Biochemical Pharmacology, Vol. 35, No. 16, pp. 2789–2795, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

IN VITRO STUDIES ON THE METABOLISM AND COVALENT BINDING OF [^{14}C]1,1-DICHLOROETHYLENE BY MOUSE LIVER, KIDNEY AND LUNG

LAUD K. OKINE* and THEODORE E. GRAM

Laboratory of Pharmacology and Experimental Therapeutics, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, U.S.A.

(*Received* 1 *June* 1985; *accepted* 24 *January* 1986)

Abstract—The metabolism and covalent binding of 1,1-dichloro[1,2-^{14}C]ethylene (DCE) to subcellular fractions of liver, kidney and lung of C57BL/6N mice have been investigated *in vitro*. Covalent binding was NADPH- and cytochrome P-450-dependent. The microsomal fraction bound more radiolabel than any other subcellular fraction, and the levels of covalent binding in cell fractions correlated well with their cytochrome P-450 content. Covalent binding by mouse liver and lung microsomes also reflected their cytochrome P-450 content. However, although mouse kidney microsomes contained twice as much total cytochrome P-450 as the lung, no detectable covalent binding of DCE-derived radioactivity occurred in kidney. Omission of NADPH, heat inactivation of microsomes, carbon monoxide, addition of SKF-525A, piperonyl butoxide or reduced glutathione (GSH), all inhibited (40–90%) covalent binding of radiolabel to liver and lung microsomes. The absence of O_2 (incubation under N_2) did not greatly affect the metabolism and covalent binding. Pretreatment of mice with various inducers, phenobarbital (PB), β-naphthoflavone (β-NF), pregnenolone 16α-carbonitrile (PCN) and 3-methylcholanthrene (3-MC), evoked increases in total liver microsomal cytochrome P-450 content (2-fold) and corresponding increases in covalent binding (3-fold). However, microsomes from PCN-treated mice showed only a 50% increase in DCE binding. Kidney microsomes from control, PB-, and β-NF-pretreated mice were incapable of covalent binding of radiolabel but those from PCN- and 3-MC-pretreated mice showed levels of binding similar to untreated mouse lung microsomes. It is proposed that the nephrotoxicity of DCE may be due to translocation of reactive metabolites from the liver to the kidney.

1,1-Dichloroethylene (DCE), a compound widely used in the manufacture of plastics, has been found to be toxic *in vivo* in rats and mice [1–6], and its toxicity has been suggested to be due to covalent binding of some reactive intermediate(s) to target organs like the kidney, liver and lung [7–9]. Studies indicate that its toxicity is increased, unaffected or decreased by pretreatment with inducers or inhibitors of the microsomal monooxygenases [8, 10–12] but is exacerbated by depletion of reduced glutathione (GSH) which is involved in its detoxification [13–19]. DCE has also been shown to be mutagenic [20–24] and carcinogenic [25–28] in laboratory animals.

The metabolism of DCE has been shown to be NADPH dependent and to involve the hepatic microsomal cytochrome P-450 system [19, 29]. However, recent studies have implicated the kidney as a possible site of metabolism [6], suggesting that toxicity in the kidney and liver may be due to metabolism and covalent binding of some toxic metabolite(s) *in situ*. Our recent studies on the distribution and covalent binding of DCE-derived material (nmole equiv./mg protein) to liver, kidney and lung of C57BL/6N mice *in vivo* indicated that the highest covalent binding occurred in kidney and the lowest in lung [9], organs which contain 6–400 times less cytochrome P-450 and its dependent monooxygenase activities than the liver [5, 30]. This seemed to suggest that the covalently bound DCE-derived materials might be produced in the liver and transported via the blood to other target organs like the kidney and lung.

Earlier studies [31] with microsomes from rats pretreated with phenobarbital (PB) and β-naphthoflavone (β-NF) led to the conclusion that multiple forms of cytochrome P-450 metabolize DCE and that the PB-induced enzyme played a minor role while the β-NF-induced enzyme was not involved in these processes. However, recent studies of DCE metabolism [32] using purified cytochrome P-450 isozymes from rats pretreated with various inducers [e.g. PB, β-NF and phenobarbital/pregnenolone 16α-carbonitrile (PB/PCN)] indicated that all isozymes produce monochloroacetic acid at about the same rate whereas only the isozymes of untreated and PB-treated rats show measurable rates of dichloroacetaldehyde formation, both products of DCE metabolism. Studies on the metabolism and covalent binding of DCE *in vitro* with tissues of mice, the species thought to be most susceptible to its toxic effects [7, 12], have not yet been investigated.

We report in this paper the covalent binding of

*Address all correspondence to: L. K. Okine, Ph.D., National Cancer Institute, National Institutes of Health, Building 37, Room 5B-22, Bethesda, MD 20892.

DCE-derived material by various tissue fractions *in vitro* from control and drug-pretreated mice in relation to their total cytochrome P-450 content. We have also investigated optimum *in vitro* conditions for studying covalent binding.

MATERIALS AND METHODS

Chemicals. 1,1-Dichloro[1,2-^{14}C]ethylene (DCE) in mineral oil (sp. act. 350 μCi/mmole, >98% radiochemical purity) was obtained from Moravek Biochemicals (Brea, CA). Aquasol liquid scintillation solution and Tween 80 were purchased from New England Nuclear (Boston, MA) and the Sigma Chemical Co. (St. Louis, MO) respectively. 2-Diethylaminoethyl-2,2-diphenylvalerate (SKF-525A) and PCN were gifts from Smith Kline & French Laboratories (Philadelphia, PA) and the Upjohn Co. (Kalamazoo, MI) respectively. Piperonyl butoxide was obtained from ICN Pharmaceuticals Inc. (Plainview, NY) and 3-methylcholanthrene (3-MC) from the Eastman Kodak Co. (Rochester, NY). β-NF was purchased from the Aldrich Chemical Co. Inc. (Milwaukee, WI). Sodium phenobarbital (PB), hexane (HPLC grade) and all other chemicals, reagent or analytical grade, were obtained from the Fisher Scientific Co. (Silver Spring, MD).

Animals and treatment. Male C57BL/6N mice (20–25 g body wt) obtained from the Frederick Cancer Research Facility (Frederick, MD) were used. They were allowed free access to standard laboratory chow and water.

Animals receiving pretreatments were dosed with test compounds as follows: PB (80 mg/kg/day, i.p., in saline, for 4 days), 3-MC (40 mg/kg/day, i.p., in olive oil, for 2 days), β-naphthoflavone (80 mg/kg/day, i.p., in olive oil, for 2 days) or PCN (100 mg/kg/day, orally, in H_2O containing 1 drop Tween 80/10 ml H_2O, for 2 days). Animals were killed 24 hr after the last dose in each treatment regimen. Control animals were either untreated or received equivalent volumes of the test vehicles, as for test compounds (5 ml/kg body wt), and killed 24 hr after the last administration.

Tissue preparation and subcellular fractionation. Tissues (kidney, liver and lung) of animals killed by cervical dislocation were removed, and homogenates (20%, w/v) were prepared in 150 mM KCl, 50 mM Tris–HCl buffer (pH 7.4) (for microsomal preparation) or 0.25 M sucrose, 50 mM Tris–HCl buffer (pH 7.4) (for subcellular fractionation) using a motor-driven Teflon–glass homogenizer. Microsomes were prepared by centrifugation of the post-mitochondrial supernatant fraction at 106,000 *g* for 60 min [33] and subcellular fractionation by the method of Bend *et al.* [34]. Protein concentration was measured by method of Lowry *et al.* [35] with bovine serum albumin as standard. Unless indicated otherwise, the suspensions of microsomes or other fractions were diluted to give a protein concentration of 4 mg/ml.

Incubations. Oxygenated microsomes or other subcellular fractions (1.0 mg/ml, unless indicated otherwise) were incubated with [1,2-^{14}C]DCE (0.2 to 1.1 mM final concentration) in the presence (complete) or absence of an NADPH-generating system (1.64 mM $NADP^+$, 17.2 mM glucose-6-phosphate, 7.5 mM $MgCl_2$ and 1 unit/ml glucose-6-phosphate dehydrogenase) in a final volume of 2.00 ml. Incubations were carried out in closed screw-capped tubes in a shaking water bath for 10 min at 37° after a 5-min preincubation period. The reactions were started by adding the appropriate amount of radiolabeled DCE (5 μl containing 0.6 μCi in a mixture of mineral oil and hexane (40:60, v/v)). Preliminary experiments indicate that this amount of the mixture of mineral oil and hexane had no effect on microsomal cytochrome P-450 content.

In cases where other substances were added and preincubated with the microsomes prior to addition of radiolabeled DCE, these were made to give the same final volume as above as follows: reduced glutathione (GSH) was added in 0.1 ml buffer (1.0 or 5.0 mM) and preincubated with microsomes for 30 sec at 37°, piperonyl butoxide in 0.01 ml methanol or SKF-525A in 0.1 ml KCl–Tris buffer (0.5 or 2.0 mM in each case) and preincubated for 5 min at 37°. These were further incubated for 10 min after the addition of radiolabeled DCE.

The effects of various gaseous atmospheres of $CO:O_2$ (4:1) and nitrogen on covalent binding were also examined. The appropriate gas or gas mixture was bubbled through the incubation mixture for 2 min and preincubated for 5 min at 37° prior to the addition of radiolabeled DCE, with further incubation for 10 min at 37°. In another experiment, microsomes were heat-inactivated by placing them in a boiling water bath for 5 min and cooling before use.

All incubations were done in duplicate, and the reactions were stopped by addition of 2 ml of 20% (w/v) trichloroacetic acid (TCA) and kept at 4° overnight for sedimentation of precipitates.

Assay of covalently bound radioactivity. The precipitates were assayed for covalently bound radioactivity after extractions with 2 ml of 20% TCA, 3 ml of 15% TCA, and six times with 5 ml methanol–ether (4:1, v/v). Tubes were centrifuged between each step. Each extracted pellet was solubilized in 1 ml of 1 N NaOH. Protein concentration and radioactivity were measured using 0.2-ml and 0.5-ml aliquots respectively. The samples were counted in a Packard Tricarb liquid scintillation counter model 460 CD after thorough mixing with 10 ml Aquasol liquid scintillation fluid.

Other assay. The cytochrome P-450 content of microsomes and other subcellular fractions was measured using the carbon monoxide-dithionite reduced difference spectra method [36].

RESULTS

Covalent binding of [^{14}C]*DCE to microsomal protein.* The NADPH-dependent covalent binding of DCE-derived radioactivity to liver microsomal protein was linear with time (0–30 min), and with protein concentration (0.5 to 1.25 mg/ml) over a 10-min incubation period (results not shown). A maximum final substrate concentration of 1.1 mM was used in this and all subsequent experiments due to limited

Table 1. NADPH-dependent covalent binding of [^{14}C]DCE to subcellular fractions of mouse liver*

Fraction†	Cytochrome P-450 (nmole/mg protein)	Covalent binding (nmole equiv./mg protein/10 min)	Covalent binding (nmole equiv./nmole P-450/10 min)
Nuclei	0.17 ± 0.01	0.18 ± 0.02	1.06 ± 0.15
Mitochondria	0.19 ± 0.01	0.15 ± 0.01	0.77 ± 0.08‡
Microsomes	0.65 ± 0.03	0.49 ± 0.03	0.75 ± 0.07‡
Cytosol	<0.02	0.05 ± 0.01	

Results are means ± S.E.M. of four (N = 4) determinations.
* See Materials and Methods for experimental details.
† Added at a final protein concentration of 1.0 mg/ml.
‡ Value is not significantly different from nuclear fraction.

solubility. Thus, experimental values obtained may be lower due to unsaturating conditions.

Capacity of liver subcellular fractions to catalyze the NADPH-dependent covalent binding of [^{14}C] *DCE to protein.* Studies with hepatic subcellular fractions (see Materials and Methods) indicated that microsomes covalently bound DCE to a greater extent than nuclear, mitochondrial (3-fold) or cytosolic fractions (10-fold). The degree of covalent binding appeared to correlate generally with the microsomal cytochrome P-450 levels in each fraction (Table 1). Microsomes were therefore used in all subsequent experiments.

NADPH-dependent covalent binding of [^{14}C]*DCE by microsomes from various tissues.* Results from these studies are presented in Table 2. Liver and lung microsomes covalently bound radiolabel, and the binding appeared to correlate with their cytochrome P-450 content. However, kidney microsomes consistently failed to exhibit any detectable covalent binding although their cytochrome P-450 content was twice that of the lung.

Effects of inducing treatments of mice on the NADPH-dependent covalent binding of [^{14}C]*DCE to isolated microsomes.* Pretreatment of mice with various inducing agents led to a 2-fold increase in liver microsomal cytochrome P-450 and to a 3-fold increase in covalent binding of DCE to microsomes *in vitro* (Table 3). However, PCN caused an equivalent increase in cytochrome P-450 but only a 50% increase in covalent binding. When the covalent binding was normalized to cytochrome P-450 content, the binding by PB, β-NF and 3-MC microsomes was about 40% above control, whereas that by PCN microsomes was about 30% below control. Although kidney microsomes from control animals did not show measurable covalent binding, kidney microsomes from PCN- and 3-MC-pretreated mice

Table 2. NADPH-dependent covalent binding of [^{14}C]DCE to microsomes isolated from various mouse tissues*

Tissue†	Cytochrome P-450 (nmole/mg protein)	Covalent binding (nmole equiv./mg protein/10 min)	Covalent binding (nmole equiv./nmole P-450/10 min)
Kidney	0.26 ± 0.01	<0.001	
Liver	0.65 ± 0.03	0.81 ± 0.06	1.24 ± 0.09
Lung	0.13 ± 0.01	0.23 ± 0.04	1.77 ± 0.21‡

Results are means ± S.E.M. of four (N = 4) determinations.
* See Materials and Methods for experimental details.
† Microsomes were added at a final protein concentration of 1.0 mg/ml.
‡ Value is not significantly different from that of liver microsomes.

Table 3. NADPH-dependent covalent binding of [^{14}C]DCE to liver microsomes from mice pretreated with various inducing agents*

Treatment	Cytochrome P-450 (nmole/mg protein)	Covalent binding (nmole equiv./mg protein/10 min)	Covalent binding (nmole equiv./nmole P-450/10 min)
Control	0.55 ± 0.04 (100)	0.81 ± 0.05 (100)	1.50 ± 0.12 (100)
PB	1.20 ± 0.05† (218)	2.44 ± 0.15† (301)	2.04 ± 0.13† (136)
β-NF	1.06 ± 0.06† (193)	2.33 ± 0.14† (288)	2.20 ± 0.07† (147)
PCN	1.07 ± 0.03† (195)	1.14 ± 0.05† (141)	1.06 ± 0.06† (71)
3-MC	1.33 ± 0.05† (242)	2.73 ± 0.04† (337)	2.06 ± 0.06† (137)

Results are means ± S.E.M. of five (N = 5) determinations. Figures in parentheses represent percent of control.
* See Materials and Methods for dosage regimens and experimental details.
† Values are significantly different from controls, $P < 0.02$ (Student's *t*-test).

showed levels of covalent binding similar to control lung microsomes (Table 4). Pretreatments with PB, PCN, 3-MC, and β-NF failed to affect cytochrome P-450 content of mouse lung microsomes. The latter agent caused a slight increase in covalent binding.

Effects of various incubation conditions on the covalent binding of [^{14}C] *DCE to liver and lung microsomes.* The optimum conditions for covalent binding of [^{14}C]DCE to liver and lung microsomes are shown in Table 5. Covalent binding of radiolabel was maximum when microsomes (1.0 mg/ml) were incubated with buffer, an NADPH-generating system and substrate at 37° under an atmosphere of oxygen. Carbon monoxide, SKF-525A, and piperonyl butoxide (inhibitors of cytochrome P-450) markedly reduced the covalent binding of radiolabeled DCE to both liver and lung microsomes (40–80%). Covalent binding to microsomes was reduced dramatically in the absence of NADPH (80–90%) and was reduced, but not abolished, with boiled microsomes (55–75%). Absence of O_2 (presence of N_2) had no effect on the covalent binding of DCE by lung microsomes but slightly reduced the binding of liver microsomes (20%). Reduced glutathione (GSH) significantly decreased the covalent binding by microsomes (35–65%), suggesting the intermediacy of an activated electrophilic species.

Table 4. NADPH-dependent covalent binding of [^{14}C]DCE to kidney and lung microsomes from mice pretreated with various inducing agents*

	Kidney		Lung	
Treatment	Cytochrome P-450 (nmole/mg protein)	Covalent binding (nmole equiv./mg protein/10 min)	Cytochrome P-450 (nmole/mg protein)	Covalent binding (nmole equiv./mg protein/10 min)
Control	0.20	<0.001	0.14	0.23 (1.6)
PB	0.22	<0.001	0.13	0.23 (1.7)
β-NF	0.14	<0.001	0.12	0.30 (2.5)
PCN	0.14	0.20 (1.5)	0.10	0.14 (1.4)
3-MC	0.16	0.25 (1.6)	0.12	0.15 (1.3)

Values represent the means of two (N = 2) determinations. Deviations from these means averaged ± 10%. Figures in parentheses represent nmole equiv./nmole P-450/10 min.

* See Materials and Methods for dosage regimens and experimental details.

DISCUSSION

In our previous studies on the covalent binding of DCE in tissues of C57BL/6N mice *in vivo,* we reported that covalent binding is evenly distributed among subcellular fractions in the kidney, liver and lung [9]. It is known that liver microsomes and purified reconstituted systems metabolize DCE *in vitro,* in the presence of an NADPH-generating system, to DCE oxide, 2-chloroacetyl chloride, 2-chloroacetic acid and 2,2-dichloroacetaldehyde [31, 32]. Our finding that the NADPH-dependent covalent binding of DCE to the microsomal fraction was 3- to 10-fold higher than any other subcellular fraction (Table 1) and dependent on their cytochrome P-450 contents suggests that DCE may be metabolized predominantly by microsomes. This is in accord with studies of the pneumotoxic agent, 4-ipomeanol, whose metabolism and covalent binding *in vitro* occur almost exclusively with microsomes [37].

Studies on the optimum conditions for covalent binding of DCE to microsomes *in vitro* (Table 5) confirm earlier reports with rat microsomes that the binding is mainly an NADPH-dependent enzymatic reaction [19, 29], since absence of NADPH, heat-inactivated microsomes and the presence of CO, SKF-525A and piperonyl butoxide dramatically reduced the covalent binding. However, there is a suggestion of some NADPH-dependent non-enzymatic binding as indicated by differences between binding with heat-inactivated microsomes, and in the presence of CO, SKF-525A and piperonyl butoxide on the one hand and that in the absence of NADPH on the other. It is also apparent that O_2-lack did not affect the metabolism and covalent binding of DCE. This finding was both unexpected and consistent and, at present, cannot be explained. In this connection,

Table 5. Effects of alterations in incubation conditions on the covalent binding of [^{14}C]DCE to liver and lung microsomes isolated from mice*

System	Covalent binding (nmole equiv./mg protein/10 min)	
	Liver	Lung
Complete system	0.77 ± 0.06	0.28
Microsomes boiled	0.18 ± 0.04† (77)	0.13 (54)
− NADPH	0.06 ± 0.01† (92)	0.05 (82)
+ N_2	0.60 ± 0.07† (22)	0.35 (—)
+ CO	0.25 ± 0.05† (68)	0.07 (75)
+ SKF-525A (0.5 mM)	0.40 ± 0.03† (48)	0.16 (43)
+ SKF-525A (2.0 mM)	0.39 ± 0.04† (49)	0.17 (39)
+ Piperonyl butoxide (0.5 mM)	0.43 ± 0.04† (44)	0.15 (46)
+ Piperonyl butoxide (2.0 mM)	0.34 ± 0.05† (56)	0.13 (54)
+ GSH (1.0 mM)	0.41 ± 0.01† (47)	0.19 (32)
+ GSH (5.0 mM)	0.26 ± 0.03† (66)	0.17 (39)

The complete system comprised an NADPH-generating system (1.64 mM $NADP^+$, 17.2 mM glucose-6-phosphate, 7.5 mM $MgCl_2$ and 1 unit/ml glucose-6-phosphate dehydrogenase), microsomes (1 mg/ml), and [1,2-^{14}C]DCE (1.1 mM) in KCl–Tris buffer, pH 7.4, in a final volume of 2.005 ml and in the presence of O_2. Results represent means ± S.E.M. of four (N = 4) determinations for liver and means of two (N = 2) determinations for lung. Deviations from means for lung averaged ± 10%. Figures in parentheses represent percent inhibition.

* See Materials and Methods for experimental details.

† Values are significantly different from complete system, $P < 0.01$ (Student's *t*-test).

it is of interest to note that thiourea binds covalently to lung proteins and that binding *in vitro* occurs under both aerobic and anaerobic conditions and is associated with GSH depletion [38]. That, in this study, GSH significantly reduced the covalent binding confirms that GSH is important in the detoxification of DCE or its metabolites and further suggests that endogenous GSH or protein thiol groups may bind DCE or its metabolites leading to modification of proteins with possible toxicological consequences.

The kidney, liver and lung have been shown to be major targets of covalent binding of DCE-derived radioactivity *in vivo,* with the highest covalent binding occurring in the kidney and the least in the lung [9]. In view of the fact that mouse liver possesses the highest level of microsomal cytochrome P-450 and its dependent monooxygenase activities [5, 30], it was proposed that the liver might be the primary site of DCE metabolism from which metabolites migrate to other target organs like the kidney and lung and bind covalently [9]. There is growing evidence in support of the concept of translocation of reactive metabolites produced predominantly in the liver to the kidney where they may be further activated to more toxic products. For example, tetrafluoroethylene, unlike most halogenated alkenes, does not undergo cytochrome P-450-dependent oxidation. Its toxicity is believed to be derived from a hepatic glutathione conjugate. Following excretion and degradation of this conjugate in bile, the cysteine conjugate is reabsorbed and further metabolized in the kidney by the enzyme β-lyase to a cytotoxic sulfur-containing species [39]. This renal activation due to β-lyase has been proposed to account for the nephrotoxicity of the cysteine conjugates of chlorotrifluoroethylene and chlorodifluoroethylene [40]. This mode of metabolism and the relationship between hepatic glutathione conjugation and renal damage are analogous to that reported for hexachlorobutadiene [41–43]. Also, recent studies have suggested that 2-bromohydroquinone or a conjugate thereof (metabolites of bromobenzene metabolism) may be formed in the liver and transported to the kidney to cause nephrotoxicity [44].

Cytochrome P-450 content in control mouse liver was found in this study to be about twice that of kidney and four times that of lung (Table 2). Since the NADPH-dependent covalent binding of DCE is mainly enzymatic and cytochrome P-450 dependent, one would have expected higher levels of covalent binding with control kidney microsomes than lung, inferred from cytochrome P-450. The fact that kidney microsomes from control animals showed no detectable binding of DCE *in vivo* confirms the earlier suggestion that covalent binding in kidney *in vivo* is due to metabolites produced predominantly in the liver. However, kidney microsomes from mice pretreated with PCN and 3-MC showed levels of binding similar to control lung microsomes (Table 4), suggesting that these pretreatments induced kidney cytochrome P-450 isozymes capable of activating DCE to covalently bound species. This may explain the observed mutagenicity of DCE species in the Ames' Salmonella test using renal microsomes from 3-MC-pretreated animals [20]. Treatment of mice with DCE (125 mg/kg, i.p.) is known to induce some kidney cytochrome P-450-dependent monooxygenases (5- to 10-fold) within 1–2 days [5, 30], similar to those shown by 3-MC [45, 46]. Thus, the possibility of DCE-induced renal cytochrome P-450 isozymes metabolizing DCE to covalently bound metabolites *in vivo* cannot be ruled out. Liver and lung microsomes from control animals, on the other hand,

metabolize and covalently bind DCE proportional to their cytochrome P-450 content.

PB is known to preferentially induce at least one specific cytochrome P-450 in liver (form 2) and its dependent monooxygenase activities without any effect on kidney or lung hemoproteins [47, 48], whereas 3-MC selectively induces a different P-450 species (form 4) and its dependent monooxygenase activities in all three tissues [45, 46]. It is shown that the 2-fold increase in total cytochrome P-450 which resulted from pretreatment of mice with various inducers (PB, 3-MC, β-NF and PCN) (Table 3) produced 3-fold increases in covalent binding of DCE to liver microsomes with the exception of PCN treatment which increased binding only about 50%. The lung and kidney microsomes did not show similar trends (Table 4), suggesting that the effects of these pretreatments on the microsomal cytochrome P-450 isozymes in these organs were different from those in liver. That covalent binding, expressed per nmole cytochrome P-450, was lower in liver microsomes of PCN-treated mice than in controls suggests that the cytochrome P-450 isozymes induced by PCN did not metabolize DCE to covalently bound species. It has been shown that PCN induces a form of cytochrome P-450 in rat liver different from those induced by PB and 3-MC [49]. These results are at variance with earlier findings [31] which suggested that hepatic cytochrome P-450 isozymes induced by β-NF cannot metabolize DCE and those of PB play only a minor role, but confirm recent findings using purified rat liver cytochrome P-450 isozymes [32].

In summary, DCE appeared to be mainly metabolized by mouse liver and lung microsomes in an NADPH-dependent enzymatic reaction to covalently bound species which may be detoxified by conjugation with GSH. The metabolism involved different isozymes of cytochrome P-450 which may not require O_2. Kidney microsomes from control mice cannot metabolize DCE to covalently bound species but, in conditions where renal cytochrome P-450 isozymes are induced, renal metabolism of DCE to covalently bound species may occur. These findings suggest that the liver and lung can metabolize and covalently bind DCE *per se*, whereas covalent binding in the kidney is likely to be due to reactive intermediates transported from the liver to the kidneys via the blood.

Acknowledgement—Laud K. Okine is thankful for the support of Hoffmann-LaRoche, Inc., Nutley, NJ.

REFERENCES

1. L. J. Jenkins, M. J. Trabulus and S. D. Murphy, *Toxic. appl. Pharmac.* **23**, 501 (1972).
2. E. S. Reynolds, M. T. Moslen, S. Szabo, R. V. Jaegar and S. D. Murphy, *Am. J. Path.* **81**, 219 (1975).
3. M. E. Andersen, J. E. French, M. L. Gargas, R. A. Jones and L. J. Jenkins, *Toxic. appl. Pharmac.* **47**, 385 (1979).
4. L. J. Jenkins and M. E. Andersen, *Toxic. appl. Pharmac.* **46**, 131 (1978).
5. K. R. Krijgsheld, M. C. Lowe, E. G. Mimnaugh, M. A. Trush, E. Ginsburg and T. E. Gram, *Toxic. appl. Pharmac.* **74**, 201 (1984).
6. Y. Masuda and N. Nakayama, *Toxic. appl. Pharmac.* **71**, 42 (1983).
7. M. J. McKenna, P. G. Wanatabe and P. J. Gehring, *Environ. Hlth Perspect.* **21**, 99 (1977).
8. R. D. Short, J. M. Winston, J. L. Minor, J. Seifter and C. C. Lee, *Environ. Hlth Perspect.* **21**, 125 (1977).
9. L. K. Okine, J. M. Goochee and T. E. Gram, *Biochem. Pharmac.* **34**, 4051 (1985).
10. G. P. Carlson and G. C. Fuller, *Res. Commun. Chem. Path. Pharmac.* **4**, 553 (1972).
11. M. E. Andersen and L. J. Jenkins, Jr., *Environ. Hlth Perspect.* **21**, 157 (1977).
12. R. J. Jaeger, L. G. Shoner and L. Coffman, *Environ. Hlth Perspect.* **21**, 113 (1977).
13. R. J. Jaeger, R. B. Conolly and S. D. Murphy, *Expl molec. Path.* **20**, 187 (1974).
14. M. E. Andersen, O. E. Thomas, M. L. Gargas, R. A. Jones and L. J. Jenkins, Jr., *Toxic. appl. Pharmac.* **52**, 422 (1980).
15. M. J. McKenna, J. A. Zempel, E. O. Madrid, W. H. Braun and P. J. Gehring, *Toxic. appl. Pharmac.* **45**, 821 (1978).
16. D. Reichert, H. W. Werner, M. Metzler and D. Henschler, *Archs Toxic.* **42**, 159 (1979).
17. E. S. Reynolds, M. P. Moslen, P. J. Boor and R. J. Jaeger, *Am. J. Path.* **101**, 331 (1980).
18. B. K. Jones and D. E. Hathway, *Chem. Biol. Interact.* **20**, 27 (1977).
19. D. C. Liebler, M. J. Meredith and F. P. Guengerich, *Cancer Res.* **45**, 186 (1985).
20. H. Bartsch, C. Malaeille, R. Montesano and L. Tomatis, *Nature, Lond.* **225**, 641 (1975).
21. L. Henschler and G. Bonse, *Archs Toxic.* **39**, 7 (1977).
22. H. Greim, G. Bonse, Z. Radwas, D. Reichert and D. Henschler, *Biochem. Pharmac.* **24**, 2013 (1975).
23. H. Bartsch, C. Malaveille, A. Barbin and G. Plance, *Archs Toxic.* **41**, 249 (1979).
24. G. Bonse, Th. Urban, D. Reichert and D. Henschler, *Biochem. Pharmac.* **24**, 1829 (1975).
25. C. Maltoni, G. Cotti, L. Morisi and P. Chieco, *Medna Lav.* **68**, 241 (1977).
26. C. Maltoni, *Environ. Hlth Perspect.* **21**, 1 (1977).
27. C. C. Lee, J. C. Bhandari, J. M. Winston, W. B. House, P. J. Peters, R. L. Dixon and J. S. Woods, *Environ. Hlth Perspect.* **21**, 25 (1977).
28. *IARC Monograph on the Evaluation of the Carcinogenic Risks of Chemicals to Man* **19**, 439 (1979).
29. K. C. Leibman and E. Ortiz, *Environ. Hlth Perspect.* **21**, 91 (1977).
30. K. R. Krijgsheld and T. E. Gram, *Biochem. Pharmac.* **33**, 1951 (1984).
31. A. K. Costa and K. M. Ivanetich, *Biochem. Pharmac.* **31**, 2083 (1982).
32. D. C. Leibler and F. P. Guengerich, *Biochemistry* **22**, 5482 (1983).
33. C. L. Litterst, E. G. Mimnaugh, R. L. Reagan and T. E. Gram, *Drug Metab. Dispos.* **3**, 259 (1975).
34. J. R. Bend, G. E. R. Hook and T. E. Gram, *Drug Metab. Dispos.* **1**, 358 (1973).
35. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
36. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
37. M. R. Boyd, L. T. Burka, B. J. Wilson and H. A. Sasame, *J. Pharmac. exp. Ther.* **207**, 677 (1978).
38. M. A. Hollinger and S. N. Giri, *Res. Commun. Chem. Path. Pharmac.* **26**, 609 (1979).
39. J. Odum and T. Green, *Toxic. appl. Pharmac.* **76**, 306 (1984).
40. A. J. Gandolfi, R. B. Nagle, J. J. Soltis and F. H. Plescia, *Res. Commu. Chem. Path. Pharmac.* **33**, 249 (1981).
41. G. A. Lock and J. Ishmael, *Toxic. appl. Pharmac.* **57**, 79 (1981).

42. J. B. Hook, M. S. Rose and E. A. Lock, *Toxic. appl. Pharmac.* **65**, 373 (1982).
43. J. A. Nash, L. J. King, E. A. Lock and T. Green, *Toxic. appl. Pharmac.* **73**, 124 (1984).
44. S. S. Lau, T. J. Monks and J. R. Gillette, *J. Pharmac. exp. Ther.* **230**, 360 (1984).
45. A. Poland, E. Glover and A. S. Kende, *J. biol. Chem.* **251**, 4936 (1976).
46. H. E. Serfried, D. J. Birkett, W. Levin, A. Y. H. Lu, A. H. Conney and D. M. Jerina, *Archs Biochem. Biophys.* **178**, 256 (1977).
47. W. H. Kluwe, K. M. McCormack and J. B. Hook, *J. Pharmac. exp. Ther.* **207**, 516 (1978).
48. B. G. Lake, R. Hopkins, J. Chakraborty, J. W. Bridges and D. V. W. Parke, *Drug Metab. Dispos.* **1**, 342 (1973).
49. N. A. Elshourbagy and P. S. Guzelian, *J. biol. Chem.* **255**, 1279 (1980).