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Metabolism of Trichloroethylene in Isolated Hepatocytes, Microsomes, and Reconstituted Enzyme Systems Containing Cytochrome P-450[1]

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ABSTRACT

The metabolism of the suspected carcinogen trichloroethylene (TCE) was studied in *in vitro* systems involving purified rat liver cytochrome P-450; rat, human, and mouse liver microsomes; rat lung microsomes; and isolated rat and mouse hepatocytes. The studies support the view that metabolism of TCE proceeds through formation of a complex with oxygenated cytochrome P-450 which, by rearrangement, can lead to: (*a*) suicidal heme destruction; (*b*) formation of chloral, which can be either reduced to trichloroethanol and conjugated to form a glucuronide or oxidized to trichloroacetic acid; (*c*) formation of TCE oxide, which decomposes to carbon monoxide and glyoxylate; and (*d*) metabolites which bind irreversibly to protein, DNA, and RNA. Studies with microsomes and reconstituted enzyme systems suggest that the contributions of the four major pathways described above vary depending upon the isozymes of cytochrome P-450 involved and that these pathways cannot be strictly correlated. Conjugation of products with glutathione does not appear to play a major role in TCE metabolism. Treatment of rats and mice with phenobarbital resulted in a number of alterations in metabolism which were more pronounced in the isolated hepatocyte system than in fortified microsomal incubations. In several cases where hepatocytes were used, the bulk of the metabolites which became irreversibly bound to DNA and protein could be trapped outside of the cells by including such macromolecules in the system, implying that metabolites which bind irreversibly must possess a reasonable degree of chemical stability. The results suggest that TCE oxide is not the TCE metabolite responsible for irreversible binding to protein and DNA. The levels of protein adducts and particularly DNA adducts formed were substantially higher in isolated C57BL/6 × C3H F_1 mouse hepatocytes than in isolated Osborne-Mendel rat hepatocytes, and these results may help to explain species differences previously reported in carcinogen bioassays.

INTRODUCTION

TCE[4] is an unsaturated, chlorinated hydrocarbon used as an industrial solvent. In selected areas of the United States, TCE is also a relatively common water pollutant. When TCE was administered by gavage in carcinogen bioassay tests, no increased incidence of hepatocellular carcinoma was observed in Osborne-Mendel rats, but sex-related differences in the extent of tumor formation were noted in C57BL/6 × C3H F_1 (hereafter called B6C3F_1) mice (24). In an 18-month chronic inhalation study conducted by Henschler *et al.* (11), TCE failed to increase the incidence of hepatocellular carcinoma in mice, rats, and hamsters. Other studies have shown increases in hepatocellular tumors in B6C3F_1 mice after administration of TCE by inhalation, while tumor increases were not observed in several strains of rats after TCE was given by gavage (18).[5] Some of the bioassays utilized TCE which also contained epichlorohydrin, a direct-acting carcinogen. However, the species and tissue specificity of epichlorohydrin is known to differ from that reported for TCE (15, 33).

The major *in vivo* metabolites of TCE are trichloroethanol (both conjugated with glucuronic acid and unconjugated), trichloroacetic acid, and chloral (3, 14). Chloral is the primary metabolite of TCE found in liver microsomal systems (16). Minor microsomal metabolites include CO (22, 30), glyoxylic acid (22), and TCE oxide (22). TCE is metabolized to reactive intermediates which bind irreversibly to nucleic acids (32) and proteins (1), and TCE oxide has been postulated to be the metabolite responsible for this binding. We have previously presented evidence that TCE is not metabolized to chloral via an epoxide intermediate (22). Instead, we have proposed that chlorine migration occurs within an oxygenated TCE-P-450 intermediate to form chloral. A proposed scheme of TCE metabolism incorporating this intermediate is shown in Chart 1.

We examined the metabolism of TCE in isolated hepatocytes and liver microsomes prepared from both B6C3F_1 mice and Osborne-Mendel rats and in reconstituted rat liver P-450 systems. We were particularly interested in the levels of chloral, TCE oxide, and covalent adducts formed with both proteins and nucleic acids in these systems. The goals of this study were the determination of metabolite profiles for TCE in mouse and rat systems in order to rationalize the apparent differences in tumorigenicity of TCE observed in mice and rats, the determination of the roles of 8 rat P-450 isozymes in TCE metabolism, and evaluation of the ability of human liver microsomes to metabolize TCE.

MATERIALS AND METHODS

Chemicals. [1,2-^{14}C]TCE (9.0 mCi/mmol) was purchased from New England Nuclear, Boston, Mass., and radiochemical purity was judged to be >99% as determined by counting fractions recovered from GC using a Tenax column at 160° and 220°.

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[4] The abbreviations used are: TCE, 1,1,2-trichloroethylene; P-450, microsomal cytochrome P-450; GC, gas chromatography; GSH, reduced glutathione; TLC, thin-layer chromatography; PB, phenobarbital; βNF, β-naphthoflavone (5,6-benzoflavone). The rationale for the nomenclature of the individual forms of P-450 is described in the text and elsewhere (7).

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[5] Z. G. Bell, K. J. Olsen, and T. J. Benya. Final report of audit findings of Manufacturing Chemists Association (MCA) administered trichloroethylene chronic inhalation study at Industrial Bio-Test Laboratories, November, 1978.

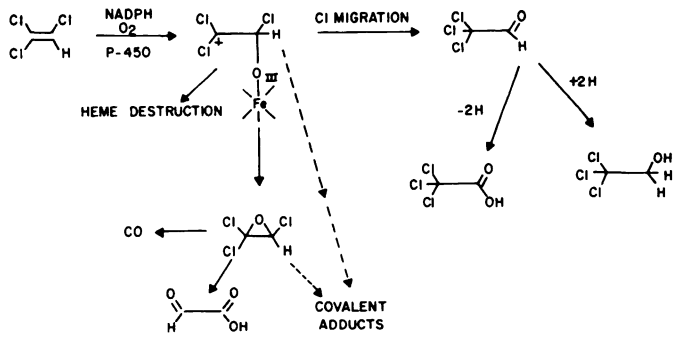

Chart 1. Postulated pathways for metabolism of TCE.

TCE, tetrachloroethylene, 2,2,2-trichloroethanol, and *p*-nitrobenzylpyridine were purchased from Aldrich Chemical Co., Milwaukee, Wis. Chloral hydrate was obtained from Mallinckrodt Chemical Works, Inc., St. Louis, Mo. Calf thymus DNA was purchased from Calbiochem-Behring Corp., La Jolla, Calif. Hanks' balanced salt solution (without Ca^{2+}, Mg^{2+}, phenol red, and sodium bicarbonate), Joklik-modified minimal essential medium, and horse serum (heat inactivated) were obtained from Grand Island Biological Co., Grand Island, N. Y. Collagenase (type I; Lot 41A275) was purchased from Worthington Biochemical Corp., Freehold, N. J. β-Glucuronidase-arylsulfatase was purchased from Boehringer-Mannheim, Indianapolis, Ind. Protease VI and rat GSH *S*-transferase were purchased from Sigma Chemical Co., St. Louis, Mo. Residual GSH was removed from GSH *S*-transferase by gel filtration chromatography. TLC plates were purchased from Whatman, Inc., Clifton, N. J.

Enzyme Preparation. Male Osborne-Mendel rats (200 to 250 g) were obtained from Camm Research, Wayne, N. J. Male $B6C3F_1$ mice (20 to 25 g) were obtained from Harlan Industries, Indianapolis, Ind. Rats were treated with PB or βNF as described elsewhere (8), while mice were treated with PB in drinking water using the following dosing regimen: 0.02% (w/v) for 4 days; 0.03% for 2 days; and 0.04% for 2 days. Mice were treated with βNF in the same manner as were rats (8). Liver microsomes were prepared by differential centrifugation (6) and stored at −70°. The rat liver P-450 isozymes were purified, and antibodies to these P-450 isozymes were prepared as described elsewhere (7). Human livers 17s (24-year-old male), 21s (65-year-old male), and 23 (17-year-old female) were obtained within 1 hr of death from organ donor patients. Human liver 19 (63-year-old female) was an autopsy sample obtained 18 hr after death.

Preparation and Incubation of Hepatocytes. Rat hepatocytes were prepared by the collagenase perfusion technique of Moldeus *et al.* (23). Hepatocytes were isolated from $B6C3F_1$ mice by retrograde perfusion with collagenase solution after cannulating the inferior vena cava (19). The hepatocytes were washed 3 times with the incubation medium before the viability was determined by trypan blue exclusion. Only preparations with an initial viability >90% were used. The incubation medium was Joklik medium containing 2.5% (v/v) horse serum. In some experiments where the DNA-containing medium was used to determine extracellular covalently bound metabolites, calf thymus DNA was first dissolved in the incubation medium and then added to hepatocytes to give a final concentration of 0.25 mg/ml. The hepatocytes were preincubated for 30 min at 37° before addition of TCE and then incubated in stoppered 50- and 100-ml Erlenmeyer flasks at 37° with shaking. At the end of incubations, the hepatocytes were separated from the medium by centrifugation (175 × *g* for 2 min). The method of Irving and Veazey (12) was used to isolate nucleic acids from hepatocytes. Intracellular and extracellular protein adducts and extracellular DNA adducts were determined as described elsewhere (6, 13). Extracellular DNA was further purified by treatment with protease VI (1 mg/ml) for 60 min at 37°. No DNA was added to incubations in which only noncovalently bound metabolites were determined. These incubations were carried out in 20-ml glass scintillation vials with polyethylene-lined caps and terminated by addition of $HClO_4$ to a final concentration of 5% (w/v). Following centrifugation, each supernatant was extracted with diethyl ether, concentrated under a nitrogen stream, and analyzed by GC.

Assays. P-450 concentrations were determined by difference spectroscopy (20). An aliquot of hepatocytes was centrifuged (175 × *g* for 2 min), and the supernatant was discarded. Two to 4 volumes of solubilizing buffer [0.1 M potassium phosphate buffer (pH 7.25) containing 1 mM EDTA, 40% (v/v) glycerol, 0.5% (w/v) sodium cholate, and 0.4% (w/v) Renex 690] were added to the cellular pellet. After mixing, the sample was maintained at 23° for 15 to 20 min before P-450 was assayed. Protein concentrations were determined by the method of Lowry *et al.* (17) unless indicated otherwise. Total hepatocyte protein was determined by a biuret assay (5) after the hepatocytes were solubilized by heating for 1 hr at 60° in 10% (w/v) NaOH containing 0.4% (w/v) cholic acid. GSH was quantified using 5,5′-dithiobis-2,2′-nitrobenzoic acid (26). Cellular suspensions were centrifuged, and the supernatant containing the medium was discarded. The pelleted cells were placed in 5% (w/v) trichloroacetic acid, mixed, and recentrifuged to remove cellular protein. An aliquot of the supernatant was assayed for GSH. The formation of GSH adducts was assayed using either LK5D silica TLC (70% *n*-propyl alcohol-30% NH_4OH, v/v) or K2 cellulose TLC (upper phase of 45% *n*-butyl alcohol-45% H_2O-10% CH_3CO_2H, v/v/v). Hepatocyte incubations were stopped by adding trichloroacetic acid to a final concentration of 5% (w/v) and were centrifuged to remove precipitated protein. An aliquot (25 or 50 μl) of the supernatant was applied to a lane of a TLC plate and developed in the described buffer systems. Standard samples of GSH were chromatographed in adjacent channels and were visualized with either 5,5′-dithiobis-2,2′-nitrobenzoic acid or ninhydrin. The TLC plates were divided into 0.5-cm zones, and radioactivity in each was determined using liquid scintillation counting.

All incubations were carried out under conditions in which product formation was linear throughout the incubation period. Incubations involving mixed-function oxidative activities of microsomes or purified P-450 isozymes included 0.1 M potassium phosphate buffer (pH 7.7) and a NADPH-generating system composed of 10 mM glucose 6-phosphate, 1.0 mg IU yeast glucose-6-phosphate dehydrogenase per ml, and 0.5 mM NADPH. TCE was dissolved in acetone and added to each vial to give the indicated final concentration [final concentration of acetone, ≤1% (v/v)]. Incubations that were carried out to quantify soluble products were stopped by the addition of $ZnSO_4$ (10%, w/v) to 30 mM, centrifuged at 2000 × *g* for 10 min to precipitate protein, acidified with 12 N H_2SO_4, and extracted with ether. Microsomal metabolites bound irreversibly to DNA were determined by either the method of Kadlubar *et al.* (13) or a modification of the procedure: the microsomal incubations (1.0 ml) were terminated by adding sodium dodecyl sulfate to 1% (w/v); 9 ml of 50 mM Tris-HCl buffer (pH 7.7) were added; and the samples were centrifuged at 10^5 × *g* and 25° for 18 hr (4). The precipitated DNA was dissolved in 2 ml of 1.5 mM sodium citrate buffer (pH 6.5) containing 15 mM NaCl, extracted with phenol containing 0.3% (w/v) 8-hydroxyquinoline, and reprecipitated with ethanol. The DNA was redissolved in the sodium citrate-NaCl buffer and incubated with 1 mg of protease VI for 1 hr at 30°. The DNA was then isolated by the method of Kadlubar *et al.* (13). Metabolites irreversibly bound to protein were determined by the method of Wallin *et al.* (34). Filter paper discs were washed 5 to 8 times in ethanol, twice in methanol, and twice in acetone. Each wash was for 30 min with gentle shaking in 20 ml of solvent. After the methanol wash, the samples remained in methanol overnight before washing with acetone. The filter paper discs were dried, and the radioactivity was determined using ACS scintillation cocktail (Amersham, Arlington Heights, Ill.).

Chloral, trichloroethanol, and trichloroacetic acid were quantified using a modification of a method described elsewhere (22). The GC oven temperature was programmed in a linear manner from 150° to 165° at a rate of 3°/min. Glyoxylic acid and CO were quantified as

described elsewhere (22). The glucuronide conjugate of trichloroethanol was quantified by GC following hydrolysis of each sample with β-glucuronidase-arylsulfatase. One-half of the $HClO_4$-terminated incubation mixture was neutralized with 1 N KOH, and 1 ml of 0.2 M sodium acetate (pH 5.0) was added before incubation with 0.1 ml of β-glucuronidase-arylsulfatase for 24 hr at 37°. Following enzymatic hydrolysis, trichloroethanol was extracted into ethyl ether for GC analysis.

CO_2 production was quantified by incubating hepatocytes and [1,2-^{14}C]TCE in 50-ml sealed Erlenmeyer flasks with center wells containing 2 N KOH and filter paper wicks. The incubations were terminated at various times by addition of 1 ml of 20% (v/v) $HClO_4$. The samples were incubated for an additional 60 min to ensure quantitative trapping of evolved CO_2. The filter paper wick and an aliquot of the KOH were added to 10 ml of scintillation cocktail, and radioactivity was determined.

Enzymatic TCE oxide formation was determined as described elsewhere (22).

RESULTS

Metabolism of TCE to Free Metabolites in Hepatocytes. Isolated rat hepatocytes maintained ≥75% viability for at least 6 hr at TCE concentrations up to 5 mM (Chart 2). Control incubations maintained >90% viability for up to 6 hr. Hepatocyte viability decreased rapidly at TCE concentrations >5 mM, and at a level of 25 mM TCE none of the cells were viable after 15 min. All subsequent incubations were carried out with either 0.1 or 1 mM TCE to ensure >80% viability throughout incubations.

TCE, when added at concentrations which did not decrease cellular viability, did not cause a decrease in GSH levels in the cells (Chart 3). Only at TCE concentrations that caused a rapid loss of viability was a decrease in GSH levels observed. A GSH conjugate of TCE was tentatively identified by TLC. This putative TCE-GSH conjugate represented approximately 1% of the total TCE metabolites formed (data not shown). When equimolar concentrations (1 mM) of TCE oxide and GSH were incubated together at 37° in 0.1 M potassium phosphate buffer

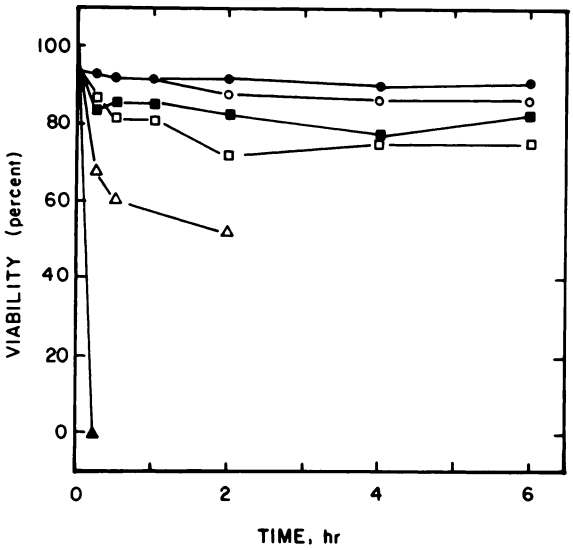

Chart 2. Effect of substrate concentration on hepatocyte viability. The hepatocytes were preincubated for 30 min at 37° before varying amounts of TCE dissolved in acetone were added: ●, control; ○, 0.1 mM TCE; ■, 1 mM TCE; □, 5 mM TCE; △, 10 mM TCE; ▲, 25 mM TCE. At selected time points, 100-μl aliquots were taken, and 25 μl of a 1.1% solution of trypan blue dye in 0.9% (w/v) NaCl were added. Cells excluding the dye were considered to be viable.

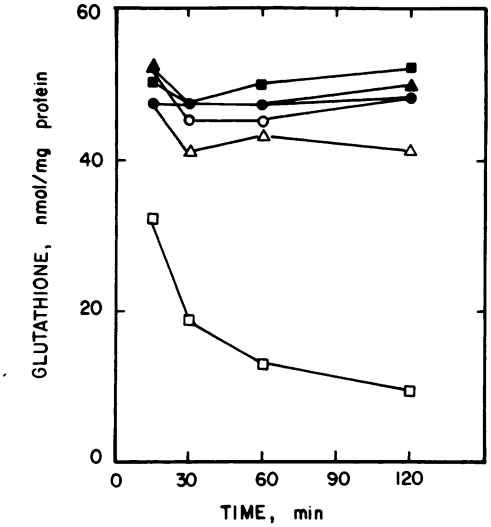

Chart 3. Effect of TCE concentrations on GSH levels. Aliquots (0.4 ml) of each incubate were taken at various times and analyzed for GSH content as described in "Materials and Methods." TCE concentrations used were: ●, 0 mM; ■, 0.1 mM; ▲, 1 mM; ○, 5 mM; △, 10 mM; □, 25 mM.

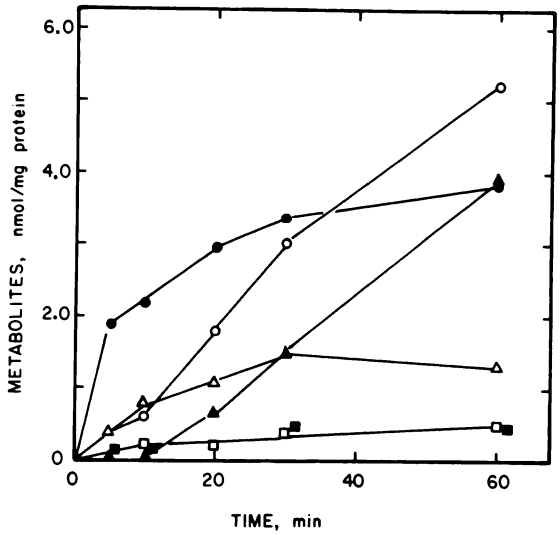

Chart 4. Kinetic course of TCE metabolite formation in isolated rat hepatocytes. Each incubate contained 3 ml of hepatocytes and 1 mM TCE. The samples were terminated at various times and analyzed as described in "Materials and Methods." Unconjugated trichloroethanol (△) was determined by sample analysis prior to enzymatic hydrolysis of the glucuronide conjugate. Total trichloroethanol content (○) was determined following enzymatic hydrolysis of the conjugate. Conjugated trichloroethanol (▲) was quantitated by determining the difference between total trichloroethanol and unconjugated trichloroethanol. Other metabolites which were quantified included chloral (●), trichloroacetic acid (□), and CO_2 (■). Results are expressed as means of duplicate determinations.

(pH 7.7) for 5 min, only 5% of the GSH was lost. In the presence of liver cytosol isolated from PB-treated rats, the epoxide caused a 28% decrease in GSH levels (data not shown). Thus, low levels of a TCE-GSH conjugate may be formed as a result of the reaction between TCE oxide and GSH.

The kinetic course of metabolite formation is shown in Chart 4. Hepatocytes prepared from one rat were used to determine the formation of chloral, trichloroethanol (conjugated and unconjugated), and trichloroacetic acid. Separate incubations were carried out to determine the formation of CO_2 and glyoxylic acid. The kinetics of TCE metabolite formation is consonant

with the postulated scheme of TCE metabolism (Chart 1). Initially, the formation of chloral was quite rapid but appeared to reach a plateau after about 30 min. The formation of trichloroethanol resembled the course of chloral formation in that trichloroethanol resembled the course of chloral formation in that trichloroethanol formation reached a plateau after 20 to 30 min. A brief lag was noted in the appearance of conjugated trichloroethanol, consistent with the metabolic scheme postulated. CO_2 and trichloroacetic acid were formed in nearly equivalent amounts while glyoxylic acid was barely detectable after 60 min (0.02 nmol/mg). Formic acid and CO could not be detected in these incubations.

Metabolism of TCE to Covalently Bound Metabolites in Hepatocytes. Experiments were carried out to determine the effect of pretreatment with either PB or βNF on TCE metabolism by both isolated rat and mouse hepatocytes. In addition, these experiments were designed to assess the ability of reactive metabolites to migrate out of hepatocytes and bind irreversibly to DNA and protein. Treatment of animals with PB resulted in elevated levels of P-450 in both rat and mouse hepatocytes (Table 1). The specific content of P-450 in mouse hepatocytes was less than in the corresponding rat hepatocytes, both before and after PB treatment. The rates of chloral and trichloroethanol formation were elevated in both types of hepatocytes, but the level of induction was less in the mouse hepatocytes than in the rat hepatocytes. Treatment of the rats with βNF did not result in an increase in TCE metabolism as judged by chloral and trichloroethanol formation (data not shown).

The data presented in Table 1 provide strong evidence that an active metabolite of TCE is stable enough to migrate through the plasma membrane and bind irreversibly to both protein and DNA. A large increase in binding to both protein and nucleic acids in isolated rat hepatocytes was detected following treatment of the rats with PB.

The levels of covalent adducts formed in mouse hepatocyte systems were severalfold higher than those formed in rat hepatocyte systems, when untreated animals were used as sources. The greatest difference was in the formation of nucleic acid adducts where extracellular DNA binding was 50-fold greater and RNA binding was 6-fold greater in mouse hepatocytes than in rat hepatocytes. Protein binding increased only 3- to 4-fold over that observed in rat hepatocyte systems. Treatment of the mice with PB increased the P-450 content of the cells but did not affect the levels of covalent adducts formed.

Metabolism of TCE in Liver Microsomal Systems. Liver microsomes prepared from control, βNF-treated, and PB-treated $B6C3F_1$ mice and Osborne-Mendel rats were used to examine the effects of these agents on the enzymes involved in the metabolism of TCE to both free and covalently bound metabolites. In both the mouse and the rat microsomal systems, βNF treatment of the animals had little effect on TCE metabolism, while PB treatment increased the rates of metabolism of TCE (Table 2). TCE oxide formation was approximately 4-fold higher in both mice and rats following PB treatment, while covalent binding was increased only 1.2- to 1.7-fold. Chloral formation was increased 3-fold in rats but only 1.4-fold in mice. The point should be stressed, however, that TCE metabolism in mouse microsomes (based on chloral, TCE oxide, and DNA adduct formation) was 2.5- to 3-fold higher than in rat microsomes. Also, the levels of TCE oxide formed were not corre-

Table 1

Effect of PB or βNF treatment of animals on TCE metabolism by isolated hepatocytes

Osborne-Mendel rats and $B6C3F_1$ mice were treated with either PB or βNF as described under "Materials and Methods" prior to hepatocyte preparation. The isolated hepatocytes were suspended in Joklik minimal essential medium, 2.5% (v/v) horse serum, and 25 mM *N*-hydroxyethyl-*N'*-2-piperazineethanesulfonate buffer (pH 7.4). Incubations conducted to determine DNA adducts also contained 0.25 mg calf thymus DNA per ml. The concentration of [1,2-^{14}C]TCE (specific activity, 9.0 mCi/mmol) used for determination of covalent adduct formation was 0.1 mM. These incubations were carried out with shaking for 2 hr at 37°. Chloral and trichloroethanol were quantified using unlabeled TCE at a concentration of 1 mM. Isolated rat hepatocytes were prepared from one animal/treatment group, while mouse hepatocytes were prepared from 4 mice/treatment group, which were pooled for the incubations. Samples were analyzed as described under "Materials and Methods."

	P-450 (nmol/mg protein)	Protein adducts (pmol/mg protein/2 hr)		DNA adducts (pmol/mg DNA/2 hr)		Intracellular RNA adducts (pmol/mg DNA/2 hr)	Chloral (nmol/mg protein/5 min)	Trichloroethanol (nmol/mg protein/5 min)
		Intracellular	Extracellular	Intracellular	Extracellular			
Untreated rats	0.26	65.4 ± 10.5[a] (0.9)[b]	75.2 ± 18.3 (5.4)	<0.2	1.35 ± 0.38 (0.9)	4.00 ± 0.80 (12)	0.46 ± 0.06	0.07 ± 0.01
PB-treated rats	1.22	534 ± 30 (7.5)	163 ± 118 (12)	26.0 ± 4.3 (18)	24.8 ± 9.2 (43)	21.3 ± 5.4 (66)	1.69 ± 0.14	0.25 ± 0.01
Untreated mice	0.19	215 ± 21 (3.0)	184 ± 26 (13)	11.2 ± 4.3 (7.8)	70.5 ± 0.1 (120)	26.3 ± 0.4 (82)	0.70 ± 0.04	0.06 ± 0.01
PB-treated mice	0.49	229 ± 11 (3.2)	128 ± 23 (9.1)	6.95 ± 0.78 (4.9)	61.0 ± 2.3 (110)	8.04 ± 0.28 (25)	1.00 ± 0.20	0.28 ± 0.09

[a] Mean ± S.D. of triplicate determinations.
[b] Numbers in parentheses, total amount of adduct formed per incubation.

Table 2

Metabolism of TCE by human, mouse, and rat liver microsomes

Incubations were carried out at 37° in a total volume of 1.0 ml in the presence of 0.8 mM TCE and 2 mg of both calf thymus DNA and rat liver microsomal protein per ml for 60 min to determine DNA and protein binding. Chloral formation was determined at 37° in 1.0-ml incubations which were carried out for 10 min and contained 1 mM TCE and 2 mg rat liver microsomal protein per ml. Separate 1.0-ml incubations containing 10 mg rat liver microsomal protein per ml and 25 mM TCE were carried out for 45 sec to determine TCE oxide formation. DNA binding, protein binding, chloral formation, and epoxide formation were quantified as described under "Materials and Methods."

Microsomal incubation system	P-450 (nmol/mg protein)	DNA adducts (pmol/mg/hr)	Protein adducts (nmol/mg/hr)	Chloral (nmol/mg/10 min)	TCE oxide (nmol/mg/45 sec)
Untreated rat	0.83	41.7 ± 6.9[a]	6.4 ± 0.3	3.2 ± 0.6	0.2 ± 0
βNF-treated rat	1.49	35.5 ± 11.1	3.9 ± 0.4	3.0 ± 1.1	0.3 ± 0.1
PB-treated rat	2.13	72.8 ± 13.7	8.7 ± 0.7	9.4 ± 1.1	0.8 ± 0.1
Untreated mouse	1.21	102 ± 33	7.2 ± 0.4	11.5 ± 0.8	0.6 ± 0.1
PB-treated mouse	2.19	127 ± 30	9.2 ± 0.7	15.8 ± 0.5	2.6 ± 0.2
βNF-treated mouse	1.66	59.9 ± 6.2	8.5 ± 0.3	11.6 ± 1.2	1.1 ± 0
Human liver 17s	0.41	39.0 ± 2.5	6.6 ± 1.3	8.7 ± 2.6	<0.1
Human liver 19	0.27	21.4 ± 8.7	4.8 ± 0.3	2.9 ± 0.4	<0.1
Human liver 21s	0.41	51.6 ± 12.5	8.5 ± 0.6	3.9 ± 0.3	<0.1
Human liver 23	0.39	33.2 ± 5.0	6.7 ± 0.4	6.9 ± 0.1	<0.1
PB-treated rat lung	0.02	ND[b]	1.0 ± 0.2	0.7 ± 0.1	ND

[a] Mean ± S.D. of duplicate incubations.
[b] ND, not determined.

lated to the amounts of either chloral or DNA or protein binding. Although DNA binding was generally higher in preparations which formed more epoxide, some samples (*i.e.*, human liver) produced substantial levels of DNA-bound metabolites but no detectable TCE oxide. An additional microsomal metabolite of TCE not previously identified was CO_2 (0.5 nmol per mg protein per 30 min).

Microsomes prepared from 4 different human livers were also examined for their ability to metabolize TCE to TCE oxide, chloral, and reactive intermediates capable of binding to DNA and protein. No TCE oxide was detected in any of the human liver microsomal systems. Some variation (approximately 3-fold) was detected in the rates of chloral formation among human liver samples. One sample, from human liver 17s, metabolized TCE to chloral at nearly the same rate as did microsomes isolated from PB-treated rats. More importantly, the levels of DNA and protein adducts formed in the human liver microsomal systems approximated those observed with liver microsomes prepared from untreated rats. Adducts formed by microsomes prepared from human liver 21s approached the levels obtained with microsomes prepared from PB-treated rats.

Anti-P-$450_{PB\text{-}B}$ blocked about two-thirds of the chloral formation in microsomes prepared from PB-treated rats. However, no effect on binding of TCE metabolites to protein or DNA was observed. When the epoxide hydrolase inhibitor 3,3,3-trichloropropylene oxide (0.1 mM) was included in microsomal incubations, formation of chloral and DNA adducts was not significantly affected (data not shown).[6]

[6] In further attempts to determine if TCE oxide plays a role in modification of DNA, we incubated excess TCE oxide (0.1 M) with guanine or adenine (10 mM) for 2 min in 0.1 N NaOH at 23°. High-pressure liquid chromatography of the products using an octadecylsilane column and a CH_3OH-aqueous ammonium phosphate (pH 5.1) gradient showed that in each case at least 5 peaks appeared after the TCE oxide incubation that were not detected with the authentic guanine and adenine, or with TCE oxide alone under these conditions. These peaks were eluted in each case before the parent nucleic acid bases and do not correspond to any *N*- or *O*-acetylated or -ethylated bases, which are all eluted after adenine and guanine. A_{254} measurements indicated that the putative products account for about 1% of the total bases. The significance of these observations, in relationship to enzymatic production of DNA adducts, is under further investigation.

Previous work in this laboratory indicated that rat lung microsomes contain a substantial level of a protein which is immunochemically similar to rat liver P-$450_{PB\text{-}B}$ and P-$450_{PB\text{-}D}$ and which has the same apparent monomeric molecular weight (10). The level of this protein was high in lung microsomes of untreated rats and was not altered when rats were treated with PB or βNF. Thus, the lung protein appears to be P-$450_{PB\text{-}D}$ or P-$450_{PB\text{-}D}$ (7), although we have not verified this hypothesis using other techniques. Since other data indicated that TCE metabolism to chloral and protein and DNA adducts by liver microsomes was enhanced by PB treatment and that purified P-$450_{PB\text{-}B}$ was the most active form of P-450 involved in TCE metabolism (see below), we were led to examine the hypothesis that rat lung P-450 is rather active in TCE metabolism. The data indicated that the lung microsomes were even more active than liver microsomes were in metabolizing TCE, when the data are expressed as nmol of metabolites formed per nmol of P-450.

Metabolism of TCE in Reconstituted P-450 Systems. The ability of 8 different isozymes of P-450 to metabolize TCE to chloral, TCE oxide, and protein adducts was examined in reconstituted systems (Table 3). The objective was to determine which P-450 isozymes are most important in TCE metabolism.

Briefly, liver microsomes isolated from untreated rats contain substantial levels of P-$450_{UT\text{-}A}$, P-$450_{PB\text{-}C}$, and P-$450_{PB/PCN\text{-}E}$ and low levels (≤0.1 nmol/mg protein) of the other P-450 isozymes (7). Treatment of rats with PB induces P-$450_{PB\text{-}B}$ and P-$450_{PB\text{-}D}$ 20- to 100-fold and P-$450_{PB\text{-}C}$ and P-$450_{PB/PCN\text{-}E}$ about 2-fold. Treatment of rats with βNF, 3-methylcholanthrene, or isosafrole induces levels of P-$450_{\beta NF\text{-}B}$ and P-$450_{\beta NF/ISF\text{-}G}$ 20- to 100-fold. P-$450_{UT\text{-}F}$ is refractory to treatment with these classic inducers, and levels of P-$450_{UT\text{-}A}$ are lowered by treatment with all of these agents.

P-$450_{PB\text{-}B}$ was the P-450 isozyme that metabolized TCE to the greatest extent, based on chloral formation. Other P-450 isozymes involved to a significant degree in conversion of TCE to chloral were P-$450_{\beta NF\text{-}B}$ and P-$450_{\beta NF/ISF\text{-}G}$. All of the P-450 isozymes formed similar levels of protein adducts, with the exception of P-$450_{UT\text{-}A}$. Other experiments yielded similar re-

Table 3

Metabolism of TCE in reconstituted P-450 systems

Incubations were carried out at 37° in the presence of 0.33 μM P-450, 0.50 μM NADPH-P-450 reductase, and 37 μM L-α-dilauroylglyceryl-3-phosphorylcholine. The protein binding studies were carried out using a total volume of 150 μl, a substrate concentration of 0.8 mM [1,2-^{14}C]TCE (8.5 mCi/mmol), and an incubation time of 10 min. The formation of chloral was determined with a total incubation volume of 0.75 ml, a substrate concentration of 1 mM TCE, and an incubation time of 3 min. TCE oxide formation was assayed with a total incubation volume of 0.5 ml in the presence of 25 mM TCE with an incubation time of 1 min. Samples were analyzed as described under "Materials and Methods."

P-450 isozyme	Protein binding (pmol/nmol P-450/10 min)	Chloral (nmol/nmol P-450/3 min)	TCE oxide (nmol/nmol P-450/min)
$P\text{-}450_{UT\text{-}A}$	272 ± 23[a]	3.4 ± 0.1	<0.1
$P\text{-}450_{PB\text{-}B}$	406 ± 11	22.5 ± 0.5	0.66 ± 0.06
$P\text{-}450_{\beta NF\text{-}B}$	350 ± 25	9.6 ± 4.3	<0.1
$P\text{-}450_{PB\text{-}C}$	423 ± 40	2.4 ± 0.1	<0.1
$P\text{-}450_{PB\text{-}D}$	515 ± 16	<0.1	<0.1
$P\text{-}450_{PB/PCN\text{-}E}$	503 ± 21	<0.1	<0.1
$P\text{-}450_{UT\text{-}F}$	450 ± 73	<0.1	<0.1
$P\text{-}450_{\beta NF/ISF\text{-}G}$	430 ± 80	16.0 ± 0.7	<0.1

[a] Mean ± S.D. of duplicate incubations.

sults when incubations were carried out for 3 or 60 min. Further, formation of protein adducts by $P\text{-}450_{PB\text{-}B}$ was linear with respect to time to at least 30 min, and both formation of chloral and protein adducts stopped when 5 mM menadione and excess CO were added to incubates and the incubates were maintained at 37° prior to analysis. Only in the reconstituted system containing $P\text{-}450_{PB\text{-}B}$ was TCE oxide detected.

DISCUSSION

Isolated hepatocytes were used to study the metabolism of TCE because cellular systems more closely simulate *in vivo* conditions than do other *in vitro* systems such as microsomes or purified enzymes. These hepatocyte systems complement microsomal and purified enzyme systems in elucidation of the various steps involved in the bioactivation and detoxication of TCE and in the determination of the importance of each. Using the battery of systems, we examined the metabolism of TCE in an attempt to understand the reported difference in tumor susceptibility between mice and rats.

Conditions were used in all hepatocyte incubations to maintain ≥80% cell viability throughout the incubation period. The products detected in the hepatocyte systems were similar to those observed *in vivo*. The kinetic course of metabolite formation (Chart 3) was consistent with the postulated metabolic pathways for TCE (Chart 1). TCE is apparently metabolized to chloral through an oxygenated TCE-P-450 intermediate, and chloral is subsequently either reduced to trichloroethanol or oxidized to trichloroacetic acid by cytosolic dehydrogenases. Trichloroethanol, either conjugated or unconjugated, is the major metabolite in both the *in vitro* hepatocyte system and *in vivo*.

Cellular GSH levels were not reduced appreciably by TCE as long as cell viability was maintained. Further analysis by TLC suggested that a TCE-GSH adduct was formed at very low levels. The apparent lack of involvement of GSH is further substantiated by the observation that no urinary metabolites of TCE, such as mercapturic acids, have been reported that would indicate the formation of GSH adducts. The lack of a role for GSH in TCE metabolism was confirmed using rat liver microsomes and purified GSH *S*-transferase. GSH plays a general protective role in the cell by reacting with electrophilic metabolites to reduce covalent binding to DNA, RNA, and proteins (2, 27). However, GSH had little effect on the level of DNA adducts. The limited role of GSH in TCE metabolism is in direct contrast to the situation with 2 closely related haloalkenes, vinyl chloride and vinylidene chloride, which are metabolized to mercapturic acids and excreted in the urine (21, 35).

Metabolism of TCE, based on chloral formation, was increased in rat hepatocyte (Table 1) and microsomal systems (Table 2) when rats were treated with PB. The most significant effect of PB pretreatment in hepatocyte systems was the nearly 20-fold increase in DNA binding while the amount of P-450, the rate of chloral formation, and the amount of RNA adducts were increased only approximately 4-fold over control levels. In contrast, hepatocytes prepared from rats treated with βNF did not show an increased rate of chloral formation, nor were the levels of nucleic acid adducts increased. Similar results were obtained in microsomal systems, where PB treatment increased the formation of DNA and protein adducts, chloral, and TCE oxide. In contrast, βNF treatment did not induce the formation of any microsomal metabolites. These results would suggest that the forms of P-450 inducible by PB are primarily involved in TCE metabolism while the βNF-inducible forms of P-450 have only a minor role in TCE metabolism. The effects of treatment of rats with PB on metabolism of TCE to irreversibly bound metabolites were considerably more dramatic in isolated hepatocytes than in fortified microsomal systems (Table 1). These discrepancies suggest that factors other than simple induction of P-450 are involved in influencing the pathways of xenobiotic metabolism in hepatocytes, as discussed by Thurman and Kauffman (29). The data indicate that hepatocytes may be more reflective of factors regulating metabolism *in vivo* and also suggest that under certain conditions (*e.g.*, enzyme induction) rats could be considerably more susceptible to carcinogenic effects of TCE.

Similar changes were observed upon treatment of animals with PB in both hepatocytes (Table 1) and liver microsomes (Table 2) prepared from mice. The P-450 content in both hepatocytes and microsomes were increased 2- to 2.5-fold, but total TCE metabolism, as estimated by chloral formation, was increased only 30%. In addition, the formation of DNA and protein adducts was increased only 25% in mouse microsomal systems by PB treatment and not at all in mouse hepatocytes. In contrast, the formation of TCE oxide in microsomes was increased 4-fold by PB treatment and 2-fold by βNF treatment. The presence of the epoxide hydrolase inhibitor 3,3,3-trichloropropylene oxide did not affect the level of DNA adducts formed in microsomal incubations. These results are not consonant with the report of Banerjee and van Duuren (1). We have previously reported that the half-life of TCE oxide is only modestly decreased by concentrations of epoxide hydrolase as high as 3 mg/ml (60 μM) (22). All of these results suggest that the epoxide is probably not involved in TCE binding to either nucleic acids or protein.

In comparing the data obtained from the systems derived from untreated rats and mice, one of the most significant results was the higher level of covalent adducts formed in the mouse systems. Apparently, TCE metabolism occurs in the mouse systems to generate relatively more of the species that form covalent adducts. A recent *in vivo* study using $B6C3F_1$ mice and Osborne-Mendel rats demonstrated that covalent binding of TCE to protein was approximately 3-fold higher in mice than

in rats. (28). The increased protein and DNA binding observed here may be important in explaining the differential susceptibility of mice and rats to tumor formation following TCE exposure.

The high level of extracellular covalently bound adducts indicated that reactive metabolites were formed that were stable enough to migrate through the hepatocyte plasma membrane. In the mouse hepatocytes, 94% of the DNA adducts formed were extracellular, while nearly 80% of the protein adducts were extracellular. This laboratory has previously demonstrated the ability of reactive metabolites of vinyl chloride to leave hepatocytes and to bind to either protein or DNA outside the cell (9). Similar results have been reported with benzo-(*a*)pyrene (27) and *N,N*-dimethylnitrosamine (31). Thus, the possibility exists that a reactive metabolite(s) could be formed in a cell or tissue with high metabolic activity and migrate to a target cell or tissue with low metabolic activity to bind and cause damage.

The metabolism of TCE by the 8 different P-450 isozymes was rather selective (Table 3). TCE was metabolized primarily by P-$450_{PB\text{-}B}$ and P-$450_{\beta NF/ISF\text{-}G}$, using chloral formation as an index of total TCE metabolism. Other isozymes, particularly P-$450_{PB\text{-}D}$, P-$450_{UT\text{-}F}$, and P-$450_{PB/PCN\text{-}E}$, appear to have only minor roles in TCE metabolism. However, all of the isozymes were capable of metabolizing TCE to species which formed protein adducts. No significant correlation existed between chloral formation and the level of protein binding for the P-450 isozymes studied. Further, anti-P-$450_{PB\text{-}B}$ inhibited chloral formation in microsomes prepared from PB-treated rats but did not inhibit formation of protein and DNA adducts. These results are consistent with the view that, in livers of PB-treated rats, P-$450_{PB\text{-}B}$ plays a major role in conversion of TCE to chloral but formation of metabolites which are bound to protein and DNA is a function of several forms of P-450. Only P-$450_{PB\text{-}B}$ produced detectable levels of TCE oxide, suggesting that the epoxide is not involved in protein binding, as all of the isozymes were able to generate covalent protein adducts. Similar conclusions can be drawn from the microsomal systems (Table 2), where TCE oxide levels were induced 4-fold but DNA and protein binding increased only 25%.

In a separate study, the metabolism of vinyl bromide and vinyl chloride to products irreversibly bound to DNA and protein was examined in rat liver microsomes, reconstituted P-450 systems, and isolated hepatocytes (8). This laboratory concluded that 2-haloethylene oxides were the major alkylating agents bound to DNA and 2-haloacetaldehydes were the major alkylating agents bound to protein. However, the aldehyde under consideration here (chloral) is probably not involved in protein binding because 3 of the P-450 isozymes did not metabolize TCE to chloral but did form TCE protein adducts.

We previously concluded that TCE is metabolized via an oxygenated TCE-P-450 intermediate where chlorine migration occurs to form chloral (22). The results obtained with the reconstituted P-450 systems suggest that the ability to catalyze chlorine migration is selectively carried out by certain P-450 isozymes. Apparently, P-$450_{PB\text{-}B}$ and P-$450_{\beta NF/ISF\text{-}G}$ are most proficient, while P-$450_{PB\text{-}D}$, P-$450_{PB/PCN\text{-}E}$, and P-$450_{UT\text{-}F}$ are much less proficient in catalyzing chlorine migration. Furthermore, these results indicate that the production of electrophiles does not vary much for the different P-450 isozymes. The nature of these electrophiles is unknown, and further work will be required to elucidate the structures of these compounds.

In summary, the scheme presented in Chart 1 is reviewed in light of the evidence available. With the postulated iron-oxygen-TCE intermediate (22, 25), one can rationalize the known metabolites of TCE. (*a*) Suidical P-450 heme destruction occurs with TCE to the extent of 40% of the heme in a microsomal system in 30 min at 37°. The heme destruction was determined by TLC analysis of radioactive heme remaining in liver microsomes prepared from rats in which P-450 was labeled by injection of δ-[4-^{14}C]aminolevulinic acid. The destruction probably occurs by attack of pyrrolic nitrogen of the heme on the intermediate, as demonstrated by Ortiz de Montellano *et al.* (25) for other olefins. (*b*) Transfer of chlorine to the adjacent carbon, accompanied by movement of the electrons of the Fe—O bond to the C—O bond, results in chloral formation. Apparently, factors which determine the kinetics of chlorine migration are specific to individual P-450 isozymes. (*c*) Trichloroethanol results from pyridine nucleotide-dependent reduction of chloral by alcohol dehydrogenases or related enzymes. The alcohol is conjugated with glucuronic acid through the action of UDP-glucuronyltransferases. (*d*) Chloral is oxidized by aldehyde dehydrogenases to form trichloroacetic acid. (*e*) TCE oxide is formed by transfer of electrons from the Fe—O bond (of the enzyme intermediate) to the carbonium ion. We have presented evidence elsewhere that TCE oxide is neither chemically nor catalytically competent as an intermediate in the formation of chloral from TCE (22). (*f*) CO can be formed from TCE oxide (22) or possibly from bond scission within the oxygenated enzyme intermediate. Formyl chloride is also a possible intermediate in the formation of CO. While CO can be observed bound to ferrous P-450 during the microsomal metabolism of TCE (data not shown), the level is not sufficient to cause the kinetic course of metabolism to become nonlinear. (*g*) Glyoxylate can be detected in *in vitro* incubations with rat liver microsomes, reconstituted P-450 systems, or hepatocytes. This compound can be formed during decomposition of TCE oxide (22) or may result from hydration of the oxygenated enzyme-TCE intermediate. (*h*) CO_2 was detected as a minor metabolite in rat liver microsomes, suggesting that phosgene may be a TCE metabolite (possibly involved in irreversible binding), inasmuch as phosgene undergoes hydrolysis to form CO_2. (*i*) The protein and DNA adducts have not yet been characterized. Several lines of evidence (see above) suggest that TCE oxide is not the major compound involved in formation of these adducts. The hydrolysis and rearrangement of the oxygenated enzyme-TCE complex probably produce unstable intermediates which attack nucleophilic sites in proteins and nucleic acids. These intermediates must possess sufficient stability to migrate for considerable distances. Moreover, these intermediates must differ from those involved in the decomposition of TCE oxide, which give rise to CO and glyoxylate. Elucidation of the nature of these intermediates should lead to a better understanding of the chemical mechanism and the risk potential of haloalkenes and will be the subject of further investigation.

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REFERENCES

1. Banerjee, S., and Van Duuren, B. L. Covalent binding of the carcinogen trichloroethylene to hepatic microsomal proteins and to exogenous DNA *in vitro*. Cancer Res., *38:* 776–780, 1978.
2. Boyd, M. R. Biochemical mechanisms in chemical-induced lung injury: roles of metabolic activation. CRC Crit. Rev. Toxicol., *7:* 103–176, 1980.
3. Defalque, R. J. Pharmacology and toxicology of trichloroethylene: a critical review of the world literature. Clin. Pharmacol. Ther., *2:* 665–688, 1961.
4. Elgin, S. C. R., and Bonner, J. Limited heterogeneity of the major nonhistone chromatin proteins. Biochemistry, *9:* 4440–4447, 1970.
5. Gornall, A. G., Bardawill, C. J., and David, M. M. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., *177:* 751–766, 1949.
6. Guengerich, F. P. Studies on the activation of a model furan compound: toxicity and covalent binding of 2-(*N*-ethylcarbamoylhydroxymethyl)furan. Biochem. Pharmacol., *26:* 1909–1915, 1977.
7. Guengerich, F. P., Dannan, G. A., Wright, S. T., Martin, M. V., and Kaminsky, L. S. Purification and characterization of liver microsomal cytochromes P450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-naphthoflavone. Biochemistry, *21:* 6019–6030, 1982.
8. Guengerich, F. P., and Martin, M. V. Purification of cytochrome P-450, NADPH-cytochrome P-450 reductase, and epoxide hydratase from a single preparation of rat liver microsomes. Arch. Biochem. Biophys. *205:* 365–379, 1980.
9. Guengerich, F. P., Mason, P. S., Stott, W. T., Fox, T. R., and Watanabe, P. G. Roles of 2-haloethylene oxides and 2-haloacetaldehydes derived from vinyl bromide and vinyl chloride in irreversible binding to protein and DNA. Cancer Res., *41:* 4391–4398, 1981.
10. Guengerich, F. P., Wang, P., and Davidson, N. K. Estimation of multiple forms of microsomal cytochrome P-450 in rats, rabbits, and humans using immunochemical staining coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biochemistry, *21:* 1698–1706, 1982.
11. Henschler, D., Romen, W., Elsässer, H. M., Reichert, D., Eder, E., and Radivon, Z. Carcinogenicity study of trichloroethylene by long term inhalation in three animal species. Arch. Toxicol., *43:* 237–248, 1980.
12. Irving, C. C., and Veazey, R. A. Isolation of deoxyribonucleic acid and ribosomal ribonucleic acid from rat liver. Biochim. Biophys. Acta, *166:* 246–248, 1968.
13. Kadlubar, F. F., Miller, J. A., and Miller, E. C. Microsomal *N*-oxidation of the hepatocarcinogen *N*-methyl-4-aminoazobenzene and the reactivity of *N*-hydroxy-*N*-methyl-4-aminoazobenzene. Cancer Res., *36:* 1196–1206, 1976.
14. Kimmerle, G., and Eben, A. Metabolism, excretion and toxicology of trichloroethylene after inhalation: I. Experimental exposure on rats. Arch. Toxicol., *30:* 115–126, 1973.
15. Laskin, S., Sellakumor, A. R., Kuschner, M., Nelson, N., La Mendola, S., Rusch, G. M., Katz, G. V., Dulak, N. C., and Albert, R. E. Inhalation carcinogenicity in noninbred Sprague-Dawley rats. J. Natl. Cancer Inst., *65:* 751–757, 1980.
16. Leibman, K. C. Metabolism of trichloroethylene in liver microsomes, I: characteristics of the reaction. Mol. Pharmacol., *1:* 239–246, 1965.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265–275, 1951.
18. Maltoni, C., Lefemine, G., Ciliberti, A., Cotti, G., and Carretti, D. Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. Environ. Health Perspect., *41:* 3–29, 1981.
19. Massey, T. E., and Racz, W. J. Effects of *N*-acetylcysteine on metabolism, covalent binding, and toxicity of acetaminophen in isolated mouse hepatocytes. Toxicol. Appl. Pharmacol., *60:* 220–228, 1981.
20. Matsubara, T., Masahiro, K., Akira, T., Yoshihiro, T., and Koichi, S. Quantitative determination of cytochrome P-450 in rat liver homogenate. Anal. Biochem., *75:* 596–603, 1976.
21. McKenna, M. J., Zempel, J. A., Madrid, E. O., Braun, W. H., and Gehring, P. J. Metabolism and pharmacokinetic profile of vinylidene chloride in rats following oral administration. Toxicol. Appl. Pharmacol., *45:* 821–835, 1978.
22. Miller, R. E., and Guengerich, F. P. Oxidation of trichloroethylene by liver microsomal cytochrome P-450: evidence for chlorine migration in a transition state not involving trichloroethylene oxide. Biochemistry, *21:* 1090–1097, 1982.
23. Moldeus, P., Högberg, J., and Orrenius, S. Isolation and use of liver cells. Methods Enzymol., *52:* 60–71, 1978.
24. National Cancer Institute. Carcinogenesis bioassay of trichloroethylene. CAS No. 79-01-6. DHEW Publication No. (NIH) 76-802. Washington, D. C.: Government Printing Office, 1976.
25. Ortiz de Montellano, P. R., Kunze, K. L., Beilan, H. S., and Wheeler, C. Destruction of cytochrome P-450 by vinyl fluoride, fluoroxene, and acetylene. Evidence for a radical intermediate in olefin oxidation. Biochemistry, *21:* 1331–1339, 1982.
26. Sedlak, J., and Lindsay, R. H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem., *25:* 192–205, 1968.
27. Shen, A. L., Fahl, W. E., and Jefcoate, C. R. Metabolism of benzo(*a*)pyrene by isolated hepatocytes and factors affecting covalent binding of benzo(*a*)pyrene metabolites to DNA in hepatocyte and microsomal systems. Arch. Biochem. Biophys., *204:* 511–523, 1980.
28. Stott, W. T., Quast, J. F., and Watanabe, P. G. The pharmacokinetics and macromolecular interactions of trichloroethylene in mice and rats. Toxicol. Appl. Pharmacol., *62:* 137–151, 1982.
29. Thurman, R. G., and Kauffman, F. C. Factors regulating drug metabolism in intact hepatocytes. Pharmacol. Rev., *31:* 229–251, 1980.
30. Traylor, P. S., Nastainczyk, W., and Ullrich, V. Conversion of trichloroethylene to carbon monoxide by microsomal cytochrome P-450. *In:* Microsomes Drug Oxidation, Proceedings of the 3rd International Symposium, pp. 615–621, 1976.
31. Umbenhauer, D. R., and Pegg, A. E. Alkylation of intracellular and extracellular DNA by dimethylnitrosamine following activation by isolated rat hepatocytes. Cancer Res., *41:* 3471–3474, 1981.
32. Van Duuren, B. L., and Banerjee, S. Covalent interaction of metabolites of the carcinogen trichloroethylene in rat hepatic microsomes. Cancer Res., *36:* 2419–2422, 1976.
33. Van Duuren, B. L., Goldschmidt, B. M., Katz, C., Seidman, I., and Paul, J. S. Carcinogenic activity of alkylating agents. J. Natl. Cancer Inst., *53:* 695–700, 1974.
34. Wallin, H., Schelin, C., Tunek, A., and Jergil, B. A rapid and sensitive method for determination of covalent binding of benzo[*a*]pyrene to proteins. Chem.-Biol. Interact. *38:* 109–118, 1981.
35. Watanabe, P. G., McGowan, G. R., and Gehring, P. J. Fate of [^{14}C]vinyl chloride after single oral administration in rats. Toxicol. Appl. Pharmacol., *36:* 339–352, 1976.