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4,4′-Methylene-bis(2-chloroaniline) (MOCA): Comparison of Macromolecular Adduct Formation after Oral or Dermal Administration in the Rat[1]

KENNETH L. CHEEVER, DONALD E. RICHARDS, WALTER W. WEIGEL, KAREN B. BEGLEY, D. GAYLE DEBORD, TERRI F. SWEARENGIN, AND RUSSELL E. SAVAGE, JR.

Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, 4676 Columbia Parkway, Cincinnati, Ohio 45226

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4,4′-Methylene-bis(2-chloroaniline) (MOCA): Comparison of Macromolecular Adduct Formation after Oral or Dermal Administration in the Rat. CHEEVER, K. L., RICHARDS, D. E., WEIGEL, W. W., BEGLEY, K. B., DEBORD, D. G., SWEARENGIN, T. F., AND SAVAGE, R. E. JR. (1990). *Fundam. Appl. Toxicol.* **14,** 273–283. The macromolecular binding of 4,4′-methylene-bis(2-chloroaniline) (MOCA), a suspect human carcinogen, was studied in the adult male Sprague–Dawley rat after both oral and dermal administration. Rats were euthanized 1, 3, 7, 10, 14, and 29 days after a single 281 μmol/kg body wt dose of [^{14}C]MOCA (oral, 213 μCi/kg; dermal, 904 μCi/kg). DNA from various tissues and hemoglobin were isolated for determination of the time course of MOCA macromolecular binding. After oral administration adduct formation was rapid with maximum levels appearing at 24 hr. The 24-hr covalent binding associated with the globin was 7.84 pmol/mg globin ($t_{\frac{1}{2}}$ = 14.3 days). More extensive 24-hr covalent binding was detected for liver DNA with 49.11 pmol/mg DNA ($t_{\frac{1}{2}}$ = 11.1 days). After dermal administration of MOCA the major portion of the dose, 86.2%, remained at the application site throughout the study. For these rats the 24-hr covalent binding determined for liver DNA was 0.38 pmol/mg DNA ($t_{\frac{1}{2}}$ = 15.6 days). Although lower levels were detected after dermal application, similar stability of MOCA–DNA adducts indicates that quantification of such MOCA adducts may be useful for the long-term industrial biomonitoring of MOCA exposure and for the evaluation of human DNA–MOCA adduct formation, a lesion thought to be associated with the production of cancer.

The compound 4,4′-methylene-bis(2-chloroaniline) (MOCA; 3,3′-dichloro-4,4′-methylene-dianiline; CAS Registry No. 101-14-4) has been widely used as a curative extender in the production of isocyanate-based polymers and epoxy resins (NIOSH, 1977, 1978). An estimated 1.0–3.5 million pounds of MOCA are imported annually into the United States (Versar, Inc., 1982), and the use of this compound was the subject of a recent review (Ward *et al.*, 1987). MOCA has been reported to produce tumors in rats (Russfield *et al.*, 1975; Stula *et al.*, 1975; Kommineni *et al.*, 1979), mice (Russfield *et al.*, 1975), and dogs (Stula *et al.*, 1977), and, because of structural similarity to arylamine carcinogens, is considered a suspect human carcinogen (Ward *et al.*, 1987). The majority of the chemical carcinogens are thought to act by covalently

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binding, either directly or by production of electrophilically active metabolites, to nucleophilic sites in target cell DNA (Kriek and Westra, 1979; Hemminki *et al.*, 1986; Perera, 1988). Several investigators have reported finding a correlation between such covalent binding and carcinogenic potency (Brooks and Lawley, 1964; Lutz, 1979; Kommineni *et al.*, 1979; Phillips *et al.*, 1984; Gupta *et al.*, 1988). Several traditional biomonitoring techniques have been developed for estimation of exposure to MOCA by determination of urinary MOCA or MOCA metabolite levels (Nieminen *et al.*, 1983; Gristwood *et al.*, 1984; Groth *et al.*, 1984; Thomas and Wilson, 1984; Ducos *et al.*, 1985; Trippel-Shulte *et al.*, 1986; Ward *et al.*, 1986). However, the use of protein or DNA for long-term MOCA biomonitoring of macromolecular adducts of MOCA, a model halogenated aromatic amine chosen to extend previous studies and ongoing worker cohort evaluation, has not been evaluated. The application of such molecular dosimetry would allow a more accurate estimation of the target dose. Recent work on the formation of adducts with hemoglobin (Hb) (Beland *et al.*, 1983; Eyer, 1983; Neumann, 1984; Green *et al.*, 1984; Ringe *et al.*, 1988; Neumann, 1988) or tissue DNA (Gupta *et al.*, 1982; Gupta and Dighe, 1984; Reddy *et al.*, 1984) using other related aromatic amines suggested that the quantification of MOCA macromolecular adducts may be of value in risk assessment by allowing a more accurate estimation of the cumulative target dose.

The objectives of this study were to determine whether rat Hb and tissue DNA macromolecular adducts are formed after oral or dermal administration of MOCA. Such adducts may serve as time-integral indices for the evaluation of exposure and be useful in risk assessment for exposed workers.

METHODS

Chemicals and solutions. The test compound, MOCA, was provided by the Anderson Development Co. (Adrian, MI).[2] The MOCA was recrystallized from methanol–water and dried over anhydrous H_2SO_4 prior to analysis for chemical purity (>99.5%) by high-pressure liquid chromatography (HPLC) as described previously (Groth *et al.*, 1984). [^{14}C]MOCA, having a specific activity of approximately 50 mCi/mmol and a radiochemical purity of greater than 99%, was obtained from Chemsyn Science Laboratories (Lenexa, KS). The radiochemical purity of this compound was verified by HPLC in conjunction with a radioactivity detector prior to use.

A dosing solution was prepared for the oral studies by dissolving appropriate amounts of MOCA and [^{14}C]-MOCA in corn oil to give a final concentration of 56 μmol/ml (sp act 84.8 μCi/ml). A second dosing solution was prepared for dermal application by dissolving MOCA and [^{14}C]MOCA in acetone to give a final concentration of 562 μmol/ml (sp act 212.0 μCi/ml).

Animals and doses. Male, Sprague–Dawley (Crl:CD(S-D)BR outbred), cesarean-derived rats, weighing 51 to 75 g, were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) and were maintained in an AAALAC-accredited facility throughout the course of the study. Laboratory temperatures, 22 to 25°C, and relative humidity, 45 to 50%, as well as a 12-hr light–dark cycle were controlled throughout the course of the studies. Animals were assigned to 13 groups of five rats and provided with NIH-07 rat and mouse diet (Ziegler Brothers, Inc., Gardners, PA), except for an 18-hr period immediately prior to treatment by gavage, and tap water *ad libitum*. For the dermal studies, a 6 × 6-cm area on the back of each rat was clipped 18 hr prior to dosing with an electric clipper equipped with a No. 40 blade. Animals weighing 190–220 g were administered [^{14}C]MOCA at 281 μmol/kg body wt either by gavage at a constant 5-ml volume of dosing solution/kg body wt or as a single 0.5 ml/kg body wt dermal application to a 3.1-cm^2 area. The rats were 45 days old on the day of treatment, and the average administered radioactivity values in the oral and dermal doses were 78 and 206 μCi/rat, respectively. Rats within a specified time group were anesthetized with pentobarbital and euthanized by exsanguination 1, 3, 7, 10, 14, or 29 days after the treatment. Blood was obtained by cardiac puncture, and tissues were immediately frozen in liquid nitrogen and stored at −70°C prior to DNA extraction.

Isolation of lymphocytes. Blood, collected after treatment with MOCA, was transferred immediately to EDTA-containing Vacutainers. A 3-ml aliquot of the whole blood was mixed at room temperature with 5 ml of pH 7.4 phosphate-buffered saline (PBS) and carefully layered onto 3 ml of Histopaque-1077 (Sigma Chemical Co., St. Louis, MO). The lymphocytes were separated by centrifugation at 400*g* for 30 min. The opaque lympho-

[2] Mention of company or product names is not to be considered an endorsement by the National Institute for Occupational Safety and Health.

cyte layer was transferred to a clean centrifuge tube and twice washed with PBS followed by recentrifugation at 250*g* for 10 min. After the washings, the lymphocytes were resuspended in 1 ml of 0.1 M Tris/0.01 M EDTA (pH 9.2) and stored at −70°C prior to DNA extraction.

Isolation of globin and hemin. After removal of the lymphocytes the packed erythrocytes (RBCs) were washed three times with 10 ml of ice-cold PBS and repacked by centrifugation at 400*g* for 10 min. The cells were then lysed by addition of 20 ml of ice-cold distilled water, and the hemolysate was centrifuged at 25,000*g* for 25 min at 4°C to eliminate cellular debris. The concentration of Hb was determined at 540 nm using a Model 554 spectrophotometer (Perkin–Elmer Corp., Norwalk, CT), with crystalline rat Hb (Sigma) as a standard (Rice, 1967). Globin was precipitated using a modification of the procedure of Anson and Miraky (1930). A 10-ml volume of hemolysate was added dropwise at 0°C to 50 ml of 1% HCl in acetone. After standing overnight at 4°C, the globin precipitate was removed by centrifugation at 1000*g* for 10 min and washed three times sequentially with 1% HCl in acetone and acetone to remove any residual heme or unbound chemical. Aliquots of the hemolysate, solvent washes, and soluble hemin were counted separately by liquid scintillation spectrometry (LSC). Globin was dried under a stream of nitrogen and weighed, and the bound ^{14}C was quantified by LSC.

Isolation of DNA. For the determination of DNA covalent binding the frozen tissues were thawed in 2 vol of cold 0.1 M Tris/0.01 M EDTA (pH 7.7) and homogenized for 15 sec using a Polytron Model PCU-1 homogenizer set at 10 and equipped with a PC-10 probe (Kinematica GmbH, Luzern, Switzerland). Homogenates were adjusted to 1.5% with 0.1 vol of 15% sodium dodecyl sulfate and incubated for 30 min at 37°C with 5 mg/ml Pronase E (Sigma). Samples were extracted by shaking for 10 min at 37°C successively with 2 vol of water-saturated phenol: chloroform:isoamyl alcohol (25:24:1), followed by chloroform:isoamyl alcohol (24:1). Aqueous layers were separated by centrifugation at 3500*g* for 10 min and transferred to a clean test tube containing 5 ml of 5 M NaCl. After mixing, 100 ml of cold absolute ethanol was added to precipitate DNA. The crude DNA was redissolved in 10 ml of 0.015 M NaCl:0.0015 M trisodium citrate:0.01 M EDTA buffer. Subsequently 100 μl of heat-denatured RNase was added and the DNA was incubated for 15 min at 37°C. The RNase concentration per sample was 1.5 mg RNase XII-A and 750 units RNase T_1 grade V (Sigma) in 1 M Tris–HCl (pH 7.4). Samples were mixed with 625 μl of 1 M Tris–HCl (pH 8) and extracted sequentially with 15 ml of phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1). DNA was then reprecipitated by the addition of 30 ml of cold ethanol and washed with 20-ml volumes of 70% ethanol, ethanol:acetone (1:1), and ethyl ether. Samples were dried under vacuum and stored at −70°C. For analysis, a portion of the DNA was redissolved at 2 mg/ml in 0.1 M Tris–HCl (pH 7.7) and diluted 1:10 with 1 N NH_4OH. Aliquots of DNA and appropriate calf thymus ultrapure DNA standards (Sigma) were dried overnight at 60°C. The samples were incubated for 30 min at 60°C with 200 μl of a 300 mg/ml 3,5-diaminobenzoic acid and mixed with 3.8 ml of 0.6 N perchloric acid. DNA was quantified (Setaro and Morley, 1976) using a Model RF-540 spectrofluorophotometer (Shimadzu Corp., Kyoto, Japan) set at an excitation wavelength of 405 nm and an emission wavelength of 491 nm. Protein contamination (<1%) was evaluated by the micro method of Bradford (1976) using a Response II UV-VIS spectrophotometer (Ciba Corning Diagnostics Corp., Medfield, MA). RNA contamination of DNA samples (<5%) was evaluated by the procedure of Alexander *et al.* (1985), and covalently bound [^{14}C]MOCA was determined by LSC as described by Martin and Garner (1987).

Subcellular localization of liver MOCA–adducts. In a separate experiment, the levels of [^{14}C]MOCA or MOCA metabolites were determined for the major liver subcellular fractions (nuclear, mitochondrial, microsomal, and 100,000*g* supernatant). Doses of [^{14}C]MOCA (281 μmol/kg body wt by gavage) were administered to five rats. The rats were anesthetized 24 hr after dosing and euthanized by exsanguination. The livers were immediately weighed and homogenized with 3 vol of 0.25 M ice-cold sucrose solution. The subcellular fractions were separated by differential centrifugation (Mazel, 1971) using a Model L5-50*E* preparative ultracentrifuge equipped with an SW-27 rotor (Beckman Instruments Co., Fullerton, CA), and ^{14}C levels were analyzed by LSC. Covalent binding was determined as described by Martin and Garner (1987).

Liquid chromatography. MOCA purity was determined using a Hewlett–Packard Model 1090M HPLC equipped with a Binary DR5 solvent delivery system (Hewlett–Packard, Co., Waldbronn Analytical Division, Waldbronn, FRG). Components were separated on a 25-cm-long × 7.6-mm-i.d. stainless-steel column packed with 5-μm LC-C18-DB silica packing (Supelco, Inc., Bellefonte, PA). The HPLC column temperature was controlled at 40°C for precise separations, and a gradient solvent system programmed from 10% methanol in water to 90% methanol over a 15-min period was used. Components were detected and quantified using a Model 1040M UV diode array detector and a Model 1046A programmable fluorescence detector (Hewlett–Packard, Co.), at 254 nm or ex = 280 nm, em = 391 wavelength settings, respectively. Radioactive components were determined using a Trace II Model 7150 radioactivity monitor (Packard Instruments, Downers Grove, IL). HPLC data were acquired and processed using a HP 9000 Model 310 HPLC ChemStation (Hewlett–Packard).

Liquid scintillation spectrometry. Tissues were solubilized using methanolic 1 N NaOH (Weigel *et al.*, 1978). These solubilized samples were dissolved in 10-ml quantities of ScintiVerse II scintillation medium (Fisher Sci-

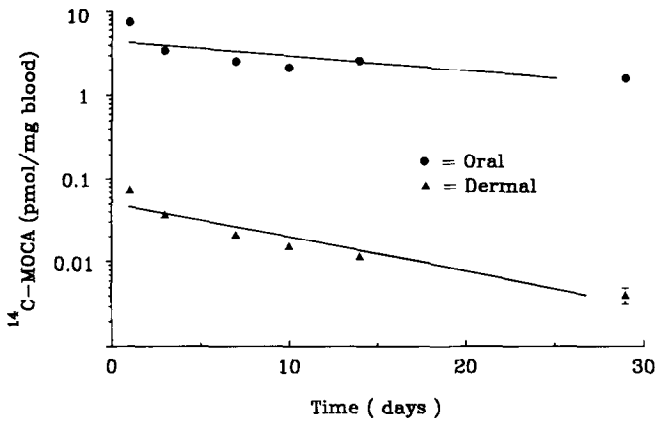

FIG. 1. Comparison of ^{14}C linear regression plots for whole blood of rats euthanized at 1, 3, 7, 10, 14, and 29 days after either oral (●) or dermal (▲) administration of a single 281 μmol/kg dose of 4,4′-methylene-bis(2-chloroaniline) (MOCA) (means ± SE, $n = 5$).

entific Co., Fairlawn, NJ) and ^{14}C levels determined using a Model 8011 liquid scintillation spectrometer (Beckman Instruments Co., Fullerton, CA). Counting efficiencies were calculated using the external standard method of Horrocks (1974).

Statistical analysis. Statistical differences between group means were determined using one-way analysis of variance. Data were processed using the HP 98820A Statistical Library Revision B (Hewlett–Packard, Co., Fort Collins, CO), installed on the HP 9000 Model 310 HPLC ChemStation. A probability level of $p < 0.05$ was considered significant. Linear regression lines were determined and fit through the data for each plot using Sigma-Plot Version 3.1 (Jandel Scientific, Sausalito, CA). Biological half-lives were calculated by the method of Rumack and Lovejoy (1986).

RESULTS

Tissue covalent binding and adduct persistence. The radioactivity present in blood, 24 hr after either a single po injection or dermal application of [^{14}C]MOCA was 7.45 and 0.07 pmol/mg blood, respectively (Fig. 1). These values, which could represent unadducted MOCA or MOCA metabolites, decreased over the 29-day experimental period to 1.58 pmol/mg blood for the oral and 0.01 pmol/mg blood for the dermal exposures. Levels of the radioactivity present in the livers of these animals at 24 hr was 11.30 pmol/mg liver for the orally and 0.10 pmol/mg liver for the dermally exposed rats (Fig. 2). The radioactivity was eliminated more rapidly from the livers than from the blood but was still detectable after 29 days at 0.12 pmol/mg liver for the oral and <0.01 pmol/mg liver after dermal exposure. The covalent binding of [^{14}C]-MOCA to the DNA of liver and urinary bladder, possible target organs for arylamine tumor formation, as well as to the DNA isolated from peripheral lymphocytes was determined. The presence of [^{14}C]MOCA–DNA adducts in the rat liver is indicated by the high activity in the isolated liver DNA in relation to that of the unextracted liver. The bound radioactivity associated with the liver DNA at 24 hr was highest for rats given the compound by the oral route, averaging 49.11 pmol/mg DNA. Because of the amount of tissue required for the isolation of DNA from the bladders, these tissues were combined for the five rats euthanized at each time point. In like manner, the peripheral lymphocytes for each exposure group were combined for DNA extraction. The order of covalent binding determined for these tissues was liver > bladder > lymphocytes (Fig. 3). The adduct formation after dermal MOCA exposure was approximately 100-fold less than that found in the corresponding tissues after oral administration, but the order of binding

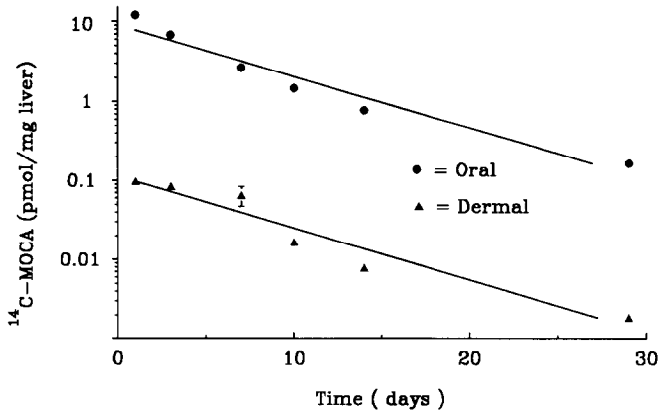

FIG. 2. Comparison of ^{14}C linear regression plots for whole liver of rats euthanized at 1, 3, 7, 10, 14, and 29 days after either oral (●) or dermal (▲) administration of a single 281 μmol/kg dose of 4,4′-methylene-bis(2-chloroaniline) (MOCA) (means ± SE, n = 5).

was the same. For dermally exposed rats, the liver 24-hr covalent binding was 0.38 pmol/mg DNA, and the elimination of bound [^{14}C]MOCA from the liver DNA, comparing *in vivo* oral and dermal values, is shown in Fig. 4. The lower tissue adduct levels present for the dermal [^{14}C]MOCA exposures are explained, in part, by the finding that the major portion of the dermal dose, averaging 86.2% of the radioactivity, remained at the skin application site throughout the study. Thus, less than 14% of the dermal dose could have been available for distribution to the tissues. The [^{14}C]MOCA remaining at the application site was not readily extractable from skin samples with acetone, and attempts to isolate skin DNA for evaluation of binding as described for liver DNA were unsuccessful.

The ^{14}C binding was determined for globin, a potentially useful marker for adduct formation by MOCA. For comparison, the binding associated with two additional fractions from the blood, the hemin and erythrocyte debris, was tested. At 24 hr after the oral

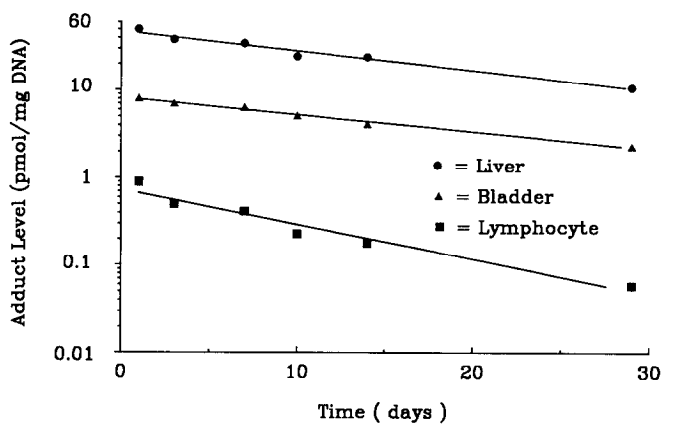

FIG. 3. Comparison of ^{14}C linear regression plots for liver (●), bladder (▲), and lymphocyte (■) DNA covalent binding of rats euthanized at 1, 3, 7, 10, 14, and 29 days after oral administration of a single 281 μmol/kg dose of 4,4′-methylene-bis(2-chloroaniline) (MOCA) (means ± SE, n = 5).

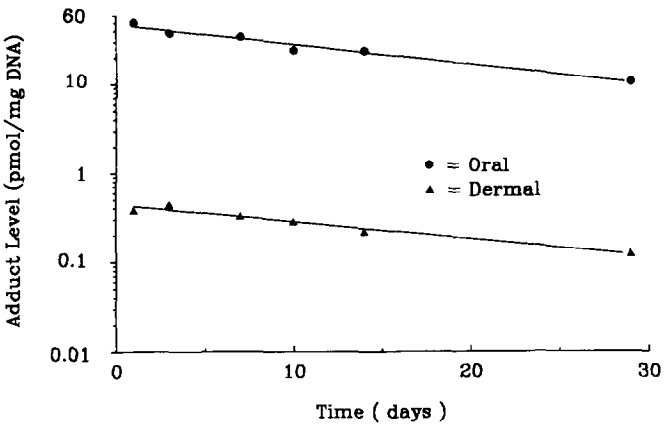

FIG. 4. Comparison of ^{14}C linear regression plots for liver DNA of rats euthanized at 1, 3, 7, 10, 14, and 29 days after either oral (●) or dermal (▲) administration of a single 281 μmol/kg dose of 4,4'-methylene-bis(2-chloroaniline) (MOCA) (means ± SE, $n = 5$).

administration of MOCA, the hemin contained 0.14 pmol of [^{14}C]MOCA/mg hemin, a significantly ($p < 0.05$) lower amount than that found in either the cell debris (6.35 pmol/mg) or the globin (7.84 pmol/mg). The elimination of bound [^{14}C]MOCA from the globin after *in vivo* oral and dermal administration is shown for comparison (Fig. 5). The ratio of the ^{14}C activity present in liver DNA and unprocessed liver increased from 4.3 to 70.8 over the 29-day period examined. However, there was no apparent change in the DNA to globin ^{14}C activity (5.4 ± 0.3) over the same period. The persistence of the MOCA protein and DNA adducts was evaluated by calculation of the biological half-life of the radioactivity present in the tissues and tissue fractions analyzed (Table 1). The biological half-lives for adducted [^{14}C]MOCA in rat globin, liver, and bladder DNA are shown to be similar.

Subcellular distribution and in vivo covalent binding. The subcellular distribution of [^{14}C]MOCA and covalent binding in those fractions was evaluated at 24 hr after oral ad-

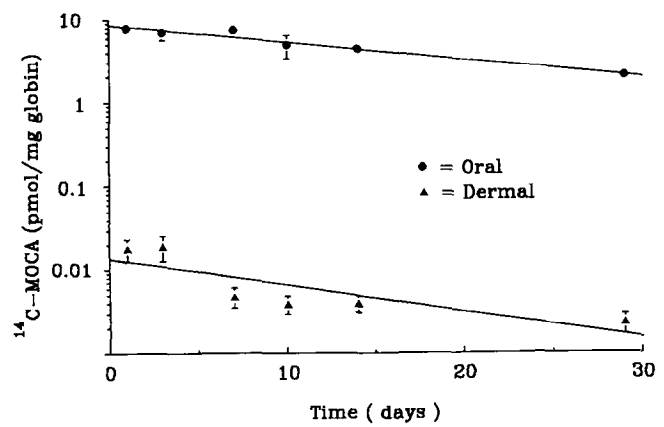

FIG. 5. Comparison of ^{14}C linear regression plots for globin of rats euthanized at 1, 3, 7, 10, 14, and 29 days after either oral (●) or dermal (▲) administration of a single 281 μmol/kg dose of 4,4'-methylene-bis(2-chloroaniline) (MOCA) (means ± SE, $n = 5$).

TABLE 1

BIOLOGICAL HALF-LIVES FOR ^{14}C-LABELED 4,4'-METHYLENE-BIS(2-CHLOROANILINE) (MOCA)

Tissue	Biological half-life (days)[a]	
	Oral administration	Dermal administration
Liver DNA	13.3	15.6
Bladder DNA	13.1	ND
Lymphocyte DNA	7.3	ND
Whole blood	16.7	7.9
Globin	14.3	9.6
Hemin	8.6	ND
Liver	4.4	4.3
Bladder	4.5	4.4
Lymphocyte	4.5	4.6

[a] Comparison of [^{14}C]MOCA biological half-lives calculated for tissues taken from rats euthanized at 1, 3, 7, 10, 14, and 29 days after either oral or dermal administration of a single 281 μmol/kg dose of MOCA. Biological half-lives were calculated by the method of Rumack and Lovejoy (1986).

ministration of a single 281 μmol/kg dose of MOCA. The radioactivity present in the nuclear, mitochondrial, microsomal, and 100,000*g* supernatant fractions was 169.24, 89.49, 244.45, and 282.64 pmol/mg protein, respectively. After precipitation and extraction, the amounts of covalently bound [^{14}C]-MOCA present in the nuclear, mitochondrial, microsomal, and 100,000*g* fractions was 71.47, 19.10, 238.65, and 188.68 pmol/mg protein, respectively (Fig. 6).

DISCUSSION

MOCA is a symmetrically substituted aromatic amine which is structurally similar to other compounds shown to cause cancer in laboratory animals (Messerly *et al.*, 1987). Percutaneous absorption of MOCA in rats (Groth *et al.*, 1984), dogs (Manis *et al.*, 1984), and cultures of human skin (Chin *et al.*, 1983) has been demonstrated, and the skin is considered to be a major route of exposure in workers (Linch *et al.*, 1971). The results of the current study indicate that exposure of rats to MOCA at 281 μmol/kg body wt by both oral and dermal routes resulted in significant covalent binding with blood protein and tissue DNA. Previously, MOCA has been shown to produce gene mutations in the Ames test using *Salmonella typhimurium* strain TA100 with rat liver S9 mix (Hesbert *et al.*, 1985). Mori *et al.* (1988) who used rat hepatocytes to evaluate DNA repair detected genotoxicity. Additionally, recent experiments conducted to detect the formation of MOCA–DNA adducts *in vitro* by rat liver slices (Silk *et al.*, 1986) and both dog and human bladder cells (Shivapurkar *et al.*, 1987) were reported to be positive. Shivapurkar and his co-workers incubated bladder explant cultures with MOCA at concentrations up to 10 μM for 24 hr. The resulting DNA adduct formation ranged from 2 to 58 pmol MOCA/mg DNA for the dog cells and 3 to 61 pmol MOCA/mg DNA for the human cells.

In the present study the 24-hr *in vivo* MOCA–DNA adduct formation was highest after oral administration of the compound, ranging from a high of 49.11 pmol MOCA/mg DNA for the liver to 0.89 pmol MOCA/mg DNA for the peripheral lymphocytes. The corresponding 24-hr globin–adduct level was 7.84 pmol MOCA/mg protein after oral dosing. Detectable adduct levels in both DNA and globin were noted after 29 days, but half-lives were shorter than that estimated for the rat erythrocyte (Neumann, 1988). The levels of MOCA–DNA adduct formation after the dermal exposures were lower than after the oral administration, ranging from 0.38 pmol MOCA/mg for the liver DNA to undetectable levels for DNA isolated from the urinary bladder or peripheral lymphocytes. The 24-hr globin–adduct level was 0.02 pmol MOCA/mg protein after dermal dosing. Dermal absorption of MOCA by human skin *in vitro* has been previously demonstrated (Chin *et al.*, 1983). However, the low levels of systemic DNA and protein adducts formed in the current studies after dermal exposure were not surprising since the major portion

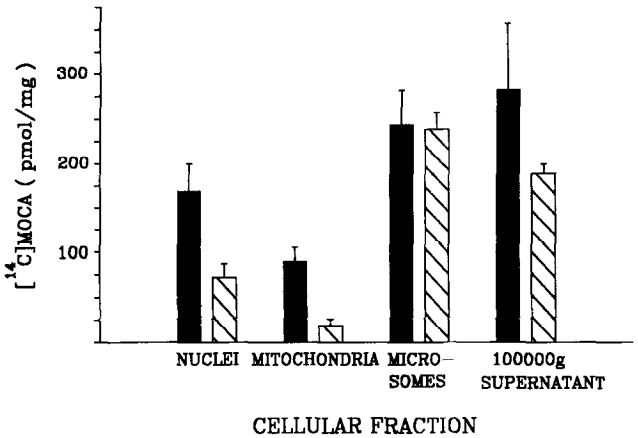

FIG. 6. Subcellular distribution of [^{14}C]MOCA and covalent binding in rat liver of rats euthanized 24 hr after oral administration of a single 281 μmol/kg dose of 4,4′-methylene-bis(2-chloroaniline) (MOCA) (means ± SE, n = 5). The solid bars show the distribution of [^{14}C]MOCA as picomoles per milligram protein prior to precipitation, and the broken bars show covalent binding after precipitation and extraction.

of the administered MOCA, 86.2%, remained at the application site. Such findings are consistent with previous low (1.9 to 2.5%) *in vivo* MOCA dermal absorption reported for rats (Groth *et al.*, 1984) and dogs (Manis *et al.*, 1984). The subcellular distribution of radiolabeled MOCA in rat liver, a target organ for tumor formation, was evaluated only at 1 hr after iv administration of MOCA by Tobes *et al.* (1983). Those investigators reported 28.2% in the nuclear fraction, 19.6% in the mitochondrial fraction, 20.1% in the microsomal fraction, and 31.6% in the 100,000*g* supernatant. Results of the current study show a similar distribution of radioactivity at 24 hr after administration of MOCA by gavage. Additionally, the majority of the radioactivity present in the microsomal and 100,000*g* supernatant fractions was covalently bound.

The metabolism and elimination of MOCA has been studied extensively (Glowinski *et al.*, 1978; Farmer *et al.*, 1981; Tobes *et al.*, 1983; Groth *et al.*, 1984; Manis and Braselton, 1984; Manis *et al.*, 1984; Yoneyama and Matsumura, 1984; Hesbert *et al.*, 1985; Morton *et al.*, 1988; Butler *et al.*, 1989), and various metabolic pathways, including *N*-acetylation, *N*-oxidation and hydroxylation, and ring oxidation have been demonstrated. Possible covalent binding of MOCA was suggested by Farmer *et al.* (1981). Subsequently, Hesbert *et al.* (1985) compared the mutagenic activity of MOCA with that of the *N*-acetyl and *N,N*-diacetyl metabolites and suggested that *N*-acetylation does not account for the mutagenic effectiveness of MOCA *in vitro*. Silk *et al.* (1986) later reported that, unlike benzidine, which requires *N*-acetylation for DNA binding in the rat, the major MOCA–DNA adduct found in rat liver cells was apparently related to formation of the *N*-hydroxy derivative of MOCA. Those investigators reported that the *in vitro* reaction of *N*-hydroxy MOCA with DNA resulted in formation of an identical DNA nucleoside adduct consisting of a single aromatic nucleus attached to a deoxyadenosine residue via the C8 position. The long-term stability of such MOCA adducts has been demonstrated in the current study and, significantly, globin–MOCA adducts have similar biological half-lives in the rat. However, the elimination of unbound MOCA and MOCA metabolites from that species is nearly complete within a 48-hr period (Farmer *et al.*, 1981; Tobes *et al.*, 1983; Groth *et al.*, 1984).

In summary, the results of this study show that significant macromolecular adduct formation occurs in various tissues after either oral or dermal administration of MOCA. In the rat globin and liver DNA biological half-

lives for adducted [^{14}C]MOCA were similar. These results suggest that parallel studies be conducted with human tissues to determine whether quantification of the globin–MOCA adducts may provide a valuable tool for determining industrial exposure.

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