o copper content and kidney
tt. 5, 131–138.
)NOSAKA, S., AND OKAHARA,
d-labeled metallothionein in
macol. 33, 258–266.
ASSEN, C. D. (1985). Concen-
n in major organs of rats after
s metals. *Fundam. Appl. Tox-*

logy of cadmium-thionein. In
nistry, and Biology of Cad-
pp. 423–431. Elsevier/North
sterdam.
tallothioneins. In *The Chem-*
l Biology of Cadmium (M.
66. Elsevier/North Holland.

um-thionein and the nephro-
Kadmium Symposium (M.
r. Eds.). pp. 101–107. Freid-
Jena.

TOXICOLOGY AND APPLIED PHARMACOLOGY 92, 103–112 (1988)

The Role of Trichloroacetic Acid and Peroxisome Proliferation in the Differences in Carcinogenicity of Perchloroethylene in the Mouse and Rat

J. ODUM, T. GREEN,[1] J. R. FOSTER, AND P. M. HEXT

Imperial Chemical Industries PLC. Central Toxicology Laboratory. Alderley Park. Nr Macclesfield. Cheshire SK10 4TJ. United Kingdom

Received June 8, 1987; accepted September 21, 1987

The Role of Trichloroacetic Acid and Peroxisome Proliferation in the Differences in Carcinogenicity of Perchloroethylene in the Mouse and Rat. ODUM, J., GREEN, T., FOSTER, J. R., AND HEXT, P.M. (1988). *Toxicol. Appl. Pharmacol.* 92, 103–112. Fischer 344 rats and B6C3F1 mice of both sexes were exposed to 400 ppm perchloroethylene (PER) by inhalation, 6 hr/day for 14, 21, or 28 days or to 200 ppm for 28 days. Increased numbers of peroxisomes were seen under the electron microscope and increased peroxisomal cyanide-insensitive palmitoyl CoA oxidation was measured (3.6-fold increase in males and 2.1-fold increase in females) in the livers of mice exposed to PER. Hepatic catalase was not increased. Peroxisome proliferation was not observed in rat liver or in the kidneys of either species. Trichloroacetic acid (TCA), a known carcinogen and hepatic peroxisome proliferating agent, was found to be a major metabolite of PER. Blood levels of this metabolite measured in mice and rats during and for 48 hr after a single 6-hr exposure to 400 ppm PER showed that peak blood levels in mice were 13 times higher than those seen in rats. Comparison of areas under the curves over the time course of the experiment showed that mice were exposed to 6.7 times more TCA than rats. The difference in metabolism of PER to TCA in mice and rats leads to the species difference in hepatic peroxisome proliferation which is believed to be the basis of the species difference in hepatocarcinogenicity. Peroxisome proliferation does not appear to play a role in the apparent carcinogenicity of PER in the rat kidney. © 1988 Academic Press, Inc.

Perchloroethylene (PER) (1,1,2,2-tetrachloroethylene) is a volatile liquid which is used extensively in the dry cleaning industry and as a general degreasant in manufacturing industry.

A significant increase in hepatocellular carcinoma has been observed in male and female mice but not rats in two carcinogenicity bioassays of PER. In the first study (NCI, 1977) Osborne-Mendel rats and B6C3F1 mice received PER by gavage in corn oil at doses of approximately 500 or 1000 mg/kg. Both dose groups showed about a 50% incidence of hepatocellular carcinoma in mice. In the second study (Mennear *et al.*, 1986) Fischer 344 rats and B6C3F1 mice were exposed to PER by inhalation (mice 100 or 200 ppm and rats 200 or 400 ppm 6 hr/day). Increased hepatic tumor incidence was again observed in mice, 50% in low and high dose males and 26 and 72% in low and high dose females. In the latter study a low incidence of kidney tubular adenocarcinoma was observed in male rats at the highest dose.

The species difference in hepatocarcinogenicity is similar to that seen with trichloroethylene (TRI) (NCI, 1976; NTP, 1983). TRI has been shown to induce peroxisome proliferation in mouse liver but not rat liver, after oral administration (Elcombe *et al.*, 1985). A causal relationship has been suggested between hepatic peroxisome proliferation and

[1] To whom correspondence should be addressed.

0041-008X/88 $3.00

hepatocellular carcinoma in rodents (Reddy *et al.*, 1980) although no such relationship has yet been shown between renal peroxisome proliferation and renal tubular adenocarcinoma (Reddy *et al.*, 1975, 1982). The species difference in hepatic peroxisome proliferation elicited by TRI is believed to be the basis of the species difference in carcinogenicity (Elcombe *et al.*, 1985).

Trichloroacetic acid (TCA), a major metabolite of TRI (Green and Prout, 1985) has recently been shown to be carcinogenic in the B6C3F1 mouse (Herren-Freund *et al.*, 1986). TCA has also been shown to be responsible for peroxisome proliferation in TRI-dosed mice (Elcombe, 1985). Quantitative differences in the metabolism of TRI in rats and mice and hence in circulating levels of TCA (Prout *et al.*, 1985) may lead to the species difference in peroxisome proliferation and consequent carcinogenicity. TCA is reported to be a major metabolite of PER in mice and rats (Yllner, 1961; Daniel, 1963; Dekant *et al.*, 1985) and may therefore elicit the same response when animals are exposed to PER. In view of the lack of mutagenicity of PER (Greim *et al.*, 1975; Bartsch *et al.*, 1979) this may be the basis for the species difference in carcinogenicity.

In this paper the pathological and biochemical changes in rat and mouse liver and kidney, with particular regard to peroxisome proliferation, were determined after inhalation exposure to PER. Blood levels of TCA in rats and mice exposed to PER were also determined. The animal strains and dose levels used in the 1985 PER bioassay (Mennear *et al.*, 1986) were adopted in order to assess the relevance of our results to the development of tumors in these animals.

METHODS

Materials

1,1,2,2-Tetrachloroethylene (Analar grade, 99.9%) and trichloroacetic acid (Analar grade, 99% pure) were obtained from BDH Chemicals PLC (Poole, Dorset, UK). Biochemicals were obtained from Sigma Chemical Co. (Poole, Dorset, UK).

Animals

Male and female Fischer 344 rats (160–180 g) and male and female B6C3F1 mice (23–28 g) were supplied by Charles River (Margate, Kent, UK). Animals were multiply housed in suspended stainless steel wire mesh cages in long-term inhalation exposure chambers, equipped with a 12-hr light cycle, prior to, during, and after exposure. They received food (PCD diet, Special Diets Services Ltd., Witham, Essex, UK) and water *ad libitum* before and after, but not during exposure.

Exposure to PER for up to 28 Days

Exposure. Male and female rats and mice (5 per group) were exposed to concentrations of 200 or 400 ppm of PER for 6 hr/day for 14, 21, or 28 consecutive days. Control animals were exposed to air only, but otherwise were treated in a manner similar to that of the test animals.

Exposures were whole body in stainless steel chambers (Doe and Tinston, 1981) having an internal volume of approximately 3.4 m^3. The chambers were air conditioned to have a nominal temperature of 22°C and relative humidity of 40–60%. The air flow through the chambers was 300 liters/min. Atmospheres were generated by passing vaporized PER into the input air of the chambers.

Atmospheres in the test chambers were analyzed for PER by gas chromatography (GC) on a Hewlett–Packard 5880A GC (flame ionization detector) fitted with a Porapak PS column (1.8 m × 4 mm). The column temperature was 195°C, helium carrier gas 50 ml/min.

Eighteen hours after the last exposure period, animals were killed by overexposure to halothane (Fluothane, Imperial Chemical Industries PLC, Pharmaceuticals Division) and exsanguinated. The livers and kidneys were rapidly removed, weighed, and then divided to provide tissue for light microscopy, electron microscopy, and biochemical analysis.

Light microscopy. Slices of liver and kidney were fixed in 10% neutral buffered formol saline, dehydrated through an ascending ethanol series, and embedded in paraffin wax. Sections (5 μm) were cut and stained with hematoxylin and eosin.

Electron microscopy. Tissues were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer, dehydrated, and embedded in epoxy resin. Sections (1 μm) were cut and stained with 1% toluidine blue in 1% borax for light microscopy. Areas were selected from the centrilobular regions of the livers and the S3 regions of the proximal tubules of the kidney for electron microscopy. Ultrathin sections of these areas were stained with uranyl acetate

TABLE 1

PEROXISOMAL CYANIDE-INSENSITIVE PALMITOYL COENZYME A OXIDATION IN RAT AND MOUSE LIVER AND KIDNEY AFTER EXPOSURE TO PER

Concentration (ppm)	Duration (days)	CN-insensitive palmitoyl CoA oxidation (nmol/min/mg protein)			
		Liver		Kidney	
		Mouse	Rat	Mouse	Rat
Male					
0	14–28	5.16 ± 1.06[a]	10.26 ± 0.51	5.57[b]	2.37 ± 0.29
200	28	11.19 ± 4.46**	12.95 ± 0.93*	6.92	2.98 ± 0.21**
400	14	11.98 ± 2.86**	13.68 ± 1.68**	7.69	2.97 ± 0.65
400	21	13.90 ± 3.27**	12.94 ± 0.81**	8.70	2.44 ± 0.49
400	28	18.64 ± 5.61**	13.61 ± 0.89**	8.18	2.76 ± 0.33
Female					
0	14–28	9.01 ± 1.62	12.62 ± 0.77	2.48	1.98 ± 0.21
200	28	16.68 ± 3.52**	15.76 ± 1.06**	2.85	3.11 ± 0.45**
400	14	14.40 ± 2.27**	14.90 ± 1.91*	2.59	2.56 ± 0.28**
400	21	18.74 ± 1.68**	15.31 ± 2.31*	2.28	2.68 ± 0.26**
400	28	17.99 ± 2.35**	14.14 ± 1.90	2.49	2.41 ± 0.19**

Note. Control animals were exposed to air only for 14, 21, or 28 days.

[a] Values are $\bar{x} \pm$ SD, $n = 5$ except for controls, where $n = 15$.

[b] Mouse kidneys were pooled according to group; values are $\bar{x}$.

* Statistically significant, $p < 0.05$.

** Statistically significant, $p < 0.01$.

and lead citrate and viewed and photographed in a JOEL JEM 100CX electron microscope. Morphometric analysis of peroxisomes was performed according to the general principles of Weibel *et al.* (1964) on electron micrographs of areas of cytoplasm at a magnification of 25,000.

Biochemical analysis. Sections of liver and kidney remaining after tissue had been taken for light and electron microscopy were placed in ice-cold sucrose (250 mM) EDTA (5.4 mM) Tris–HCl (20 mM) buffer, pH 7.4. Mouse kidneys were pooled according to group. Homogenates (25% w/v approximately) were prepared using a Teflon glass homogenizer at 4°C. Homogenates were centrifuged at 3000*g* for 5 min at 4°C. The supernatants from the kidney homogenates were stored at −70°C until used. Supernatants from the liver homogenates were further centrifuged at 15,000*g* for 15 min at 4°C, as described by Elcombe *et al.* (1985). The supernatants were discarded and the pellets (containing peroxisomes) were resuspended in the above buffer and stored at −70°C.

The protein content of the liver and kidney fractions was determined by the method of Lowry *et al.* (1951). The activities of the peroxisomal enzymes catalase and cyanide-insensitive palmitoyl coenzyme A oxidase were determined by the methods of Beers and Sizer (1952) and Bronfman *et al.* (1979), respectively.

TCA Concentrations in Blood after Exposure to PER

Rats and mice were exposed to 400 ppm PER for up to 6 hr. Animals killed at time points of less than 6 hr were exposed in glass desiccators at a flow rate of 5–10 liters/min. Those exposed for the full 6 hr were housed in the long-term chambers described above.

Atmospheres were generated by vaporizing PER into the air stream and were monitored by gas chromatography. Groups of three rats or three mice were killed by exposure to CO_2 and bled by cardiac puncture at intervals from the start of exposure until 48 hr postexposure (see Fig. 2). TCA was extracted from blood as described by Prout *et al.* (1985) and the methylated samples were analyzed on a Hewlett–Packard 5890A gas chromatograph fitted with an electron capture detector. A glass column (2 m × 2 mm), packed with Porapak PS and operated at 180°C with a nitrogen carrier gas flow of 25 ml/min, was used for the analysis. Under these conditions TCA had a retention time of 5.7 min. The limit of detection for TCA in blood was 0.2 μg/ml.

Statistics

Values were tested for statistical significance using the two-sided Student *t* test.

RESULTS

Effects of Exposure to PER

The mean analyzed concentrations of PER for the 28 days of exposure were 193 and 389 ppm for the rats and 196 and 395 ppm for the mice. These were close to the target levels of 200 and 400 ppm. No significant clinical abnormalities were seen in rats or mice exposed to either concentration of PER.

Liver

Exposure of B6C3F1 mice to 400 ppm PER for either 14, 21, or 28 days resulted in small but statistically significant increases in liver/body weight ratios up to 1.2- and 1.3-fold in males and females, respectively. F344 rats exposed to PER showed no changes in liver/body weight ratios.

Cyanide (CN)-insensitive palmitoyl CoA oxidase, a marker for peroxisomal β-oxidation was significantly increased in mouse liver after exposure to PER (Table 1). This enzyme increased to a similar level in males and females but the control rate of CN-insensitive palmitoyl CoA oxidation was higher in females. Therefore the increase over control rates was lower in females than males and maximum response (seen after 28 days exposure to 400 ppm) was a 3.6-fold increase in males and a 2.1-fold increase in females.

In contrast, only small increases in CN-insensitive palmitoyl CoA oxidation were observed following treatment of F344 rats with PER (Table 1) although these were sometimes statistically significant. The maximum increase (1.3-fold) was in males exposed to 400 ppm for 28 days. The basal activity of CN-insensitive palmitoyl CoA oxidation was noted to be approximately 2-fold greater in F344 rats than in B6C3F1 mice (Table 1).

Catalase, another peroxisomal enzyme, was largely unaffected in mice and rats exposed to PER (Table 2). The only increases (up to 1.4-fold) were observed in male mice exposed to 400 ppm.

TABLE 2

PEROXISOMAL CATALASE ACTIVITY IN RAT AND MOUSE LIVER AFTER EXPOSURE TO PER

Concentration (ppm)	Duration (days)	Catalase ($ksec^{-1}$ $mg\ protein^{-1}$) Mouse	Rat
Male			
0	14–28	1.05 ± 1.17[a]	1.62 ± 0.26
400	14	1.12 ± 0.19	1.88 ± 0.57
400	21	1.30 ± 0.17[b]	1.62 ± 0.21
400	28	1.44 ± 0.34[b]	1.90 ± 0.13
Female			
0	14–28	1.79 ± 0.12	1.56 ± 0.23
400	14	1.56 ± 0.09	1.46 ± 0.15
400	21	1.85 ± 0.05	1.77 ± 0.30
400	28	1.76 ± 0.28	1.59 ± 0.24

Note. Control animals were exposed to air only 14, 21, or 28 days.

[a] Values are $\bar{x} \pm SD$, $n = 5$ except for controls, where $n = 15$.

[b] Statistically significant, $p < 0.05$.

By light microscopy the livers of mice exposed to 400 ppm PER showed centrilobular eosinophilia and centrilobular fatty vacuolation. Both effects were seen to a similar extent in males and females and the numbers of animals affected increased from 14 to 28 days. Similar effects on lipid were seen at the electron microscope level in mice exposed to 200 ppm for 28 days or 400 ppm for 14, 21, or 28 days. Extensive lipid accumulation was observed in centrilobular hepatocytes. The lipid was present in the form of large droplets, 2- to 5-μm diameter, lying free in the cytoplasm of the cells (macrovesicles), and small droplets, 0.1- to 0.5-μm diameter, contained within the cisterna of the endoplasmic reticulum (microvesicles). Figure 1 shows the ultrastructural appearance of a centrilobular hepatocyte from (a) an untreated male mouse and (b) a male mouse exposed to 400 ppm PER for 28 days. Electron microscopy showed proliferation of peroxisomes in the

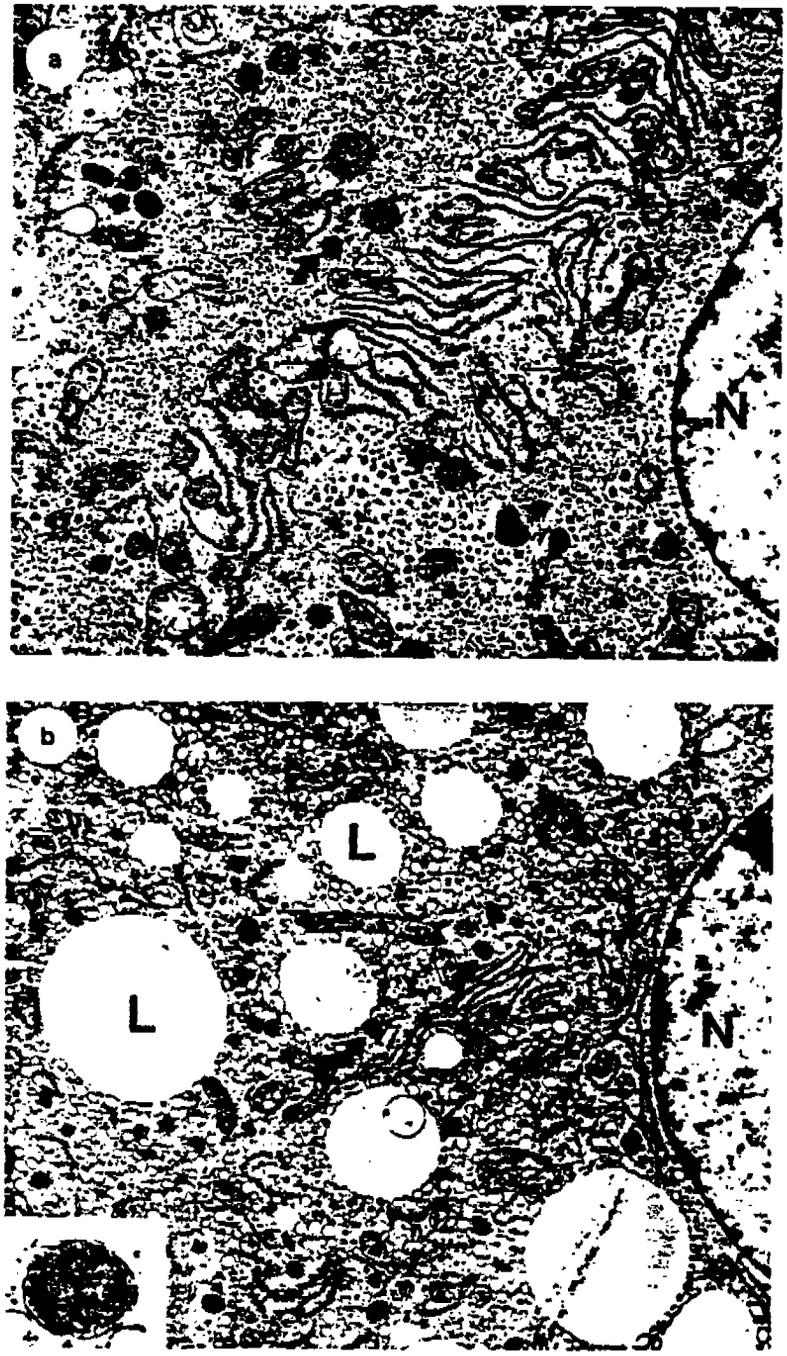

FIG. 1. (a) Ultrastructural appearance of a centrilobular hepatocyte from an untreated male B6C3F1 mouse showing nucleus (N) and peroxisomes ( ). ×6300. (b) Ultrastructural appearance of a centrilobular hepatocyte from a male mouse exposed to 400 ppm PER 6 hr/day for 28 days. The cell shows an accumulation of lipid in the form of large droplets (L) and small vesicles and a proliferation of peroxisomes ( ). The nucleus is seen at (N). ×6300. Insert: higher magnification of peroxisome showing electron dense core, ×19,800.

TABLE 3

MORPHOMETRIC ANALYSIS OF HEPATIC PEROXISOMES IN MICE AND RATS EXPOSED TO PER

Exposure		Peroxisome volume (% cytoplasm)			
		Mouse		Rat	
Concentration (ppm)	Duration (days)	Male	Female	Male	Female
0	28	2.5 ± 0.6[a]	2.5 ± 0.8	3.1 ± 0.1	3.3 ± 0.7
200	28	5.2 ± 1.5*	4.4 ± 0.6*	3.7 ± 1.3	4.7 ± 1.9
400	14	4.9 ± 0.7*	4.9 ± 1.5*	2.3 ± 0.6	2.7 ± 0.5
400	21	5.4 ± 1.3*	4.8 ± 0.9*	2.8 ± 0.7	3.2 ± 1.1
400	28	6.0 ± 1.4*	4.8 ± 1.2*	3.4 ± 1.4	3.4 ± 1.0

[a] Values ($\bar{x} \pm$ SD. $n = 5$) are calculated from three micrographs per animal, with 375 points applied to each micrograph.

* Statistically significant, $p < 0.01$.

centrilobular region of the mouse liver (Fig. 1b, Table 3). The proliferated peroxisomes were small (<0.5 μm) and the majority retained the central nucleoid (Fig. 1b, insert). Exposure to 400 or 200 ppm resulted in statistically significant increases in the volume of cytoplasm occupied by peroxisomes (Table 3).

Exposure of male mice to 200 or 400 ppm PER also resulted in a decrease in mitochondria after 14 days but this was followed by mitochondrial proliferation in those animals subsequently exposed to 400 ppm. The effect was not seen in females. Concomitant with these changes, exposure at either level for any of the time periods investigated resulted in a decrease in the amount of normal rough endoplasmic reticulum in the cells.

Light microscopic examination of livers from rats exposed to PER showed centrilobular hypertrophy in both sexes with a concomitant loss of glycogen. The effects in males were of similar intensity in both the 200- and 400-ppm dose groups and there was little evidence of progression of the lesion from 14 to 28 days in the 400-ppm group. Results suggest that the males were more sensitive to the liver hypertrophic effects of PER since no effect was seen in females exposed to 200 ppm for 28 days.

Electron microscopy showed a time-dependent proliferation of smooth endoplasmic reticulum in the liver in both sexes which correlated well with the centrilobular hypertrophy. The males were more susceptible than the females. There was no dose- or time-dependant increase in peroxisomes in the livers of either sex (Table 3).

Kidney

No increases in kidney/body weight ratios were seen in rats and mice exposed to PER.

The effect of PER on peroxisomal cyanide-insensitive palmitoyl CoA oxidation in rats and mice is shown in Table 1. Insufficient mouse kidney tissue precluded the measurement of this marker in individual animals. Consequently values could not be tested for statistical significance. Slight increases were seen in β-oxidation in male mouse kidney, the maximum being a 1.6-fold increase after 21 days exposure to 400 ppm. Small increases in this marker were also observed in female rat kidneys after exposure to PER (Table 1) up to a maximum of 1.6-fold.

There was no effect of PER on renal catalase activity in rats or mice of either sex (data not shown).

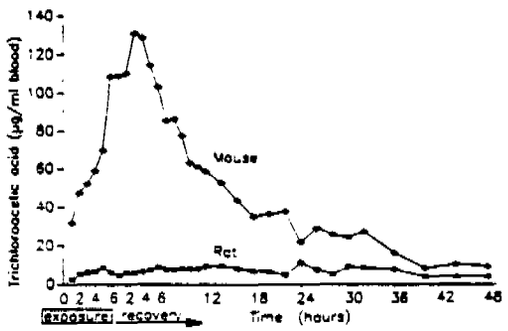

FIG. 2. Blood levels of TCA in mice and rats exposed to PER (400 ppm) for 6 hr and recovery. Values are means with three animals to each time point.

No compound-related changes were observed in the kidneys of either species at the light or electron microscope level.

Blood Levels of TCA after Exposure to PER

Blood levels of TCA in rats and mice during and after a 6-hr exposure to PER (400 ppm) are shown in Fig. 2. Peak blood levels of TCA (approximately 130 μg/ml) in mice were reached 3–4 hr after the end of the exposure period and thereafter declined with a half-life of 7–8 hr. Forty hours after exposure, levels persisted at 8–10 μg/ml. In contrast blood levels in the rat reached a plateau of approximately 7 μg/ml after 3 hr of exposure declining to 4 μg/ml 48 hr after the end of exposure. Comparison of the concentrations of TCA to which the two species were exposed, by calculation of the area under the curves, shows the mouse to have been exposed to 6.7-fold more TCA than the rat.

DISCUSSION

The hepatocarcinogenicity of PER in the mouse has been known for some years but no satisfactory mechanism, either genotoxic or epigenetic, has so far been proposed. The first step in the metabolism of PER is believed to be oxidation to an epoxide (Reichert, 1983) and the metabolites which have been identified support this assumption (Yllner, 1961; Daniel, 1963; Bonse *et al.*, 1975; Sakamoto, 1976). Alkylation of nucleotides by reactive epoxides has been described for other chlorinated alkenes such as vinyl chloride (Laib and Bolt, 1977) and vinylidene chloride (Reitz *et al.*, 1980). DNA binding has not however been demonstrated after treatment of rats and mice with PER (Schumann *et al.*, 1980) nor does PER induce gene mutations in bacteria (Bartsch *et al.*, 1979; Greim *et al.*, 1975; Bronzetti *et al.*, 1983). These observations led to proposals (Schumann *et al.*, 1980) that PER-induced liver tumors in B6C3F1 mice are a result of recurrent cytotoxicity and enhancement of the high spontaneous incidence of liver tumors found in this strain of mouse.

The results of the present study suggest an alternative hypothesis for the mechanism of PER-induced carcinogenicity, that of peroxisome proliferation leading to cancer formation via an epigenetic mechanism (Reddy *et al.*, 1980). Exposure of male and female mice to PER for up to 28 days resulted in a significant proliferation of peroxisomes in the liver as measured by peroxisomal β-oxidation (Table 1) and morphometric analysis of electron micrographs (Table 3). The induction of peroxisomal β-oxidation in this study was not accompanied by increases in catalase (Table 2). This phenomenon has been observed after administration of other peroxisome proliferators (Cohen and Grasso, 1981; Reddy and Lalwani, 1983, for review) and is believed to lead to increased levels of hydrogen peroxide in the cell, causing oxidative damage, cytotoxicity, and possibly DNA damage. However, a definite link between such changes and the eventual development of cancer remains to be established.

Although increases in the activity of peroxisomal enzymes were observed in the livers of rats after treatment with PER (Table 1), these were slight compared to the changes seen in the mouse and could not be corroborated by electron microscopy (Table 3). Sim-

ilar results after treatment of rats and mice with PER have recently been reported by Goldsworthy and Popp (1987). A sixfold increase in hepatic CN-insensitive palmitoyl CoA oxidase was observed in B6C3F1 mice dosed PER by gavage at 1000 mg/kg for 10 days. No such increase was seen in F344 rats. This species difference in peroxisome proliferation is identical to that found by Elcombe *et al.* (1985) after TRI was dosed by gavage to mice and rats. There were however differences in the pathology of treated livers between the present study and that reported by Elcombe *et al.* (1985) for TRI. PER-induced peroxisome proliferation was observed in the centrilobular region of mouse liver. The induced peroxisomes were small and retained the nucleoid core, whereas after TRI treatment they generally lacked the nucleoid core. Exposure to PER also resulted in a concomitant accumulation of lipid in centrilobular cells with periportal cells unaffected. The reasons for these differences are unknown but may be due to the effects of other metabolites or the parent chemical.

TCA, the major metabolite of PER (Dekant *et al.*, 1985), is a known hepatic peroxisome proliferator in both rats and mice (Elcombe, 1985) and is the metabolite responsible for increased peroxisomes in mice exposed to TRI in previous studies (Elcombe *et al.*, 1985). In the present study, TCA arising from metabolism of PER only induced hepatic peroxisome proliferation in mice because of the much higher concentrations of this metabolite in mouse blood. The lack of a response in rats indicates that a threshold concentration of TCA has to be reached in order to induce peroxisome proliferation in rodent liver. The low blood levels of TCA observed in rats compared with mice correlates with the lower rate of oxidative metabolism of PER in rats than mice (Schumann *et al.*, 1980; Ikeda and Ohtsuji, 1972).

Recent studies have confirmed that TCA is in fact a carcinogen in B6C3F1 mice (Herren-Freund *et al.*, 1986). TCA dosed to male mice in drinking water at 5 g/liter for 61 weeks produced a 50% tumor incidence compared to a 5% incidence in the control group. Peroxisome proliferation was observed in the livers of treated animals. Thus the species difference in the carcinogenicity of PER between rats and mice may be explained by the marked difference in blood levels of TCA and a mechanism which induces peroxisome proliferation.

The effect of PER on the kidney in mice and rats was minimal (Table 1). No compound-related changes were seen at the light or electron microscope level in regions of the nephron where peroxisomes are known to be most prevalent (Beard and Novikoff, 1969). Similarly the increases observed in peroxisomal enzymes were slight and not related to dose or exposure. Peroxisome proliferation is therefore unlikely to play a role in the carcinogenicity of PER in the rat kidney and further investigations are needed to establish an alternative mechanism.

Metabolism of PER in man is known to occur at a very slow rate (Fernandez *et al.*, 1976; Monster *et al.*, 1979). It is also a saturable process, saturation occurring at the low inhalational exposure level of 100 ppm (Ikeda *et al.*, 1972; Ohtsuki *et al.*, 1983). Consequently man is exposed to lower concentrations of TCA than mice or rats. Furthermore, TCA does not induce peroxisome proliferation *in vitro* in human hepatocytes (Elcombe, 1985); indeed the response of primates to the induction of peroxisome proliferation by other agents is generally much lower than that of rodent species (Cohen and Grasso, 1981; Reddy and Lalwani, 1983).

In conclusion this study demonstrates that quantitative differences in the metabolism of PER to TCA in mice and rats lead to proliferation of peroxisomes in the livers of mice but not rats. The known carcinogenicity of TCA in B6C3F1 mice and the correlation between hepatic peroxisome proliferation and cancer in rodents strongly suggests that TCA-induced peroxisome proliferation is the basis of the species difference in hepatocarcinogenicity of PER. The limited capacity of humans

to metabolize PER coupled with an intrinsic deficiency in response to TCA as a peroxisome proliferator indicates that PER is unlikely to cause hepatocellular carcinoma in man.

ACKNOWLEDGMENTS

The authors thank Mr. S. Millward and Mr. I. Bennett for carrying out the inhalation exposures and Mr. N. Gowans and Mr. W. M. Provan for their help with the TCA blood level study.

REFERENCES

BARTSCH, H., MALAVEILLE, C., BARBIN, A., AND PLANCHE, G. (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadiene and dichlorobutenes produced by rodent or human liver tissues. Evidence for oxirane formation by P450 linked microsomal mono-oxygenases. *Arch. Toxicol.* **41**, 249–277.

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.

BEERS, R. F., AND SIZER, J. W. (1952). A spectrophotometric method of measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **195**, 133–140.

BONSE, G., URBAN, TH., REICHERT, D., AND HENSCHLER, D. (1975). Chemical reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. *Biochem. Pharmacol.* **24**, 1829–1834.

BRONFMAN, M., INESTROSA, N. C., AND LEIGHTON, F. (1979). Fatty acid oxidation by human liver peroxisomes. *Biochem. Biophys. Res. Commun.* **88**, 1030–1036.

BRONZETTI, G., BAUER, C., CORSI, C., DEL CARRATORE, R., GALLI, A., NIERI, R., AND PAOLINI, M. (1983). Genetic and biochemical studies on perchloroethylene "*in vitro*" and "*in vivo*." *Mutat. Res.* **116**, 323–331.

COHEN, A. J., AND GRASSO, P. (1981). Review of the hepatic response to hypolipidaemic drugs in rodents and assessment of its toxicological significance to man. *Food Cosmet. Toxicol.* **19**, 585–605.

DANIEL, J. W. (1963). The metabolism of ^{36}Cl-labeled trichloroethylene and tetrachloroethylene in the rat. *Biochem. Pharmacol.* **12**, 795–802.

DEKANT, W., HANG, R., AND HENSCHLER, D. (1985). Absorption, elimination and metabolism of tetrachloroethylene. *Naunyn-Schmiedeberg's Arch. Pharmacol. (Suppl.)* **329**, R24.

DOE, J. E., AND TINSTON, D. J. (1981). Novel chambers for long term inhalation studies. In *Proceedings of the Inhalation Toxicology and Technology Symposium* (B. K. J. Leong, Ed.), pp. 77–88. Ann Arbor Science Pub., Ann Arbor, MI.

ELCOMBE, C. R. (1985). Species differences in carcinogenicity and peroxisome proliferation due to trichloroethylene: A biochemical human hazard assessment. *Arch. Toxicol. (Suppl.)* **8**, 6–17.

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.

FERNANDEZ, J., GUBERMAN, E., AND CAPEROS, J. (1976). Experimental human exposures to tetrachloroethylene vapour and elimination in breath after inhalation. *Amer. Ind. Hyg. Assoc. J.* **37**, 143–150.

GOLDSWORTHY, T. L., AND POPP, J. A. (1987). Chlorinated hydrocarbon induced peroxisome enzyme activity in relation to species and organ carcinogenicity *Toxicol. Appl. Pharmacol.* **88**, 225–233.

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, M., 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.

HERREN-FREUND, S. L., PEREIRA, M. A., OLSEN, G., AND DE ANGELO, A. B. (1986). The carcinogencity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid in mouse liver. *Proc. Amer. Assoc. Cancer Res.* **27**, 91.

IKEDA, M., AND OHTSUJI, H. (1972). A comparative study of the excretion of Fujiwara reaction-positive substances in urine of humans and rodents given trichloro or tetrachloro-derivatives of ethane and ethylene. *Br. J. Ind. Med.* **29**, 99–104.

IKEDA, M., OHTSUJI, H., IMAMURA, T., AND KOMOIKE, Y. (1972). Urinary excretion of total trichloro-compounds, trichloroethanol and trichloroacetic acid as a measure of exposure to trichloroethylene and tetrachloroethylene. *Br. J. Ind. Med.* **29**, 328–333.

LAIB, R. J., AND BOLT, H. M. (1977). Alkylation of RNA by vinyl chloride metabolites *in vitro* and *in vivo*: Formation of 1-^{6}*N*-etheno-adenosine. *Toxicology* **8**, 185–195.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

MENNEAR, J. H., CLARKE, W., RAGAN, H., AND MILLER, R. (1986). Toxicology and carcinogenicity

studies of inhaled tetrachloroethylene (PERC) in male and female F344 rats and B6C3F1 mice. *Toxicologist* 6, 135.

MONSTER, A. C., BOERSMA, G., AND STEENWEG, H. (1979). Kinetics of tetrachloroethylene in volunteers: Influence of exposure concentration and work load. *Int. Arch. Occup. Environ. Health* 42, 303–309.

National Cancer Institute (1976). *Carcinogenesis Bioassay of Trichloroethylene.* CAS No. 79-01-6. DHEW pub. No. (NIH) 76-802.

National Cancer Institute (1977). *Bioassay of Tetrachloroethylene for Possible Carcinogenicity.* DHEW pub. No. 77-813.

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

OHTSUKI, T., SATO, K., KOIZUMI, A., KUMAI, M., AND IKEDA, M. (1983). Limited capacity of humans to metabolise tetrachloroethylene. *Int. Arch. Environ. Health* 51, 381–390.

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). Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature (London)* 283, 397–398.

REDDY, J. K., AND LALWANI, N. D. (1983). Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* 12, 1–58.

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

REDDY, J. K., WARREN, J. R., REDDY, M. K., AND LALWANI, N. D. (1982). Hepatic and renal effect of peroxisome proliferators: Biological implications. *Ann. N.Y. Acad. Sci.* 386, 81–110.

REICHERT, D. (1983). Biological actions and interactions of tetrachloroethylene. *Mutat. Res.* 123, 411–429.

REITZ, R. H., WATANABE, P. G., MCKENNA, M. J., QUAST, J. F., AND GEHRING, P. J. (1980). Effects of vinylidene chloride on DNA synthesis and DNA repair in the rat and mouse: A comparative study with dimethylnitrosamine. *Toxicol. Appl. Pharmacol.* 52, 357–370.

SAKAMOTO, N. (1976). Metabolism of tetrachloroethylene in guinea pigs. *Japan. J. Health* 18, 11–16.

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.

WEIBEL, E. R., STAUBLI, W., GNAGE, H. R., AND HESS, F. (1964). Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model stereological methods and normal morphometric data for rat liver. *J. Cell Biol.* 21, 68–91.

YLLNER, S. (1961). Urinary metabolites of 14C-tetrachloroethylene in mice. *Nature (London)* 191, 820.