V. L., MORGAN, R. L.,
ts of thiosulfate on cya-
s. *Toxicol. Appl. Phar-*

n and calcium fluxes in
iol. (London) **286,** 525–

RINK, T. J. (1982). Cal-
nphocytes: Cytoplasmic
a new, intracellularly
J. Cell Biol. **94,** 325–

(1984). On the applica-
l cells in chemical toxic-
on. Health **13,** 511–520.
xication and its mecha-
v. Pharmacol. Toxicol.

TOXICOLOGY AND APPLIED PHARMACOLOGY **88,** 225–233 (1987)

Chlorinated Hydrocarbon-Induced Peroxisomal Enzyme Activity in Relation to Species and Organ Carcinogenicity

T. L. GOLDSWORTHY AND J. A. POPP

Department of Experimental Pathology and Toxicology, Chemical Industry Institute of Toxicology, P O. Box 12137, Research Triangle Park, North Carolina 27709

Received August 18, 1986; accepted December 8, 1986

Chlorinated Hydrocarbon-Induced Peroxisomal Enzyme Activity in Relation to Species and Organ Carcinogenicity. GOLDSWORTHY, T. L., AND POPP, J. A. (1987). *Toxicol. Appl. Pharmacol* **88,** 225–233. Trichloroethylene (TCE), perchloroethylene (PER), and pentachloroethane (PENT) are widely used industrial chemicals that cause an increased incidence of hepatocellular carcinoma in mice and a very low incidence of renal tubular adenocarcinoma in rats. A recent study (C. R. Elcombe, M. S. Rose, and I. S. Pratt (1985), *Toxicol. Appl. Pharmacol.* **79,** 365–376) suggested that the species difference in the hepatocarcinogenicity of TCE seen between rats and mice was due to a species difference in peroxisomal proliferation and cell proliferation. The purpose of the present investigation was to understand better the association of peroxisome proliferation in the species-specific hepatocarcinogenicity, and nephrocarcinogenicity of TCE, PER, and PENT. TCE (1000 mg/kg body wt), PER (1000 mg/kg body wt), PENT (150 mg/kg body/wt), the metabolite trichloroacetic acid (TCA; 500 mg/kg body wt) or the potent peroxisome proliferating agent Wy-14,643 (WY; 50 mg/kg body wt) was administered by gavage to male F-344 rats and B6C3F1 mice for 10 days. Cyanide-insensitive palmitoyl CoA oxidation activity (PCO) was used to measure the peroxisome proliferation response. Of the chlorinated hydrocarbons, TCE and PER elevated PCO activity in mouse liver whereas only TCE elevated rat liver and kidney PCO. All agents increased PCO activity in the kidneys of mice. None of the chlorinated hydrocarbons induced a PCO response stronger than WY. These results (1) support an association between peroxisome proliferation and hepatic tumors in mice following TCE and PER, but not PENT, administration and (2) suggest that chlorinated hydrocarbon-induced peroxisome proliferation does not correlate with species-specific renal carcinogenicity.

Trichloroethylene (TCE), perchloroethylene (PER), and pentachloroethane (PENT) are used extensively as industrial solvents. Administration of TCE (National Cancer Institute (NCI), 1976; National Toxicology Program (NTP), 1983a), PER (NCI, 1977; NTP, 1986), and PENT (NTP, 1983b) caused an increased incidence of hepatocellular carcinomas in B6C3F1 mice and a low (4–8%) but significant increase of renal tubular adenocarcinomas in male Fischer-344 rats. Although the mechanism(s) by which these chlorinated hydrocarbons cause neoplasia remains unknown, it is generally believed that these agents require metabolic activation to exert their carcinogenic effects (Henschler *et al.*, 1976; Schumann *et al.*, 1980; Bolt *et al.*, 1982; Stott *et al.*, 1982; Green and Prout, 1985; Prout *et al.*, 1985).

Recent work by Elcombe and co-workers (1985) demonstrated that short-term administration of TCE-induced peroxisome proliferation (PP) in the livers of mice but not rats. Trichloroacetic acid (TCA), a metabolite of TCE, induced peroxisome proliferation in the livers of both rats and mice. These studies

0041-008X/87 $3.00

suggested that the high rates of metabolism of TCE in mice result in concentrations of TCA (Green and Prout, 1985; Prout *et al.*, 1985) sufficient to induce peroxisome proliferation whereas low concentrations of TCA found in rats do not induce the peroxisome proliferation. These authors suggested that a species difference in metabolism and peroxisome proliferation may explain the TCE-induced species-specific hepatocarcinogenicity observed. Since TCA is also a metabolite of PER (Costa and Ivanetich, 1980) and PENT (Yllner, 1971), a similar mechanism for mouse liver tumor induction may exist for these two halogenated solvents.

Agents that induce liver peroxisome proliferation also cause hepatocellular carcinomas in both rats and mice (Reddy *et al.*, 1980; Reddy and Lalwani, 1983). On the other hand, a correlation between renal peroxisome proliferation and renal carcinogenesis has not been established. Peroxisomes are localized in the tubular epithelium of the kidneys in rats and mice (Beard and Novikoff, 1969; Reddy *et al.*, 1975). Proliferation of peroxisomes can be determined morphologically or biochemically. Since the assay for cyanide-insensitive palmitoyl CoA oxidation (PCO) is specific for peroxisomes, this assay provides a rapid quantitative indicator for peroxisome proliferation (Reddy and Lalwani, 1983; Elcombe *et al.*, 1985). In the present study we investigated the effects of short-term administration of TCE, PER, PENT, and TCA on peroxisome enzyme activity in livers and kidneys of rats and mice. For comparison to the three halogenated solvents, TCA was given in a high dose in these studies to determine if peroxisome proliferation may occur in the kidney as previously described for liver. The animal strain, dose, and route of administraton of TCE, PER, and PENT were chosen to be the same as in the chronic bioassays in order to (1) better understand the role of peroxisome proliferation in the species-specific hepatocarcinogenicity and (2) to determine if chlorinated hydrocarbon-induced peroxisome proliferation is associated with the development of the renal carcinogenicity in rats.

METHODS

Chemicals. Trichloroethylene (99+%, epoxide stabilizer free), tetrachloroethylene (99+%, epoxide stabilizer free), pentachloroethane (96%, impurities not identified), and trichloroacetic acid (99+%, ACS) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Corn oil, NAD, FAD, acetyl coenzyme A, dithiothreitol, bovine serum albumin (BSA) and palmitoyl coenzyme A were supplied by Sigma Chemical Co. (St. Louis, MO). Triton X-100 and KCN were obtained from Fisher Scientific Co. (Pittsburgh, PA). Wy-14,643 (WY) was a generous gift from Wyeth Laboratories (Radnor, PA).

Animals. Male Fischer-344 (CDF(F-344)/Crl BR) rats and male B6C3F1 (B6C3F1/Crl BR) mice from Charles River Breeding Laboratories, Inc. (Kingston, NY), were quarantined on arrival for 2 weeks and found to be free of pathogenic viruses by a standard virus titer screen (rat/mouse assessment profile, Microbiological Associates, Bethesda, MD). Animals were housed in polycarbonate shoe-box cages with filter tops at 72 ± 2°F and 50 ± 10% humidity with a 12-hr light/dark cycle. NIH-07 pelleted diet and tap water were available *ad libitum* throughout the study. At the start of the treatments, rats and mice weighed 170–200 and 20–25 g, respectively.

Experiment 1. Animals were gavaged (1 ml per rat; 0.05 ml per mouse) with test agents dissolved in corn oil for 10 consecutive days. WY was dissolved in a 1:9 dimethyl sulfoxide (DMSO)/corn oil mixture. Dosages of chemicals follow: TCE (1000 mg/kg body wt), PER (1000 mg/kg body wt), PENT (150 mg/kg body wt), TCA (500 mg/kg body wt), WY (50 mg/kg body wt). Control animals received an appropriate volume of corn oil vehicle alone.

Experiment 2. In the vehicle comparison studies, the same test agents except PENT were administered to rats at the above concentrations in either corn oil or methyl cellulose for 10 days. Naive animals received no treatment and vehicle controls received the vehicle alone.

Experiment 3. In concurrent administration studies, rats and mice were gavaged with TCE (1000 mg/kg body wt), PER (1000 mg/kg body wt), or TCE + PER (separate doses of 1000 TCE mg/kg body wt + 1000 PER mg/kg body wt administered immediately following one another) dissolved in corn oil for 10 consecutive days. All animals were killed 24 hr after the last dose.

Tissue collection and enzyme assay. Animals were weighed, anesthesized with methoxyflurane, and killed by exsanguination. After liver and kidney organ weights

TABLE 1

EFFECTS OF CHLORINATED HYDROCARBON GAVAGE TREATMENT ON HEPATIC PEROXISOMAL ENZYME ACTIVITIES OF MALE F344 RATS[a]

Treatment	n	Liver wt/body wt		CN^--insensitive palmitoyl CoA oxidation	
		Ratio	% of control	nmol/mg protein	% of control
Corn oil	5	3.68 ± 0.06[b]		3.73 ± 0.35	
Wy-14,643 (50 mg/kg)	5	7.18 ± 0.11[c]	195	20.94 ± 2.22[c]	561
Trichloroethylene (1000 mg/kg)	5	4.52 ± 0.08[c,d,e]	122	6.72 ± 0.25[d,e]	180
Perchloroethylene (1000 mg/kg)	5	4.39 ± 0.07[c,d,e]	119	5.28 ± 0.31[d,e]	142
Pentachloroethane (150 mg/kg)	5	4.27 ± 0.09[c,d,e]	116	4.73 ± 0.32[d,e]	127
Trichloroacetic acid (500 mg/kg)	6	5.18 ± 0.11[c,d]	141	10.58 ± 0.41[c,d]	284

[a] Male Fischer 344 rats were administered agents by gavage in corn oil for 10 consecutive days. Control animals received corn oil vehicle alone.

[b] $\bar{x}$ ± SE of n animals.

[c] Significantly different from corn oil control, $p < 0.05$, by Newman–Keuls "multicompare" test.

[d] Significantly different from Wy-14,643, $p < 0.05$, by Newman–Keuls "multicompare" test.

[e] Significantly different from trichloroacetic acid, $p < 0.05$, by Newman–Keuls "multicompare" test.

were taken, tissues from the left lobe of the liver and the kidney cortex were processed for enzyme analysis. Tissue from the left lobe of liver was used since no preferential peroxisome enzyme increase was seen in various liver lobes to either WY or nafenopin treatment in previous studies (T. L. Goldsworthy, and J. A. Popp, unpublished data). Tissues were placed in glass tubes with ice-cold 0.154 M KCl/50 mM Tris–HCl buffer, pH 7.4, at a volume ratio of 1:4–5 of liver to buffer and of 1:2–3 of kidney to buffer. Samples were then homogenized by two strokes of a rotating Teflon pestle using a Braun homogenizer, and 1-ml samples were frozen at −20°C.

Palmitoyl CoA oxidase activity, a measure of peroxisomal β-oxidation activity, was measured, using a modification (Butterworth *et al.*, 1984) of Lazarow's method (1981), in supernatants of thawed samples centrifuged at 2500*g* for 5 min at −4°C. Briefly, peroxisomal β-oxidation was measured in liver and kidney homogenates as palmitoyl CoA-dependent reduction of NAD^+ in the presence of CN^-. Assay media for kidney samples were identical to those described for the liver except for a 5-fold increase in the concentration of palmitoyl CoA and a 1.6-fold increase in KCN concentration, conditions that were shown to increase the sensitivity of the assay. Tissue test samples were diluted in buffer (liver 1:20, kidney 1:2) and analyzed using a 1:101 sample to total volume ratio in an Abbott VP Bichromatic Analyzer. The protein content of the samples was determined by the biuret method using a commercially available kit (Abbott Laboratories). Enzyme activity was measured as nmol NAD^+ reduced * min^{-1} * mg $protein^{-1}$ (nmol/mg). The Newman–Keuls "multicompare" test was used for comparisons between test groups at a significant p value of $p < 0.05$.

RESULTS

In Experiment 1, the administration of test agents by gavage to rats and mice for 10 days had little or no effect upon the body weight gain of the animals compared to control animals with the exception of WY-treated rats which demonstrated no body weight gain (data not shown). All agents significantly increased the liver to body weight ratio compared to controls in rats: WY > TCA > TCE = PER = PENT (Table 1). Only WY caused a significantly increased rat kidney to body

TABLE 2

EFFECTS OF CHLORINATED HYDROCARBON GAVAGE TREATMENT ON RENAL PEROXISOMAL ENZYME ACTIVITIES OF MALE F344 RATS[a]

Treatment	n	Kidney wt/body wt		CN^--insensitive palmitoyl CoA oxidation	
		Ratio	% of control	nmol/mg protein	% of control
Corn oil	5	0.75 ± 0.01[b]		0.80 ± 0.13	
Wy-14,643 (50 mg/kg)	5	0.90 ± 0.02[c]	120	7.66 ± 0.25[c]	958
Trichloroethylene (1000 mg/kg)	5	0.82 ± 0.02	109	2.39 ± 0.20[c,d,e]	300
Perchloroethylene (1000 mg/kg)	5	0.79 ± 0.01[d]	105	1.39 ± 0.09[d,f]	174
Pentachloroethane (150 mg/kg)	5	0.78 ± 0.01[d]	104	0.42 ± 0.09[d,e,f,g]	53
Trichloroacetic acid (500 mg/kg)	6	0.80 ± 0.01[d]	107	1.40 ± 0.19[d]	175

[a] Male Fischer-344 rats were administered agents by gavage in corn oil for 10 consecutive days. Control animals received corn oil vehicle alone.

[b] $\bar{x} \pm$ SE of n animals.

[c] Significantly different from corn oil control, $p < 0.05$, by Newman–Keuls "multicompare" test.

[d] Significantly different from Wy-14,643, $p < 0.05$, by Newman–Keuls "multicompare" test.

[e] Significantly different from trichloroacetic acid, $p < 0.05$, by Newman–Keuls "multicompare" test.

[f] Significantly different from trichloroethylene, $p < 0.05$, by Newman–Keuls "multicompare" test.

[g] Significantly different from perchloroethylene, $p < 0.05$, by Newman–Keuls "multicompare" test.

ratio (Table 2). All agents except PENT significantly increased the liver to body weight ratio in mice compare to controls (Table 3). In contrast, no increases in the kidney to body weight ratio were observed in treated mice (Table 4).

As anticipated, increases in hepatic cyanide-insensitive palmitoyl CoA oxidation, compared to controls, were seen following administration of WY (561% of control) and TCA (284% of control) to rats (Table 1). Hepatic PCO activity was not changed in rats following TCE, PER, and PENT treatments.

WY (958% of control value) and TCE (300% of control) caused an increase in renal PCO activity in rats (Table 2). The TCE-induced PCO activity was significantly higher than that seen following PER and PENT administration.

PCO activity was significantly elevated in the livers of mice in all treatment groups except PENT (Table 3). PCO responses varied between treatment groups: WY > TCE > PER > TCA = PENT.

All chemicals increased PCO activity in the kidneys of mice (Table 4). The elevated PCO response was similar in all treatment groups (232 to 360% of controls) with the exception of WY (689% of controls).

In Experiment 2, rats were gavaged with TCE, PER, TCA, or WY in methyl cellulose or corn oil for 10 days to determine if there was a vehicle effect of the administration of these chemicals on rat liver and kidney PCO activity. PCO activity compared to respective control values was similar in rat liver and kidney when chlorinated hydrocarbons were administered in either corn oil or methyl cellulose. In general, the data in the vehicle comparison studies confirm the results from the previous experiment (Tables 1 and 2) for the induction of PCO activity compared to con-

TABLE 3

EFFECTS OF CHLORINATED HYDROCARBON GAVAGE TREATMENT ON HEPATIC PEROXISOMAL ENZYME ACTIVITIES OF MALE B6CF31 MICE[a]

Treatment	n	Liver wt/body wt		CN^--insensitive palmitoyl CoA oxidation	
		Ratio	% of control	ng/mg protein	% of control
Corn oil	7	4.55 ± 0.13[b]		1.77 ± 0.34	
Wy-14,643 (50 mg/kg)	7	8.81 ± 0.37[c]	194	21.65 ± 1.59[c]	1223
Trichloroethylene (1000 mg/kg)	7	6.83 ± 0.13[c,d]	150	11.07 ± 0.51[c,d,e]	625
Perchloroethylene (1000 mg/kg)	7	6.48 ± 0.14[c,d]	142	7.58 ± 1.34[c,d,e,f]	428
Pentachloroethane (150 mg/kg)	7	5.06 ± 0.12[d,e,f,g]	111	2.79 ± 0.37[d,f,g]	158
Trichloroacetic acid (500 mg/kg)	8	6.36 ± 0.10[c,d]	140	4.96 ± 0.38[c,d]	280

[a] Male B6C3F1 mice were administered agents by gavage in corn oil for 10 consecutive days. Control animals received corn oil vehicle alone.

[b] $\bar{X}$ ± SE of n animals.

[c] Significantly different from corn oil control, $p < 0.05$, by Newman–Keuls "multicompare" test.

[d] Significantly different from Wy-14,643, $p < 0.05$, by Newman–Keuls "multicompare" test.

[e] Significantly different from trichloroacetic acid, $p < 0.05$, by Newman–Keuls "multicompare" test.

[f] Significantly different from trichloroethylene, $p < 0.05$, by Newman–Keuls "multicompare" test.

[g] Significantly different from perchloroethylene, $p < 0.05$, by Newman–Keuls "multicompare" test.

trol values in the livers and kidneys of rats. In contrast to Experiment 1, the TCE-induced rat liver PCO values, although relatively weak, were significantly elevated compared to control values and differed from PER-induced PCO activity. Corn oil or methyl cellulose alone had no effect on PCO.

In Experiment 3, animals were gavaged with TCE + PER daily for 10 days and the PCO response was compared to that of each agent administered alone to examine the effects of concurrent administration of chlorinated hydrocarbons on the peroxisome enzyme activity in the livers and kidneys of rats and mice. The PCO response observed by concurrent administration of TCE + PER was not additive or synergistic and did not differ from that observed with either TCE or PER alone. The values for TCE + PER, TCE, or PER, respectively, represented as a percentage of PCO activity of control values, are for rat liver 263, 239, 167; for rat kidney 319, 261, 87; for mouse liver 460, 625, 428; and for mouse kidney 232, 360, 232.

DISCUSSION

In an attempt to understand the carcinogenicity of chlorinated hydrocarbons, many studies have focused on the metabolism of the chemicals and the potential role of DNA interaction. It has been suggested that the interaction of reactive metabolites of these agents with critical cellular macromolecules, in particular DNA, may result in somatic mutations and subsequent carcinogenesis (Greim *et al.*, 1975; Simmon *et al.*, 1977). However, the low level or lack of binding of metabolites to DNA *in vivo* and the lack of

TABLE 4

EFFECT OF CHLORINATED HYDROCARBON GAVAGE TREATMENT ON RENAL PEROXISOMAL ENZYME ACTIVITIES OF MALE B6CF31 MICE[a]

Treatment	n	Kidney wt/body wt		CN^--insensitive palmitoyl CoA oxidation	
		Ratio	% of control	nmol/mg protein	% of control
Corn oil	7	1.42 ± 0.03[b]		1.11 ± 0.29	
Wy-14,643 (50 mg/kg)	7	1.49 ± 0.08	104	7.65 ± 0.56[c]	689
Trichloroethylene (1000 mg/kg)	7	1.33 ± 0.05	94	4.00 ± 0.35[c,d]	360
Perchloroethylene (1000 mg/kg)	7	1.36 ± 0.04	96	2.57 ± 0.21[c,d]	232
Pentachloroethane (150 mg/kg)	7	1.38 ± 0.05	97	3.44 ± 0.40[c,d]	310
Trichloroacetic acid (500 mg/kg)	8	1.40 ± 0.03	98	3.38 ± 0.23[c,d]	305

[a] Male B6C3F1 mice were administered agents by gavage in corn oil for 10 consecutive days. Control animals received corn oil vehicle alone.

[b] $\bar{x} \pm$ SE of n animals.

[c] Significantly different from corn oil control, $p < 0.05$, by Newman–Keuls "multicompare" test.

[d] Significantly different from Wy-14,643, $p < 0.05$, by Newman–Keuls "multicompare" test.

overtly detectable genotoxicity of these chemicals (Bolt and Filser, 1977; Schumann *et al.*, 1980; Parchman and Magee, 1982; Stott *et al.*, 1982) does not support the above hypothesis and does not implicate epigenetic mechanisms for the carcinogenicity observed. In this light it has been suggested that compounds such as TCE and PER enhance the high background incidence of hepatocellular carcinoma found in B6C3F1 mice through sustained hepatotoxicity and resultant cell division (Schumann *et al.*, 1980; Stott *et al.*, 1982). On the other hand, the recent identification in rat urine of small amounts of mercapturic acids following administration of TCE and PER suggests an alternative activation pathway in the rat the intermediates of which are mutagenic and may thus cause cellular damage (Dekant and Henschler, 1986).

Studies that evaluated the role of metabolism to account for the species difference in the hepatocarcinogenicity of TCE and PER have demonstrated that mice are more active than rats in metabolizing these agents (Schumann *et al.*, 1980; Stott *et al.*, 1982; Green and Prout, 1985; Prout *et al.*, 1985). One study has suggested that the species difference in the hepatocarcinogenicity of TCE in rats and mice is due to a species difference in peroxisomal proliferation (Elcombe *et al.*, 1985). Although peroxisome proliferation has been linked to neoplasia in rodents, the mechanism(s) involved in the development of tumors has not been established. One hypothesis suggests that the carcinogenic response of peroxisome-proliferating agents involves the interaction of increased steady-state concentrations of H_2O_2, or other reactive oxygen species resulting from carcinogen-stimulated peroxisomal metabolism, with critical cellular macromolecules (Reddy and Lalwani, 1983). However, there is no evidence that peroxisome-proliferating agents are reactive with DNA (von Daniken *et al.*, 1983, 1984; Goel *et al.*, 1985; Gupta *et al.*, 1985).

XISOMAL

nsitive palmitoyl
A oxidation

tein	% of control
,c	689
,c,d	360
c,d	232
,c,d	310
c,d	305

ays. Control animals

est.

are more active
se agents (Schu-
al., 1982; Green
al., 1985). One
pecies difference
of TCE in rats
difference in per-
nbe *et al.*, 1985).
eration has been
nts, the mecha-
elopment of tu-
d. One hypothe-
enic response of
nts involves the
dy-state concen-
reactive oxygen
ogen-stimulated
th critical cellu-
and Lalwani,
o evidence that
nts are reactive
al., 1983, 1984;
, 1985).

To further test the hypothesis that peroxisome proliferation may be associated with the carcinogenicity of chlorinated hydrocarbons (Elcombe *et al.*, 1985), we studied the PCO activity following administration of three chlorinated hydrocarbons and compared this response to their species and organ-specific carcinogenicity. Induction of PCO activity correlated to increased organ to body weight ratios. Of the chlorinated hydrocarbons tested, TCE and PER elevated PCO activity in mouse liver whereas only TCE elevated rat liver PCO activity. PENT resulted in a slight but not significant increase in the liver PCO. Unexpectedly, TCE increased mouse liver PCO activity more than TCA. The peroxisome proliferative response of TCE and PER, coupled with an increased cell proliferation observed in the mouse following administration of TCE and PER (Schumann *et al.*, 1980; Stott *et al.*, 1982; Elcombe *et al.*, 1985), may be an additional important factor in the development of mouse hepatic tumor.

Since the relationship between the extent of peroxisome proliferation and the carcinogenic response observed in rodents has not been established, it is not clear if peroxisome proliferation plays any role in PENT-induced hepatocarcinogenicity. The TCE-induced rat liver PCO response, albeit weak (1.8- to 3.2-fold increase over controls), was somewhat contrary to a previous study (Elcombe *et al.*, 1985) which did not demonstrate an increased rat liver PCO response to TCE exposure. The use of different rat strains in the two studies or potential experimental variability may explain the differences observed. If hepatic tumor formation in rodents requires both liver cell hyperplasia and increased peroxisome proliferation following TCE exposure, then the lack of TCE-induced cell proliferation in rat liver (Elcombe *et al.*, 1985) may explain the lack of hepatocellular carcinoma development in the rat. In these studies none of the chlorinated hydrocarbons induced a PCO response as strong as that by potent peroxisome proliferator and hepatocarcinogen Wy-14,643 in either species or in either organ in any of the experiments reported herein.

PCO data for the concurrent administration of TCE + PER did not show additive or synergistic effects. This result was somewhat unexpected in mouse liver since mice had shown a linear relationship up to 2000 mg/kg between dose of TCE and amount of TCA formed (Prout *et al.*, 1985). However, preferential metabolism of TCE at the dose levels used may in part explain the lack of an additive effect following combined TCE and PER treatment. Schumann *et al.* (1980) reported that only 17% of a 500-mg/kg/day dose of PER was metabolized in mice. Additive dose concentrations of TCE and PER greater than 1000 mg/kg in the rat would not be expected to increase the concentration of TCA in the liver (Prout *et al.*, 1985) and thus would not be expected to increase hepatic PCO activity.

There does not appear to be a relationship between chlorinated hydrocarbon-induced PCO activity and the renal-specific carcinogenicity. However, the very low incidence of renal tumors induced by chlorinated hydrocarbons makes it very difficult to establish a cause and effect relationship between treatment-related changes in peroxisomes and renal carcinogenicity. All agents increased mouse kidney PCO but only TCE treatment elevated rat kidney PCO activity. In contrast to these PCO results, an increased incidence of renal tubular adenocarcinomas was observed only in the male rat following administration of these agents. However, it is possible that a significant PCO activity in rat kidney was masked by the heterogeneity of cell types found in the kidney cortex. The PCO activity observed in the kidney following TCE treatment may be the result of strong induction of peroxisomes in a specific renal tubular cell population. Factors such as cell replication may be necessary along with an increased peroxisomal response before such changes affect the neoplastic response. In this context, a recent report (Goldsworthy *et al.*, 1986) noted that protein droplet accumula-

tion and increased cell replication were observed following short-term treatment with PER and PENT but not with TCE.

Under conditions similar to the chronic bioassay studies (i.e., animal strains, dose), our results support an association between increased PCO activity and the development of TCE- and PER-induced hepatocellular carcinoma in mice. However, the weak response observed in mouse liver following PENT suggests that other factors are involved in the development of species-specific hepatocarcinogenicity following chlorinated hydrocarbon administration. The role of the metabolite TCA with peroxisome proliferation and tumor induction in rats and mice remains to be established. It is clear, with the possible exception of TCE, that chlorinated hydrocarbon-induced peroxisome enzyme activity does not correlate with the species-specific renal carcinogenicity of these compounds.

ACKNOWLEDGMENTS

The authors thank Douglas A. Neptun and Otis Lyght for excellent technical assistance, and Diane Doach and Linda Smith for typing the manuscript.

REFERENCES

BEARD, M. E., AND NOVIKOFF, A. B. (1969). Distribution of peroxisomes (microbodies) in the nephron of the rat: A cytochemical study. *J. Cell. Biol.* **42**, 501–518.

BOLT, H. M., AND FILSER, J. G. (1977). Irreversible binding of chlorinated ethylene to macromolecules. *Environ. Health Perspect.* **21**, 107–112.

BOLT, H. M., LAIB, R. J., AND FILSER, J. G. (1982). Reactive metabolites and carcinogenicity of halogenated ethylenes. *Pharmacology* **31**, 1–4.

BUTTERWORTH, B. E., BERMUDEZ, E., SMITH-OLIVER, T., EARLE, L., CATTLEY, R., MARTIN, J., POPP, J. A., STROM, S., JIRTLE, R., AND MICHALOPOULOS, G. (1984). Lack of genotoxic activity of di(2-ethylhexyl)-phthalate (DEHP) in rat and human hepatocytes. *Carcinogenesis* **5**, 1329–1335.

COSTA, A. K., AND IVANETICH, K. M. (1980). Tetrachloroethylene metabolism by the hepatic microsomal cytochrome *P*-450 system. *Biochem. Pharmacol.* **29**, 2863–2869.

DEKANT, W., AND HENSCHLER, D. (1986). Metabolic conversion to tri- and tetrachloroethylene: Formation and deactivation of genotoxic intermediates. *Toxicol. Lett.* **31**(Suppl.), 17.

ELCOMBE, C. R., ROSE, M. S., AND PRATT, I. S. (1985). Biochemical, histological and ultrastructural changes in rat and mouse liver following the administration of trichloroethylene: Possible relevance to species differences in hepatocarcinogenicity. *Toxicol. Appl. Pharmacol.* **79**, 365–376.

GOEL, S. K., LALWANI, N. D., FAHL, W. E., AND REDDY, J. K. (1985). Lack of covalent binding of peroxisome proliferators nafenopin and WY-14,643 to DNA in vivo and in vitro. *Toxicol. Lett.* **24**, 37–43.

GOLDSWORTHY, T. L., LYGHT, O., MARTIN, J. T., AND POPP, J. A. (1986). Chlorinated hydrocarbon nephrotoxicity and cell proliferaton. *Pharmacologist* **28**(3), 479.

GREEN, T., AND PROUT, M. S. (1985). Species differences in response to trichloroethylene. II. Biotransformation in rats and mice. *Toxicol. Appl. Pharmacol.* **79**, 401–411.

GREIM, H., BONSE, G., RADWAN, Z., REICHERT, D., AND HENSCHLER, D. (1975). Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. *Biochem. Pharmacol.* **24**, 2013–2017.

GUPTA, R. C., GOEL, S. K., EARLEY, K., SINGH, B., AND REDDY, J. K. (1985). P-postlabeling analysis of peroxisome proliferator—DNA adduct formation in rat liver in vivo and hepatocytes in vitro. *Carcinogenesis* **6**, 933–936.

HENSCHLER, D., BONSE, G., AND GREIM, H. (1976). Carcinogenic potential of chlorinated ethylenes, tentative molecular rules. *INSERM Symp. Ser. (Paris)* **52**, 171–176.

LAZAROW, P. B. (1981). Assay of peroxisomal β-oxidation of fatty acids. *In Methods in Enzymology* (J. M. Lowenstein, Ed.), Vol. 72, pp. 315–319. Academic Press, New York.

National Cancer Institute (NCI) (1976). *Carcinogenesis Bioassay of Trichloroethyleme.* CAS No. 79-01-6, DHEW Publ. No. (NIH) 76-802.

National Cancer Institute (NCI) (1977). *Bioassay of Tetrachloroethylene for Possible Carcinogenicity,* DHEW Publ. No. (NIH) 77-813.

National Toxicology Program (NTP) (1983a). National Toxicology Program draft report: Abstracts on nine chemical carcinogenesis animal bioassays. *Chem. Regul. Rep.* **6**, 767–768.

National Toxicology Program (NTP) (1983b). *Carcinogenesis Bioassay of Pentachloroethane in F-344/N Rats and B6C3F1 Mice.* NTP Technical Report No.

232, pp. 7–8. National Institutes of Health, Bethesda, MD.

National Toxicology Program (NTP) (1986). NTP TR 232, USDHEW, Public Health Service.

PARCHMAN, L. G., AND MAGEE, P. N. (1982). Metabolism of [^{14}C]trichloroethylene to $^{14}CO_2$ and interaction of metabolite with liver DNA in rats and mice. *J. Toxicol. Environ. Health* **9**, 797–813.

PROUT, M. S., PROVAN, W. M., AND GREEN, T. (1985). Species differences in response to trichloroethylene. I. Pharmacokinetics in rats and mice. *Toxicol. Appl. Pharmacol.* **79**, 389–400.

REDDY, J. K., AZARNOFF, D. L., AND HIGNITE, C. E. (1980). Hypolipidemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature (London)* **283**, 397–398.

REDDY, J. K., KRISHNAKANTHA, T. P., AND RAO, M. S. (1975). Microbody (peroxisome) proliferation in mouse kidney induced by methyl clofenapate. *Virch. Arch. B. Cell Path.* **17**, 295–306.

REDDY, J. K., AND LALWANI, N. D. (1983). Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risks of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* **13**, 1–58.

SCHUMANN, A. M., QUAST, J. F., AND WATANABE, P. G. (1980). The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity. *Toxicol. Appl. Pharmacol.* **55**, 207–219.

SIMMON, V. F., KAUBANER, K., AND TARDIFF, R. G. (1977). Mutagenic activity of chemicals identified in drinking water. In *Progress in Genetic Toxicology* (D. Scott, B. A. Bridges, and F. H. Sobels, Eds.), pp. 249–258. Elsevier/North-Holland, Amsterdam.

STOTT, W. T., QUAST, J. F., AND WATANABE, P. G. (1982). The pharmacokinetics and macromolecular interactions of trichloroethylene in mice and rats. *Toxicol. Appl. Pharmacol.* **62**, 137–151.

VON DANIKEN, A., LUTZ, W. K., JACKH, R., AND SCHLATTER, C. (1984). Investigation of the potential for binding of di(2-ethylhexyl)phthalate (DEHP) and di(2-ethylhexyl)adipate (DEHA) to liver DNA *in vivo*. *Toxicol. Appl. Pharmacol.* **73**, 373–387.

VON DANIKEN, A., LUTZ, W. K., AND SCHLATTER, C. (1983). Lack of covalent binding to rat liver DNA of the hypolipidemic drugs clofibrate and fenofibrate. *Toxicol. Lett.* **7**, 305–310.

YLLNER, S. (1971). Metabolism of pentachloroethane in the mouse. *Acta Pharmacol. Toxicol.* **29**, 481–489.