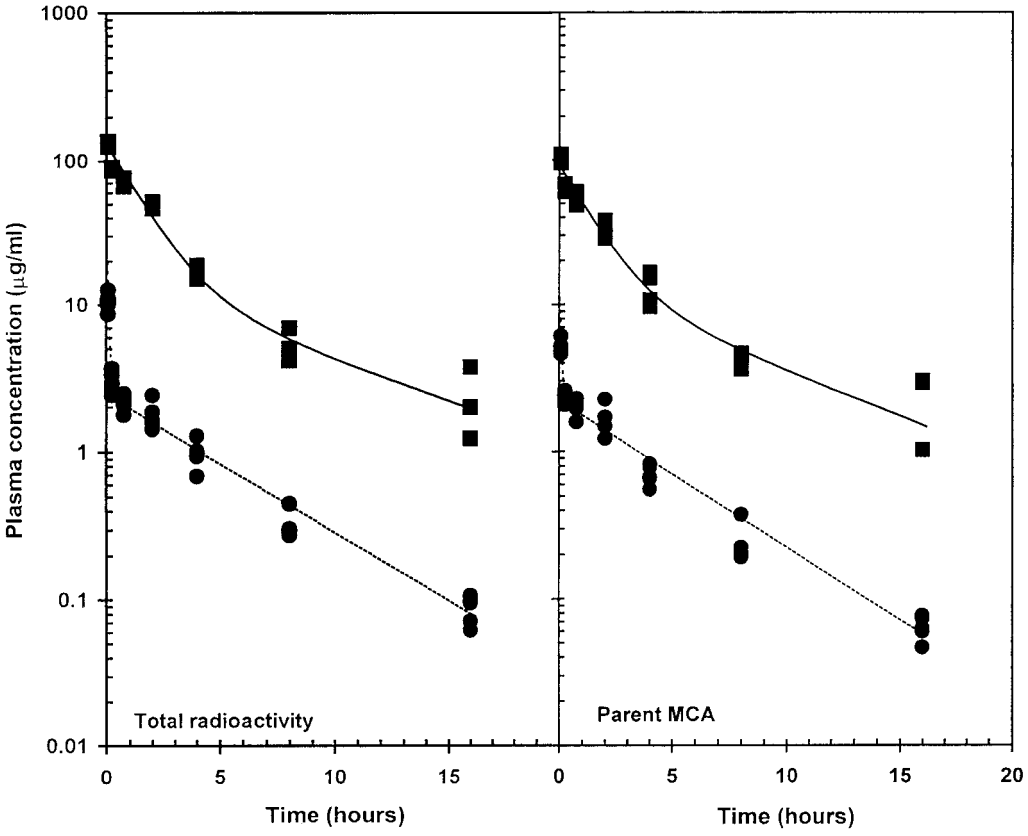

Fig. 1. Time course of total radioactivity and monochloroacetic acid concentrations in plasma of male rats following a single intravenous injection of a subtoxic dose of 10 mg/kg (●) or a highly toxic dose of 75 mg/kg (■). Observed data of the individual rats are depicted as filled circles or squares. Solid and dotted smooth lines represent model predictions for the 10- and 75-mg/kg doses, respectively.

min. The second phase of peak concentrations was reached at about 4 to 8 h (~1% dose/g in both dose groups) after administration of MCA with a slow decline in tissue concentrations. There was dose proportionality in concentrations of MCA in thymus at every time point sampled. Appreciable concentrations of MCA were also found in fat (both white and brown fat), which were comparable to concentrations observed in muscle at early time points. They were even higher than in muscle at later time points (Tables 2 and 3). Distribution of the toxic dose to fat and muscle was 13 to 19 times higher than that of the subtoxic dose (Table 4).

A very large fraction of the doses was recovered from the GI tract within 45 min (~10% of each dose at 5 min and 47 and 23% at 45 min of the subtoxic and toxic doses, respectively). Most of the radioactivity found in the GI tract was confined to the small intestine (Tables 2 and 3) and rapidly

reabsorbed. A maximum of only about 5% of dose reached the large intestine (Tables 2 and 3), where some of it was further reabsorbed, leaving very little for fecal excretion. About 2 to 3% of dose was found in the colon within 5 min (Tables 2 and 3), which could only be due to direct transport from blood across the gut wall. AUCs for the small intestine and colon reflected dose proportionality (Table 4).

Figure 2 compares the time course of the concentration profile of total radioactivity in the GI tract and its excretion into feces after the two different doses. Fecal excretion of radioactivity was very limited compared with the amount found in the GI tract. The rate of excretion of ^{14}C-labeled material into the GI tract (in the form of a metabolite) was different between the two dosages; rats treated with 75 mg/kg were 4 times less efficient in clearing MCA-related metabolite(s) through the bile than rats treated with 10 mg/kg. All of radioactivity found in the GI tract was associated with one major metabolite, which was more polar than the parent compound (probably glutathione conjugate). Figure 3 shows a characteristic HPLC radiochromatogram of bile collected from [^{14}C]MCA-treated rats compared with that of MCA.

Parent MCA and metabolite(s) were rapidly excreted with 6 to 8% of the doses recovered in urine within 45 min. Within 16 h, rats excreted 73% of the subtoxic dose, whereas excretion of the toxic dose was somewhat slower (58% of the dose) (Tables 2 and 3). HPLC analysis of the urine samples revealed that a total of 55 and 68% of the excreted radioactivity was parent MCA at the nontoxic and toxic dosages, respectively, amounting to about 40% of the injected dose of both dosages (Tables 2 and 3; Fig. 4). Figure 5 shows a representative HPLC radiochromatogram of a urine sample (Fig. 5A), and in comparison to the same sample after spiking with parent compound (Fig. 5B). Rats treated with the toxic dose became anuric about 4 h after dosing. This condition was apparently due to their inability to empty urinary bladders rather than reduction in urine production; urinary bladders of all anuric rats were filled to their capacity with no or very little output of urine.

Kinetics. Disappearance of [^{14}C]MCA as well as that of the parent compound from plasma followed a biexponential pattern (Fig. 1). Table 5 compares kinetic characteristics of total radioactivity and of parent MCA in plasma after injection of the subtoxic and toxic doses. A two-compartment model with 1/(predicted concentration)^2 weighting was used to obtain these parameters, which provided a better fit than a one- or three-compartment model. Noncompartmental models were also used to calculate $AUC_{0\rightarrow\infty}$ and MRT_{∞} for a series of tissues to compare the two doses (Table 4). Extrapolation of the AUC of the observed data ($AUC_{0\rightarrow 16\ h}$ to $AUC_{0\rightarrow\infty}$) accounted for <10% of the area for most tissues with the exception of brain, lung, and muscle of the subtoxic dose and brain, lung, muscle, liver, and colon of the toxic dose (Table 4). The subtoxic MCA dose was rapidly distributed to tissues, whereas distribution of the toxic dose to tissues was much slower. Similarly, distribution of the toxic dose of MCA from the tissue compartment back to the central compartment was also somewhat slower than at the subtoxic dose (Table 5). The apparent volume of distribution of total radioactivity in the central compartment (V_c) was 2 times higher at the toxic dose (332 versus 567 ml kg^{-1}), whereas that of the parent MCA was almost identical at both doses (825 versus 802 ml kg^{-1}). The apparent volumes (total radioactivity and parent MCA) of tissue compartments (V_t) at the

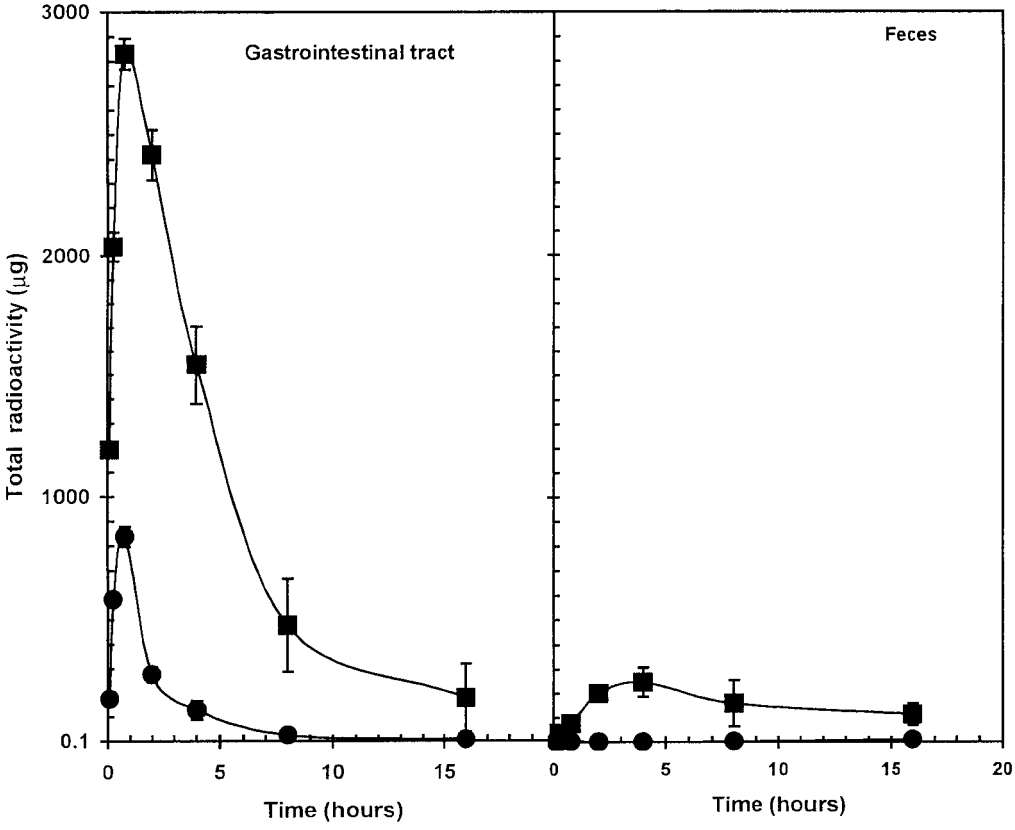

Fig. 2. [^{14}C]Monochloroacetic acid concentrations in the gastrointestinal tract (stomach + small intestine + colon) at different time points after exposure (left) and elimination through feces (right) in male rats following a single intravenous injection of a subtoxic dose of 10 mg/kg (●) or a highly toxic dose of 75 mg/kg (■). Each point represents the mean ± S.E. of three to five animals.

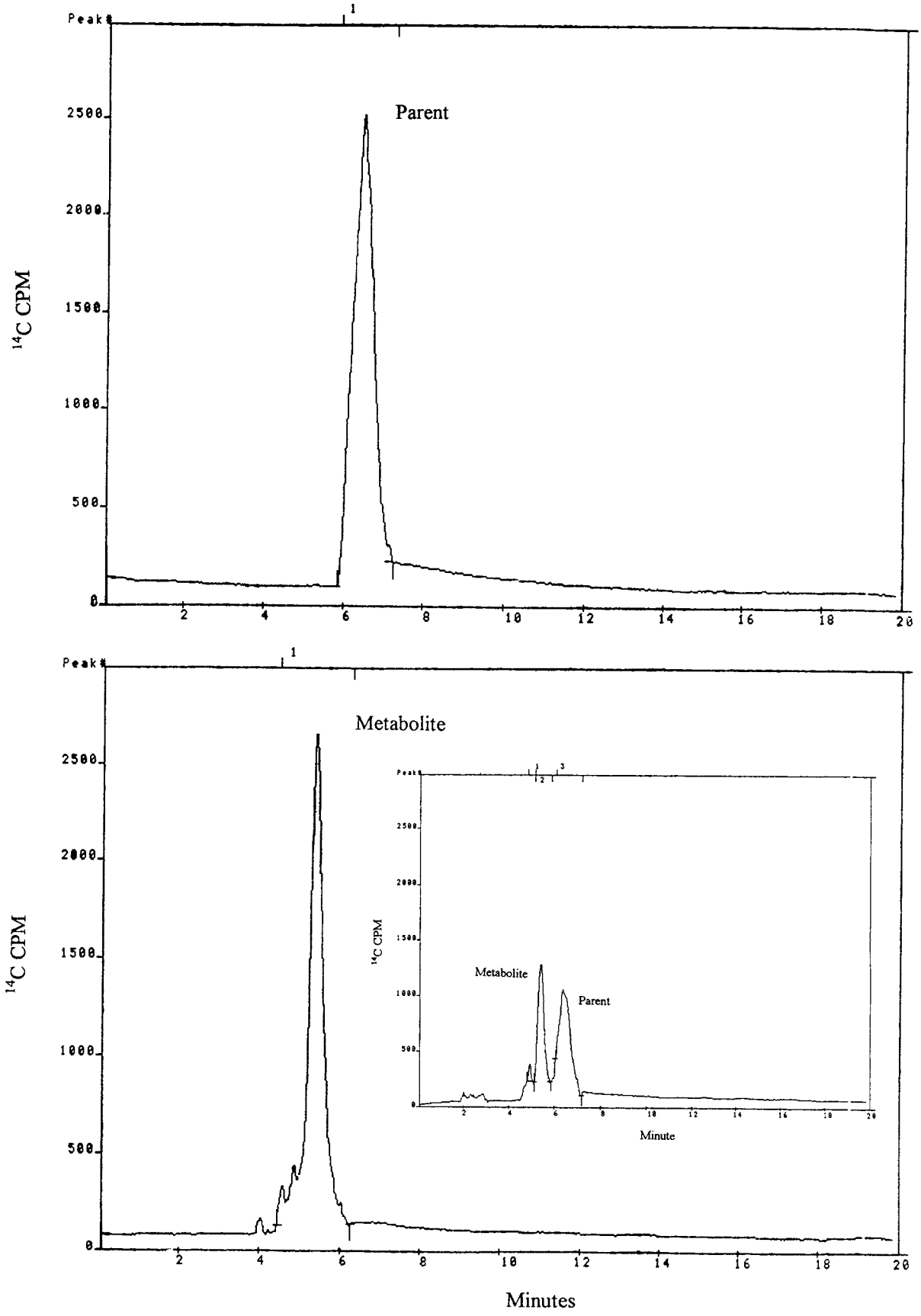

Fig. 3. HPLC radiochromatogram of bile (bottom) collected 0.5 to 1.5 h after a single intravenous injection of [^{14}C]monochloroacetic acid (10 mg/kg). Parent compound was not detected in bile samples. Radiochromatogram (top) of the dosing solution (parent [^{14}C]monochloroacetic acid) and the inset is a 1:1 mixture of the dosing solution and the bile collected after intravenous injection.

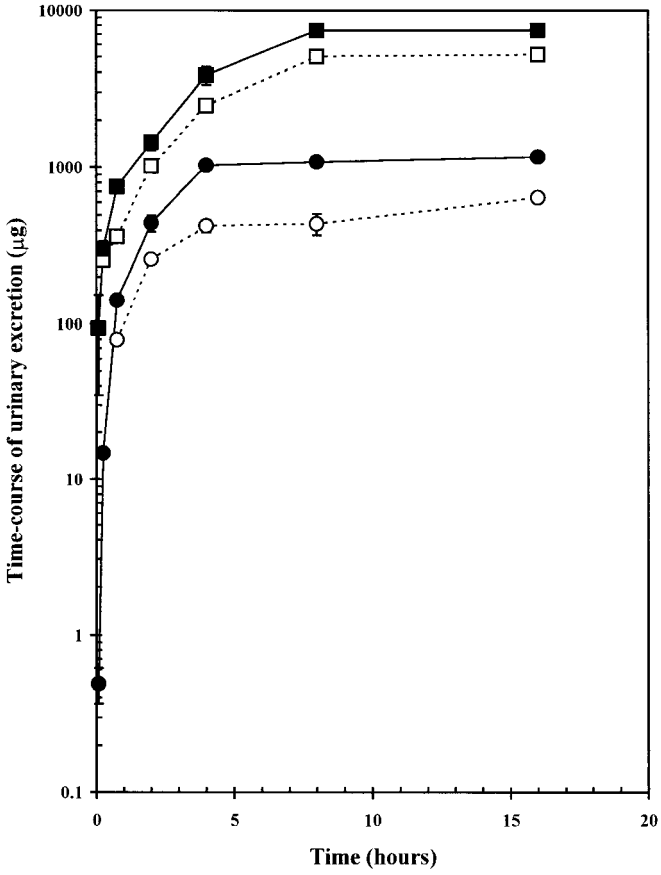

Fig. 4. Time course of total radioactivity and parent MCA concentrations in urine of male rats following a single intravenous injection of a subtoxic dose of 10 mg/kg (●) or a highly toxic dose of 75 mg/kg (■). Filled symbols and solid lines represent total radioactivity, whereas open symbols and dotted lines represent concentration of parent MCA. Each point represents the mean ± S.E. of three to five animals.

toxic dose was about 5 times lower than the V_t at the subtoxic dose (Table 5). The mean residence time of MCA in plasma was about 4 h. The apparent volume of distribution at steady state (V_{ss}) was about 3 times lower at the toxic dose than at the subtoxic dose. Total body clearance (Cl_{total}) and renal clearance (Cl_{renal}) reflected similar differences at the toxic and subtoxic doses (Table 5). Decreased biotransformation of the toxic dose was apparent at early time points (up to 2 h) from significantly lower (up to 28% lower than the subtoxic dose) biliary excretion of the metabolite(s) but higher urinary excretion of the parent MCA (Tables 2 and 3). The time course of reduced biotransformation and increased retention of the dose in tissues correlated well with the time of onset of toxicity (coma and death), which occurred between 40 and 70 min after dosing (Table 1). Animals surviving this time period had lower concentrations in most tissues and plasma/blood by 2 h after dosing than rats succumbing to the toxicity of MCA, whereas their GI contents contained a larger quantity of metabolite(s) than that of rats dying from toxicity (Tables 3 and 6). Survivors of the critical period of toxicity after the toxic dose of 75 mg/kg eliminated the remaining MCA from their body slightly slower than rats that received the subtoxic dose (Table 5).

Comparison of Tissue Levels between Dead and Surviving Rats. Table 6 compares the tissue concentrations of [^{14}C]MCA in rats that died as a result of toxicity before their scheduled termination with those sacrificed at the two closest experimental time points. Concentrations in most of tissues of rats that died were either identical or lower than those sacrificed at 45 min with the exception of muscle and fat, which were significantly higher ($p \leq 0.02$) (Table 6). Concentrations of MCA in most of the tissues of rats that were sacrificed at 2 h post dosing were lower than those found in dead rats (Table 6). It is important to note that rats dying before sacrifice were half as efficient in metabolizing and excreting MCA into the GI tract with bile than those that survived until scheduled termination ($p < 0.001$) (Table 6). Radioactivity in surviving rats represents a mixture of parent MCA and apparently less toxic metabolite(s), which was (were) reabsorbed from the GI tract. Radioactivity in tissues of rats that died consists mainly of the more toxic parent compound, because of lower metabolism/biliary excretion of MCA in rats lethally intoxicated. Urinary samples from dead rats were not saved.

Antidotal Therapy Attempts. To take advantage of the extensive biliary excretion and enterohepatic circulation of MCA observed during the kinetic studies, animals were treated with activated charcoal or cholestyramine to trap radioactivity excreted with bile and thereby prevent its reabsorption. Table 7 summarizes the results of this study. Both treatments increased rather than decreased toxicity compared with positive controls (rats treated with the same dose of MCA alone). Mortality between the two treatments was not statistically different ($p = 0.25$); however, both of the treatments significantly increased mortality compared with animals that were not treated with any trapping agent ($p = 0.017$ and 0.003 for cholestyramine and activated charcoal, respectively) (Table 7).

Discussion

There has been a proliferation in the use of the word "toxicokinetic" in recent publications. Many if not most of these reports in reality were describing the kinetics of toxic chemicals at subtoxic dose(s). Using the term kinetics in conjunction with some qualification of dose could avoid further contentious discussions.

The kinetics of MCA at a subtoxic dose harbors few surprises (Tables 2, 4, and 5). A rapid distribution was to be expected of a very small water-soluble molecule, which moves into and out of body compartments with bulk flow of water. There is a 3- to 10-fold concentration gradient between liver and other tissues at early time points (5 and 15 min), indicative of active uptake of MCA into this organ or some other trapping mechanism responsible for this finding. Biliary excretion was expected to occur because of the report of Bhat et al. (1990) showing the presence of radioactivity in the duodenum by autoradiography. However, the magnitude of biliary excretion of MCA was a surprise. A total of 71% of dose was recovered in the contents of the small intestine during the first 2 h after i.v. injection of MCA. Urinary excretion (including contents of the urinary bladder) during the first 15 min (0.85% of dose) indicates that MCA is excreted into urine at a rate of 3 to 4%/h. Low levels of MCA at 5 min suggest no active uptake into the kidneys. With a delay of 15 min, kidney concentrations of MCA begin to rise to levels previously seen in liver. The pattern in kidney is very similar to that observed in the contents of the GI tract, which is com-

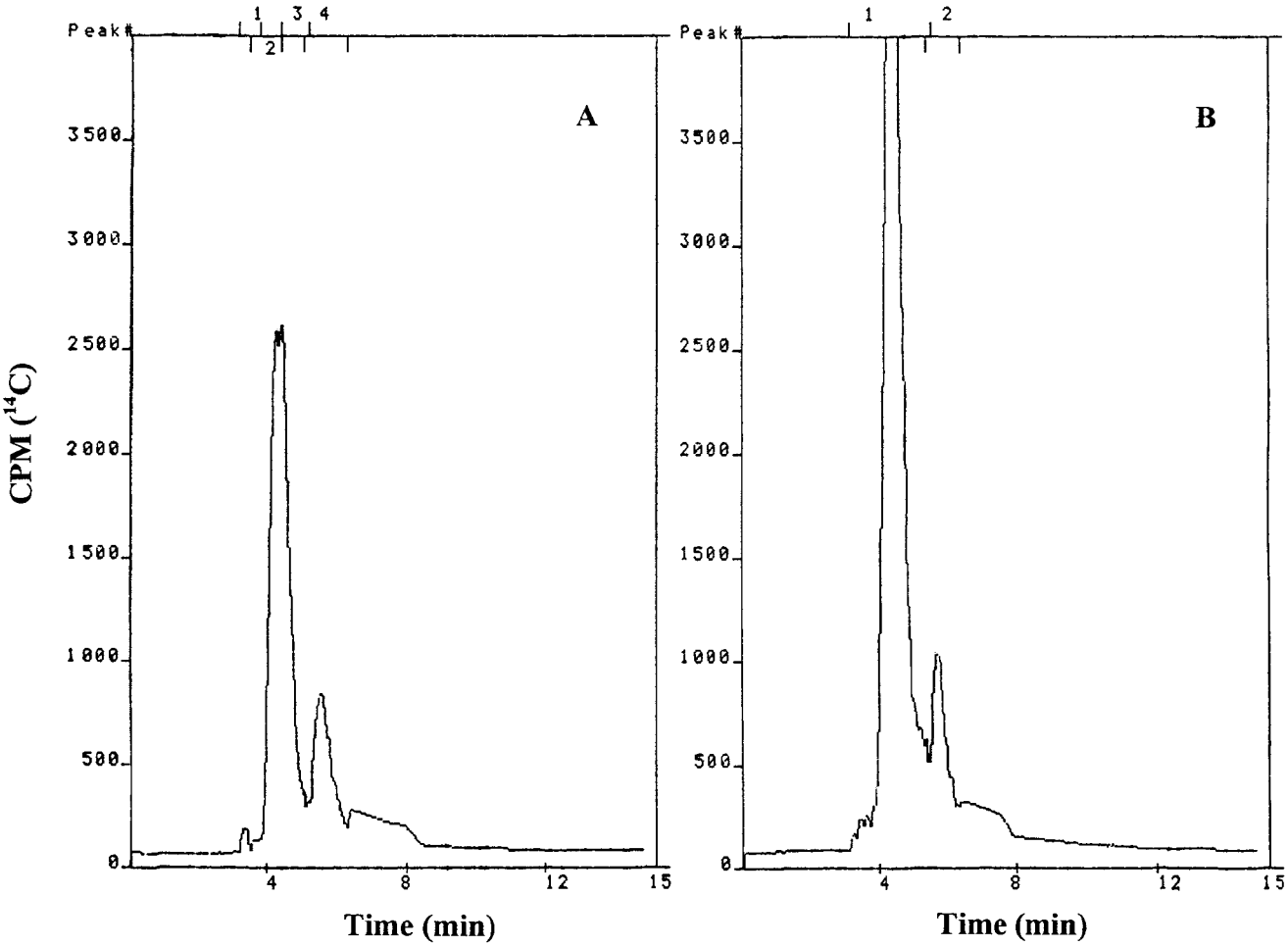

Fig. 5. A, representative HPLC radiochromatogram of urine showing MCA and some metabolites. Urinary samples were collected up to 8 h after a single intravenous injection of 10 mg/kg [^{14}C]monochloroacetic acid. B, radiochromatogram of the same sample after spiking it with [^{14}C]MCA.

TABLE 5

Kinetic parameters of total radioactivity associated with [^{14}C]monochloroacetic acid and parent compound in adult male Sprague-Dawley rats after a single intravenous injection of a subtoxic dose of 10 mg/kg and a highly toxic dose of 75 mg/kg

Parameter estimates were calculated from the average concentrations of total radioactivity and parent MCA in plasma of three to five rats at each time point using a two-compartment model with $1/(\hat{Y})^2$ weighting, with $\hat{Y}$ being the predicted concentration based on the model.

Kinetic Parameters	Radioactivity (10 mg/kg)		Radioactivity (75 mg/kg)	
	Total	Parent	Total	Parent
V_c (ml kg^{-1})	332.3	824.7	567.2	801.9
V_t (ml kg^{-1})	2700	3122	492.4	673.5
K_{10} (h^{-1})	2.257	1.17	0.462	0.445
K_{12} (h^{-1})	11.30	10.64	0.168	0.194
K_{21} (h^{-1})	1.391	2.81	0.193	0.231
α (h^{-1})[a]	14.74	14.39	0.694	0.730
β (h^{-1})[a]	0.213	0.228	0.128	0.141
A (μg/ml)[b]	27.65	9.91	117.1	79.19
B (μg/ml)[b]	2.44	2.21	15.09	14.35
C_{max} (μg ml^{-1})	30.09	12.13	132.2	93.52
MRT (h)	4.04	4.09	4.04	4.13
V_{ss} (ml kg^{-1})	3033	3946	1060	1476
$t_{1/2\beta}$ (h)	3.25	3.03	5.40	4.91
Cl_{total} (ml h^{-1} kg^{-1})	750.0	964.7	262.0	357.1
$X_{urine\ 0\to16}$ (% dose)	72.8 ± 0.9	40.2 ± 1.5	58.6 ± 4.2	39.9 ± 4.9
Cl_{renal} (ml h^{-1} kg^{-1})	546.0	387.8	153.5	142.5

[a] Dispositional rate constants.
[b] Dispositional constants.

patible with the notion that metabolite(s) (presumably glutathione conjugates) excreted with bile are being reabsorbed (very little passage of MCA or metabolites into the large intestine) and excreted with urine after further metabolism of the glutathione conjugate by the liver or the kidneys. *S*-Carboxymethylcysteine and thiodiacetic acid have been

TABLE 6

Comparison of the [^{14}C]monochloroacetic acid concentrations in selected tissues of rats that died with those that were terminated on schedule after a single intravenous injection of a highly toxic dose of 75 mg/kg

Tissue	Rats Euthanized		Rats Died (~1 h[a], n = 7)
	(45 min[a], n = 5)	(2 h[a], n = 3)	
	μg/g equivalent [^{14}C]MCA in tissues or μg/ml in blood		
Plasma[b]	70.58 ± 2.00[c]	49.23 ± 1.60	75.58 ± 7.50[†]
Liver	108.77 ± 4.73	81.11 ± 5.72	74.26 ± 3.93**
Kidney	129.40 ± 3.5	164.99 ± 20.13	90.09 ± 4.00**,[††]
Heart	63.48 ± 1.10	44.55 ± 2.58	54.36 ± 2.56**,[†]
Brain	53.96 ± 1.27	45.60 ± 2.65	53.44 ± 2.56
Lung	48.39 ± 1.35	39.11 ± 1.69	44.38 ± 1.86
Muscle	31.84 ± 0.99	21.95 ± 1.59	62.81 ± 3.99**,[††]
Brown fat	77.29 ± 5.34	94.15 ± 7.89	43.29 ± 2.20**,[††]
White fat	23.23 ± 1.46	24.86 ± 1.18	34.40 ± 3.49*
Skin	48.59 ± 1.58	33.50 ± 3.10	48.17 ± 3.41[†]
	Total % of the applied [^{14}C]MCA		
Upper GI[d]	17.61 ± 1.55	15.55 ± 0.40	9.46 ± 0.42**,[††]
Colon	5.31 ± 0.24	3.52 ± 0.53	4.63 ± 0.31[†]

[a] Mortality occurred between 50 and 70 min after dosing and thus, concentration in different tissues of the dead rats were compared with rats terminated at 45 min and 2 h.
[b] Radioactivity was determined in whole blood of the dead rats.
[c] Mean ± S.E.
[d] Upper GI represents pooled contents of stomach and small intestine.
*,** Significantly different from 45 min (* $p < 0.02$; ** $p < 0.001$).
†,†† Significantly different from 2 h († $p \leq 0.04$; †† $p < 0.001$).

TABLE 7

Comparison of the effects of attempted antidotal treatment on mortality of male Sprague-Dawley rats following a single i.v. injection of 100 mg/kg monochloroacetic acid

	Treatment Prior to Injection of 100 mg/kg Monochloroacetic Acid[a]		
	Cholestyramine (N = 18)	Activated Charcoal (N = 12)	None (N = 12)
Mortality (%)	88.9	100*	50[†,¶]

[a] Rats were gavaged either with cholestyramine (0.5 g/rat) or activated charcoal (0.3 g/rat) in an aqueous suspension 2, 3, 4, or 5 h prior to administration of MCA. Data were pooled due to lack of time-related effect of the treatment.
* Not significantly different from cholestyramine treatment: $p = 0.25$.
† Significantly different from cholestyramine treatment: $p = 0.017$.
¶ Significantly different from activated charcoal treatment: $p = 0.003$.

reported to be the major metabolites in urine comprising 66 to 85% of the total urinary excretion in mice (Yllner, 1971a). Rats appeared to be less efficient in metabolizing MCA than mice (apparent from more parent MCA in rat than mouse urine), consequently they were more sensitive to its toxicity ($LD_{50} \approx 70$–100 mg/kg for rats versus 255 mg/kg for mice) (this study; Woodard et al., 1941; Yllner, 1971a). Rapid increase in the percentage of dose excreted with urine after 45 min reflects a basal level of filtration of the parent compound combined with excretion of the glutathione derived metabolite(s) of MCA by the kidneys.

The kinetic profile of MCA at a toxic dose (Tables 3–5) tells a significantly different story. At a highly toxic dose, the apparent volume of distribution of MCA to the central compartment was 2 times higher than that at the subtoxic dose. Toxicity reduced its clearance by about 2- to 4-fold and its volume of distribution at steady state was decreased also by about 3-fold. Slower distribution of the toxic dose to most tissues and its slower release back to blood was possibly due to poor blood flow to tissues related to decreased cardiac output since MCA is known to cause myocardial damage and to reduce blood pressure at toxic doses (Bryant et al., 1992; Kulling et al., 1992). The terminal slope of plasma elimination for survivors of the toxic dose of MCA became almost parallel to that of the subtoxic dose (Table 5). This is consistent with findings of the acute toxicity part of this study, in that survivors of the critical period of toxicity (even those that went into coma) regained consciousness suddenly and recovered without showing any further signs of toxicity. This is clearly due to partial elimination of MCA by metabolism/excretion during the period of intoxication (40–70 min), lowering MCA concentrations to below acutely toxic levels.

At the toxic dose, liver did not accumulate MCA relative to other organs and tissues, indicating saturation of active uptake or another saturable dispositional step, which was not saturated at the subtoxic dose; saturation also accounted for slower distribution of MCA to liver. As a consequence the fraction of dose excreted with bile during the first 2 h remained at about 30% of dose far below that observed at the subtoxic dose (71% of dose). It is instructive to compare this rate of excretion in surviving versus nonsurviving rats of the toxic dose showing that biliary excretion was further compromised in nonsurvivors (Table 6). Delayed appearance profile in kidneys and urine is similar to the subtoxic dose but as a fraction of dose less and delayed at that. A comparison of the low-dose kinetic with the high-dose kinetic profile of MCA clearly demonstrates that the abrupt onset of coma/death in MCA exposed rats is due to a rapid overwhelming of the detoxification capacity of the liver.

Anuria seen in rats at the toxic dose was most probably due to spasm of the sphincter controlling the urinary discharge rather than damage to the kidneys; damage to kidneys was not evident in rats dosed with similarly toxic doses (Bryant et al., 1992). Spasm of the sphincter is not surprising due to the high irritating potency of MCA and/or its toxicity to the central nervous system (Berardi et al., 1987). Direct inhibition of sulfhydryl groups in kidney has also been suggested as cause of anuria (Hayes et al., 1973). However, this can be ruled out since anuric rats in this study were producing urine at a normal rate but were unable to discharge it as was apparent from the highly dilated bladders. The occurrence of anuria is dose-dependent, a slightly less toxic (~LD_{20}) dose did not cause anuria in rats (Saghir et al., 2000; Siegrist et al., 2000), however higher (≥LD_{50}) doses induced anuria in all rats (this study; Hayes et al., 1973). Anuria has also been reported in a human patient who was exposed to MCA and died 8 days later (Kulling et al., 1992).

The insights gained into the kinetics of MCA call for two possible therapeutic interventions in MCA intoxications: 1) improving the detoxification capacity of organism, and 2) enhancing its elimination from it. In light of the results of these experiments, the antidotal efforts (Table 7) were doomed from the beginning since biliary excretion of MCA metabolite(s) represent(s) a detoxification step (which was not clear at the time of the conduct of the antidotal experiments). Thus, it is obvious now that detoxification cannot be enhanced by trapping less toxic metabolite(s) in the GI tract. Increased toxicity of MCA in charcoal/cholestyramine-treated rats was, although, a surprise. It is likely to be due to decreased bile flow (=decreased detoxification) since both trapping agents used adsorb/bind bile acids, which are among the most potent choleretic compounds. Administration of bile acids would increase bile flow but it may not help

therapeutically if the rate-determining step turns out to be a saturable dispositional phenomenon rather than bile flow.

A rapid detoxification by the liver also provides an explanation for the difficulty of establishing a well defined dose response for MCA. The time course of detoxification [appearance of metabolite(s) in GI tract] is very similar to the time course of intoxication, both of them occurring on a time scale of a few hours. Thus, in rats treated with highly toxic doses of MCA, the development of toxicity and recovery from it are running concurrently. Therefore, a single i.v. injection of MCA does not measure the relationship between dose and toxicity alone but among dose, toxicity, and recovery. An ideal dose-response study, which would control the third variable (recovery), would be to administer a loading dose followed by a maintenance infusion to prevent recovery from occurring at the same time as toxicity is developing.

There are a number of case reports in the literature describing accidental exposure of humans to MCA, most of them with fatal outcome (Millischer et al., 1987; Kush et al., 1990; Kulling et al., 1992). Various antidotal treatments (e.g., ethanol and ethanol + *N*-acetylcysteine) have been tried without conclusive evidence for their effectiveness. Additional avenues in animals have been explored such as administration of dichloroacetic acid and phenobarbital, also without much success (Berardi et al., 1987). There is one case report relating recovery of an individual from severe intoxication by MCA (Kush et al., 1990). The attending physician administered a diuretic and was unsure whether recovery was due to a less-than-lethal dose or to the use of the diuretic. Insights into the high-dose kinetics of MCA described in this manuscript in combination with the description of exposure and the severity of symptoms of the recovered individual make it likely that treatment with the diuretic saved the individual's life. Therefore, an aggressive treatment with a diuretic together with infusion of $NaHCO_3$ is likely to save the life of individuals lethally intoxicated with MCA if therapeutic intervention is started early enough (within about an hour of exposure). Catheterization of the urinary bladder is recommended as a precautionary measure to prevent anuria as a result of irritation of the sphincter.

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