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The Pharmacokinetics and Macromolecular Interactions of Trichloroethylene in Mice and Rats[1]

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The Pharmacokinetics and Macromolecular Interactions of Trichloroethylene in Mice and Rats. STOTT, W. T., QUAST, J. F., AND WATANABE, P. G., (1982). *Toxicol. Appl. Pharmacol.* 62, 137–151. Male B6C3F1 mice metabolized inhaled trichloroethylene (TRI) (600 ppm/6 hr) to a greater extent (262% more) than male Osborne-Mendel rats. Mice metabolized more (332%) inhaled TRI to a hepatic macromolecular binding metabolite *in vivo* than rats. Oral administration of TRI resulted in treatment-related hepatocellular cytotoxicity in repeated dosing trials in the mouse. Hepatic effects observed in mice treated with a maximum tolerated dose of 2400 mg/kg/day TRI for 3 days were primarily centrilobular hepatocellular swelling with focal hepatocellular necrosis. These effects lead to an enhanced regenerative process as indicated by increased hepatic DNA synthesis activity (220% of control) and incidence of mitotic figures. Treatment of mice (po) with TRI for a 3-week period (5 days/week) resulted in a dose-related increase in hepatocellular swelling with giant and mineralized cells present in the 2400 mg/kg/day dosed animals. Contrasting the mouse data, rats appeared to be less sensitive to a maximum tolerated dose level of TRI, displaying enhanced hepatic DNA synthesis levels (175% of control) but no histopathology after a similar 3-week treatment with 1100 mg/kg/day TRI. Renal tissue in both species was not significantly affected by TRI. An estimate of the extent of TRI interaction with DNA was also determined by measuring the radioactivity associated with purified hepatic DNA. Only a very low level of *in vivo* TRI–DNA interaction was observed in mice given 1200 mg/kg TRI po which is reportedly tumorigenic upon chronic administration (maximum estimate = 0.62 ± 0.43 alkylation/10^6 nucleotides). When coupled with the very weak or negative responses of pure TRI in *in vitro* mutagenesis assays, the DNA alkylation data indicate a lack of genotoxic potential. These data *in toto* suggest an epigenetic mechanism of tumor formation in the B6C3F1 mouse, implying that a tumorigenic response to TRI exposure in these animals would only be evident upon chronic administration of high, cytotoxic dose levels of TRI.

Trichloroethylene (TRI) has been widely used as an industrial solvent and dry cleaning agent. The safety of trichloroethylene has been questioned on the basis of the results of a carcinogen bioassay conducted by the National Cancer Institute (NCI) (1976); an increased incidence of hepatocellular carcinoma was observed in male mice given TRI. A confounding factor in this study (NCI, 1976) was the use of TRI containing approximately 0.2% epichlorohydrin (Henschler *et al.*, 1977) which has previously been shown to cause nasal carcinomas in rats at toxic doses (Laskin *et al.*, 1980). No increased incidence of tumors was found in a recent 18-month chronic inhalation bioassay of 100 and 500 ppm nonepoxide-stabilized TRI in hamsters, rats, and mice (Henschler

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et al., 1980). In addition, a cohort study of workers exposed to TRI has not revealed any increase in cancer mortality (Axelson *et al.*, 1978).

TRI has been reported to be both negative or weakly positive in numerous mutagenicity bioassays. However, many of these studies are of questionable value because they lack enough data for critical evaluation and/or analytical data regarding the presence of mutagenic epoxide-stabilizing compounds in the TRI utilized (Duprat and Gradiski, 1980; Konietzko *et al.*, 1978; Slacik-Erben *et al.*, 1980; Fahrig, 1977; Bartsch *et al.*, 1979; Bronzetti *et al.*, 1978; Greim *et al.*, 1975; Shahin and Von Borstel, 1977; Simmon *et al.*, 1977; Waskell, 1978). Further, TRI metabolites reportedly bind to protein, RNA, and DNA *in vitro* and protein *in vivo* (Laib *et al.*, 1979; Uehleke and Poplawski-Tabarelli, 1977; Van Duuren and Banerjee, 1976; Bolt *et al.*, 1977; Bolt and Filser, 1977; Banerjee and Van Duuren, 1978).

The present study was undertaken to provide a better understanding of the bioassay results (NCI, 1976) and their relevance in terms of carcinogenic risk to humans exposed to TRI. This evaluation was approached by studying the metabolism, bioactivation, and cellular interactions of TRI in susceptible (B6C3F1) and nonsusceptible (Osborne-Mendel) mice and rats, respectively.

METHODS

Chemicals and radiochemicals. Trichloroethylene (>99.9% purity, amine stabilized) was obtained from the Dow Chemical Company (Freeport, Tex.) [UL-^{14}C]Trichloroethylene [^{14}C](TRI) (specific activity = 11.3 mCi/mmol) was purchased from Midwest Research Institute (Kansas City, Mo.). Radiochemical purity was determined to be >99% by radio-gas chromatographic/mass spectroscopic analysis. [6-^{3}H]Thymidine ([^{3}H]dT) (specific activity = 24.7 mCi/mmol) was obtained from New England Nuclear (Boston, Mass.). All enzymes, nucleosides, and nucleic acid base standards were obtained from Sigma Chemical Company (St. Louis, Mo.).

Animals. Male B6C3F1 mice (17 to 29 g) were obtained from Charles River Breeding Laboratory (Portage, Mich.) and male Osborne-Mendel rats (190 to 250 g) were obtained from CAMM Research Laboratory (Wayne, N.J.). All animals were fed rat chow (Ralston Purina, St. Louis, Mo.) and given water *ad libitum*, housed under conditions of a 12-hr light cycle, 21 ± 2°C, 50 ± 15% R.H., and acclimated at least 7 days. Animals were housed in groups except for rats on study which were housed individually.

Metabolism study. Sixteen animals were exposed to either 10 or 600 ppm [^{14}C]TRI for 6 hr in a 30-liter glass inhalation chamber supplied with an airflow either of 5 liters/min (mice) or 10 liters/min (rats). Four animals per exposure were acclimated ≥1 day in metabolic cages prior to exposure. Chamber concentrations were monitored continuously by a Wilks Infrared spectrometer (S. Norwalk, Conn.). The chamber concentrations were maintained within 10% of targeted concentrations for the entire exposure period (9.8 ± 0.5 and 613 ± 6 ppm for mice; 9.4 ± 0.7 and 630 ppm for rats). The specific activity of the chamber [^{14}C]TRI was analyzed at the beginning and end of exposure by slowly bubbling 0.5 ml of chamber atmosphere through 20 ml of ACS scintillation medium (Amersham Corp., Arlington Heights, Ill.) and counting ^{14}C in a Beckman Model No. LS9000 liquid scintillation counter (Beckman Instruments, Inc., Anaheim, Calif.). Specific activities equalled 665 μCi and 174 μCi/mmol [^{14}C]TRI for mice, and 15 μCi and 4.5 μCi/mmol [^{14}C]TRI for rats in 10 and 600-ppm exposure levels, respectively. At the termination of [^{14}C]TRI exposure, four animals were placed individually into Rothtype metabolism cages for sequential collection of excreta including expired volatiles. The remaining 12 animals were utilized for studies of macromolecular binding (see below).

Excretion of ^{14}C activity in the urine, feces, and expired air was monitored for 50 hr postexposure. Urine was collected at −80°C, carbon dioxide was trapped by bubbling the chamber effluent through a solution of 2-methoxyethanol:ethanolamine (7:3), and expired [^{14}C]TRI was trapped on activated charcoal columns (6 g). In preliminary experiments, charcoal trapping of [^{14}C]TRI was observed to be essentially complete, and the trapped ^{14}C was found by GC–MS analysis to be [^{14}C]TRI; desorption of trapped [^{14}C]TRI by toluene for 4 hr was >95%. It was also noted in preliminary experiments that >98% of gavage-administered [^{14}C]TRI was recovered in 48 hr. Therefore in the present study, at approximately 50 hr post [^{14}C]TRI exposure the animals were killed by decapitation; the liver, kidneys, and a patch of skin from the back were collected, weighed, and stored at −80°C until analyzed. Carcasses were skinned, weighed, ground, and homogenized with a measured volume of distilled water and stored at −80°C. The micromole equivalents (μmol-eq) of [^{14}C]TRI in

the samples were then determined by direct liquid scintillation counting (LSC) (urine, CO_2 trap, and toluene-desorbed TRI) or by first oxidizing samples (liver, kidney, skin, feces homogenate, and carcass homogenate) in a Harvey Biological Material Oxidizer (Hillsdale, N.J.). Results are expressed as total micromole equivalents of [^{14}C]TRI present in a sample.

Macromolecular binding. At 0, 6, and 24 hr postinhalation exposure to 10 or 600 ppm [^{14}C]TRI for 6 hr (see above), four animals were killed by decapitation. The livers and kidneys were excised, frozen on dry ice, and stored at −80°C. Tissue samples were obtained from mice or rats from the metabolism study at 50 hr postexposure. A 1:1 (tissue:water) homogenate was then prepared, and the irreversible binding of radioactivity to hepatic and renal tissue was determined (Jollow *et al.*, 1973). After exhaustive solvent extraction of the trichloroacetic acid precipitable material, the pellet was solubilized in 1 M potassium hydroxide and neutralized with 1 M hydrochloric acid; the ^{14}C content was then quantitated by LSC. Protein content of the pelleted material was assayed by the method of Lowry *et al.* (1951). The results are expressed as picomole equivalents of TRI bound per microgram of protein.

Repeated dosing experiments. For the 3-day study, mice were gavaged with 0 or 2400 mg/kg/day TRI in corn oil (USP grade, 10 ml/kg), and rats received daily doses of TRI (0 and 1100 mg/kg/day TRI) in corn oil (5 ml/kg). All animals received daily sc injections of [^{3}H]thymidine ([^{3}H]dT) 3 to 4 hr after TRI dosing. Mice were administered 1 mCi, 15.2 μg [^{3}H]dT, and 10 ml volume/kg (specific activity = 15.0 Ci/mmol) while rats were administered 0.1 mCi, 1.52 μg [^{3}H]dT, and 1 ml volume/kg (specific activity = 15.9 Ci/mmol). In the first 3-week study, mice were treated as above (5 days/week) except that animals were administered [^{3}H]dT (0.1 mCi, 6.42 μg [^{3}H]dT, and 10 ml volume/kg, specific activity = 3.77 Ci/mmol) on the last 5 days of TRI dosing. In the larger, second 3-week study, mice received either 0, 250, 500, 1200, or 2400 mg/kg/day (5 days/week) TRI in corn oil (10 ml/kg). Mice received sc injections of [^{3}H]dT as described for the 3-day experiments on the 4 days preceding termination of the experiment. Rats were dosed with TRI as above and treated with [^{3}H]dT via implantation between the shoulder blades of an osmotic pump (Alza Corp., Palo Alto, Calif.) containing 0.1 mCi and 1 μg [^{3}H]dT on Day 15 of the experiment.

Upon termination of an experiment, animals were killed by decapitation, the liver and kidneys (where noted in results) were excised, weighed, placed on dry ice, and stored at −80°C. Upon thawing, DNA was extracted from tissues after the procedure outlined by Reitz *et al.* (1980a) and quantitated by the diphenylamine method of Burton (1956). DNA synthesis indicated by [^{3}H]dT incorporation was detected by LSC. DNA synthesis activity ([^{3}H]dT incorporation) is expressed as disintegrations per minute [^{3}H]dT per microgram of DNA.

DNA alkylation in vivo: DNA isolation and high-pressure liquid chromatography analysis. Mice were dosed by gavage with 1200 mg/kg [^{14}C]TRI (>500 μCi/animal, specific activity = 2.4 mCi/mmol) in corn oil (10 ml/kg) and killed 5 hr later by decapitation. Livers were immediately excised, frozen on dry ice, and stored at −80°C (<2 days). DNA was isolated by a combination of the methods of Marmur (1961) and Kirby (1957). Isolated DNA was free of detectable RNA (orcinol reaction) and glycogen (anthrone reaction). Quantification of DNA and ^{14}C contents was accomplished as described above on small aliquots of the sample.

High-pressure liquid chromatographic (HPLC) analysis of the remaining DNA isolate was undertaken by measuring the radioactivity associated with isolated free purine bases or nucleosides. Half the DNA samples were enzymatically digested to nucleosides in 0.05 M Tris-0.01 M $MgCl_2$ buffer (pH 7.8) by 0.05 mg DNAase I, 1 mg snake venom phosphodiesterase (*Crotalus atrax*), and 1.5 U alkaline phosphatase/mg DNA at 37°C for 16 hr. The remaining half of the samples were digested in 0.1 M HCl for 30 min at 70°C, a procedure that cleaves purine bases from the phosphate–sugar backbone of DNA without significant loss of alkylated products (Lawley, 1976; Swenson and Lawley, 1978). Both types of DNA digests were analyzed by HPLC on a Partisil M-9 10/50 SCX column (Whatman Inc., Clifton, N.J.) with a Waters Model 6000A pump (Milford, Mass.) equipped with a Perkin–Elmer Model LC 55B Spectrophotometer (Oak Brook, Ill.). Nucleosides and bases were eluted from the column by an isocratic flow of 2 ml/min of 0.1 M ammonium phosphate buffer (pH 3.5) and detected at 254 nm. Fractions (1.0 ml) of the eluate were collected and following addition of 10 ml Aquasol-2 scintillation medium (New England Nuclear), the ^{14}C content was determined by LSC. Significant levels of disintegrations per minute sample were determined to be those greater than a possible two sigma counting error above the mean background disintegrations per minute sample (i.e., mean of background plus possible counting error). This procedure has given excellent separation of alkylated nucleosides or bases from normal nucleosides and bases in our laboratory. The pattern of radioactivity elution was then compared to the nucleoside or free base elution pattern allowing for the differentiation of metabolically incorporated (C-1) ^{14}C in normal bases, void volume of ^{14}C associated with trace protein or glycogen contamination (C-1 metabolic incorporation or binding) and true DNA alkylated bases.

Histopathology. Tissue samples were taken from the central region of the left lobe of the liver and/or right

kidney prior to freezing of the excised tissue. Samples were fixed in 10% neutral-buffered formalin, sectioned, processed by standard procedures, stained with hematoxylin–eosin, and observed microscopically for evidence of morphologic changes associated with treatment.

Statistics. Animals were computer randomized into treatment groups in all repeated dosing experiments and checked for group homogeneity and significant differences between groups by Bartlett's test and Dunnett's t test (Steel and Torrie, 1960). All results were compared by Dunnett's or Student's t test (Steel and Torrie, 1960) with the level of significance set at $p < 0.05$.

RESULTS

Metabolism

As shown in Table 1, 98 to 99% of the total ^{14}C body burden of male B6C3F1 mice exposed to either 10 or 600 ppm [^{14}C]TRI for 6 hr was metabolized within 50 hr. The primary route of excretion in mice was the urine constituting approximately 75% of the total TRI body burden. Approximately 9% of the [^{14}C]TRI body burden was biotransformed to $^{14}CO_2$. As shown by the general lack of change in the routes of excretion and total metabolism of [^{14}C]TRI, the metabolism of [^{14}C]TRI by the B6C3F1 mouse did not appear to be saturated at the 600-ppm-TRI exposure level. While small increases in excretion of unmetabolized [C^{14}]TRI (exhaled) and carcass ^{14}C were observed between the low- and high-exposure levels, these changes were within the potential experimental error. In contrast, the metabolism of [^{14}C]TRI by the male Osborne–Mendel rat appeared to show characteristics of saturation at the 600-ppm exposure level (Table 2). Total metabolism of [^{14}C]TRI (μmol-eq TRI/kg body wt) was decreased in the rats exposed to 600 ppm (79% [^{14}C]TRI body burden) relative to those exposed to 10 ppm (98% [^{14}C]TRI body burden). At the same time exhalation of [^{14}C]TRI increased 10-fold with increased [^{14}C]TRI exposure level. As with mice, the primary route of excretion of [^{14}C]TRI was via the urine which accounted for 62 and 55% of body burden [^{14}C]TRI in low- and high-dose rats, respectively.

TABLE 1

RECOVERY OF RADIOACTIVITY FOR 50 hr POSTINHALATION EXPOSURE OF MALE B6C3F1 MICE TO 10 OR 600 ppm 1,1,2[UL-^{14}C]TRICHLOROETHYLENE FOR 6 hr[a]

	10 ppm		600 ppm	
	μmol-eq TRI/kg body wt	%[b]	μmol-eq TRI/kg body wt	%[b]
Expired				
1,1,2-TRI	0.617 (0.182)	0.79	76.2 (27.3)	2.4
CO_2	7.38 (0.383)	9.4	299 (37.5)	9.5
Urine	58.1 (18.8)	74.0	2278 (505)	72.6
Feces	2.92 (0.542)	3.7	115 (24.6)	3.7
Cage wash[c]	1.10 (0.446)	1.4	85.5 (65.5)	2.7
Skin	2.33 (1.13)	3.0	56.3 (11.9)	1.8
Liver	2.40 (0.401)	3.1	72.2 (10.4)	2.3
Kidney	0.310 (0.310)	0.39	11.1 (3.18)	0.35
Carcass	1.50 (0.316)	1.9	146 (57.9)	4.6
Total body burden	78.5 (17.9)		3138 (616)	
Total metabolized	77.9 (18.1)	99.2	3062 (592)	97.6

[a] Average of four animals (±SD).
[b] Percentage of recovered radioactivity.
[c] Primarily due to urine.

TABLE 2

RECOVERY OF RADIOACTIVITY FOR 50 hr POSTINHALATION EXPOSURE OF MALE OSBORNE–MENDEL RATS TO 10 OR 600 ppm 1,1,2[UL-^{14}C]TRICHLOROETHYLENE FOR 6 hr[a]

	10 ppm		600 ppm	
	μmol-eq TRI/kg body wt	%[b]	μmol-eq TRI/kg body wt	%[b]
Expired				
1,1,2-TRI	0.778 (0.103)	2.1	227 (6.40)	21.1
CO_2	1.71 (0.295)	4.8	31.2 (2.10)	2.9
Urine	22.6 (5.96)	63.1	594 (79.8)	55.3
Feces	2.52 (0.253)	7.0	38.3 (3.47)	3.6
Cage wash[c]	0.357 (0.204)	1.0	15.1 (10.7)	1.4
Skin	3.78 (1.21)	10.6	76.2 (30.3)	7.1
Liver	1.01 (0.085)	2.8	16.3 (1.13)	1.6
Kidney	0.131 (0.009)	0.37	1.72 (0.083)	0.16
Carcass	2.82 (0.898)	7.9	75.1 (27.7)	7.0
Total body burden	35.8 (5.64)		1075 (87.2)	
Total metabolized	35.0 (5.64)	97.8	847 (81.6)	78.9

[a] Average of four animals (±SD).
[b] Percentage of recovered radioactivity.
[c] Primarily due to urine.

A comparison between the mouse and rat revealed that the mouse metabolized 123% ($p < 0.01$) more body burden TRI on a per kilogram body weight basis than rats after exposure to 10 ppm and 262% ($p < 0.01$) more [^{14}C]TRI/kg than rats after exposure to 600 ppm.

Macromolecular Binding

As shown in Table 3, mice activated more [^{14}C]TRI to a reactive, macromolecular binding metabolite than did rats upon exposure to 10 and 600 ppm [^{14}C]TRI. The species differences in binding, measured as picomole-equivalents as [^{14}C]TRI per microgram of protein, were considerably greater (three- to fourfold) in both hepatic and renal tissues following the 600-ppm exposure, and only a modest increase in binding was observed in hepatic tissue following the 10-ppm exposure. Presumably this finding was due to the saturation of [^{14}C]TRI metabolic activation in rats exposed to 600 ppm while the activation of [^{14}C]TRI in mice remained unsaturated. Maximum binding observed in the liver occurred immediately postexposure (3 hr for the kidneys) and decreased steadily over the next 48 hr.

Repeated Dosing Experiments

Repeated gavage administration of dosages of TRI to mice caused alterations in hepatocellular morphology and in levels of hepatic DNA synthesis but had no observable effect on renal tissue (Table 4). Mice dosed for 3 days with 2400 mg/kg/day TRI had increased liver weights (120% of control), slightly less DNA per gram of tissue (90% of control), and an increase in hepatic DNA synthesis activity (222% of control). Upon microscopic examination of hepatic tissue, histopathological changes were observed which included a generalized pattern of centrilobular hepatocellular swelling with

TABLE 3

HEPATIC AND RENAL MACROMOLECULAR BINDING OF 1,1,2[UL-^{14}C]TRICHLOROETHYLENE METABOLITE IN MALE B6C3F1 MICE AND OSBORNE-MENDEL RATS EXPOSED TO 10 OR 600 ppm FOR 6 hr[a]

Exposure (ppm)	Tissue	Test animal	Binding (pmol-eq [^{14}C]TRI/μg protein)	Ratio (mouse/rat)
10	Liver	Mouse	0.318 (0.004)[b]	1.2
		Rat	0.268 (0.013)	
	Kidney	Mouse	0.168 (0.014)	1.1
		Rat	0.153 (0.007)	
600	Liver	Mouse	20.4 (2.49)[b]	4.3
		Rat	4.72 (0.415)	
	Kidney	Mouse	5.06 (0.667)[b]	3.0
		Rat	1.77 (0.200)	

[a] Average of four animals (±SD) at time of maximum binding postexposure (liver = 0 hr, kidney = 3 hr).
[b] $p < 0.01$.

some individual hepatocellular acute necrosis and an increase in mitotic activity.

The response to TRI exposure after 3 weeks of repeated gavage dosing over a broad dose range in mouse hepatic tissue is shown in Tables 4 and 5. In an initial 3-week repeated dosing experiment (Table 4), mice dosed with 2400 mg/kg/day had a large increase in liver weight (170% of control), a substantial decrease (76% of control) in DNA content per gram of tissue (indicative of larger cell size), and an increase in hepatic DNA synthesis activity (181% of control). Histopathological changes observed were a

TABLE 4

ORGAN-TO-BODY WEIGHT RATIOS, DNA CONTENT, DNA SYNTHESIS, AND HISTOPATHOLOGY DATA FROM MALE B6C3F1 MICE ADMINISTERED 1,1,2-TRICHLOROETHYLENE BY GAVAGE FOR 3 DAYS OR 3 WEEKS (5 DAYS/WEEK)[a]

Experiment (dose level)	Percentage organ weight/body weight	mg DNA/g tissue	DNA synthesis: dpm [^{3}H]dT/μg DNA	DNA synthesis: Treated/control	Histopathology[b]
3-Day repeated gavage					
Control (corn oil)					
Liver	4.6 (0.19)	3.19 (0.20)	120.7 (37.6)		–
Treated (2400 mg/kg)					
Liver	5.5 (0.32)[c]	2.89 (0.19)[c]	268.3 (69.2)[c]	2.22	+++
3-Week repeated gavage (5 days/week)					
Control (corn oil)					
Liver	5.28 (0.25)	2.99 (0.21)	11.06 (2.25)		–
Kidney	1.49 (0.14)	5.63 (0.64)	10.35 (2.40)		–
Treated (2400 mg/kg)					
Liver	8.95 (0.72)[c]	2.27 (0.13)[c]	20.02 (6.96)[c]	1.81	+++
Kidney	1.49 (0.11)	4.98 (0.60)	8.39 (1.11)	0.81	–

[a] Average of 10–12 animals (±SD).
[b] –, No histopathology observed, +++, histopathology observed (see text for description).
[c] $p < 0.01$.

TABLE 5

LIVER-TO-BODY WEIGHT RATIOS, DNA CONTENT, DNA SYNTHESIS, AND HISTOPATHOLOGY DATA FROM MALE B6C3F1 MICE ADMINISTERED 1,1,2-TRICHLORETHYLENE BY GAVAGE FOR 3 WEEKS (5 DAYS/WEEK)[a]

Dose level (mg/kg)	Percentage liver weight/body weight	μg DNA/g hepatic tissue	DNA synthesis: dpm [³H]dT/μg DNA	DNA synthesis: Treated/control	Histopathology[b]
Control (corn oil)	4.85 (0.28)	2.72 (0.20)	128 (27.5)		–
Treated					
250	5.20 (0.53)	2.52 (0.22)	108 (34.0)	0.84	+
500	5.46 (0.61)**	2.39 (0.20)**	87.6 (15.4)*	0.68	+
1200	6.56 (0.42)**	2.14 (0.12)**	140 (41.0)	1.09	++
2400	8.57 (0.46)**	2.09 (0.36)**	156 (52.6)	1.22	+++

[a] Average of 10–12 animals (±SD).
[b] –, No histopathology observed, +++ > ++ > +, severity of observable histopathology.
* $p < 0.05$.
** $p < 0.01$.

generalized pattern of centrilobular hepatocellular swelling with giant cell inflammation and dystrophic mineralized cells in the centrilobular or midzonal regions of the lobule (Fig. 1). These mineralized (calcified) cells were indicative of earlier individual cell necrosis in these animals as seen in the 3-day repeated dosing trial. No renal effect of administered TRI was observed and body weights did not differ significantly between treated and control animals. In a second 3-week repeated TRI-dosing experiment (Table 5), mice dosed with 0, 250, 500, 1200, or 2400 mg/kg/day showed a clear dose-related hepatocellular hypertrophic response to administered TRI. A dose-related increase in liver weights (up to a maximum of 177% of control) and a decrease in DNA content per gram of tissue (77% of control) were observed in mice repeatedly dosed with ≥500 mg/kg/day TRI. Dose-related histopathological changes in hepatic tissue were observed at all dose levels. These changes ranged from slight increases in cytoplasmic eosinophilic staining of centrilobular hepatocytes (often associated with depleted glycogen or proliferation of smooth endoplasmic reticulum) at 250 and 500 mg/kg/day TRI dose levels to increased centrilobular hepatocellular swelling (1200 mg/kg/day TRI group), and to still more severe centrilobular hepatocyte swelling, giant cell inflammation, and mineralized cells (2400 mg/kg/day TRI group) (Fig. 1). While there was some indication of increased hepatic DNA synthesis at the higher-dose levels, the biological variability precluded statistical significance contrary to earlier observations.

Unlike mice, only slight, nonsignificant changes were observed in hepatic tissue of rats repeatedly dosed for 3 days with a 1100 mg/kg/day dose of TRI (Table 6). In rats repeatedly dosed for 3 weeks (5 days/week) with 1100 mg/kg/day TRI, an increase in liver weights (118% of control) with no decrease in DNA content per gram of tissue and no hepatic histopathology was observed. An increase in hepatic DNA synthesis activity (175% of control) in these animals was observed, however, indicating that the increased liver weight was apparently due to an increase in morphologically normal-looking hepatocytes. Renal tissue and body

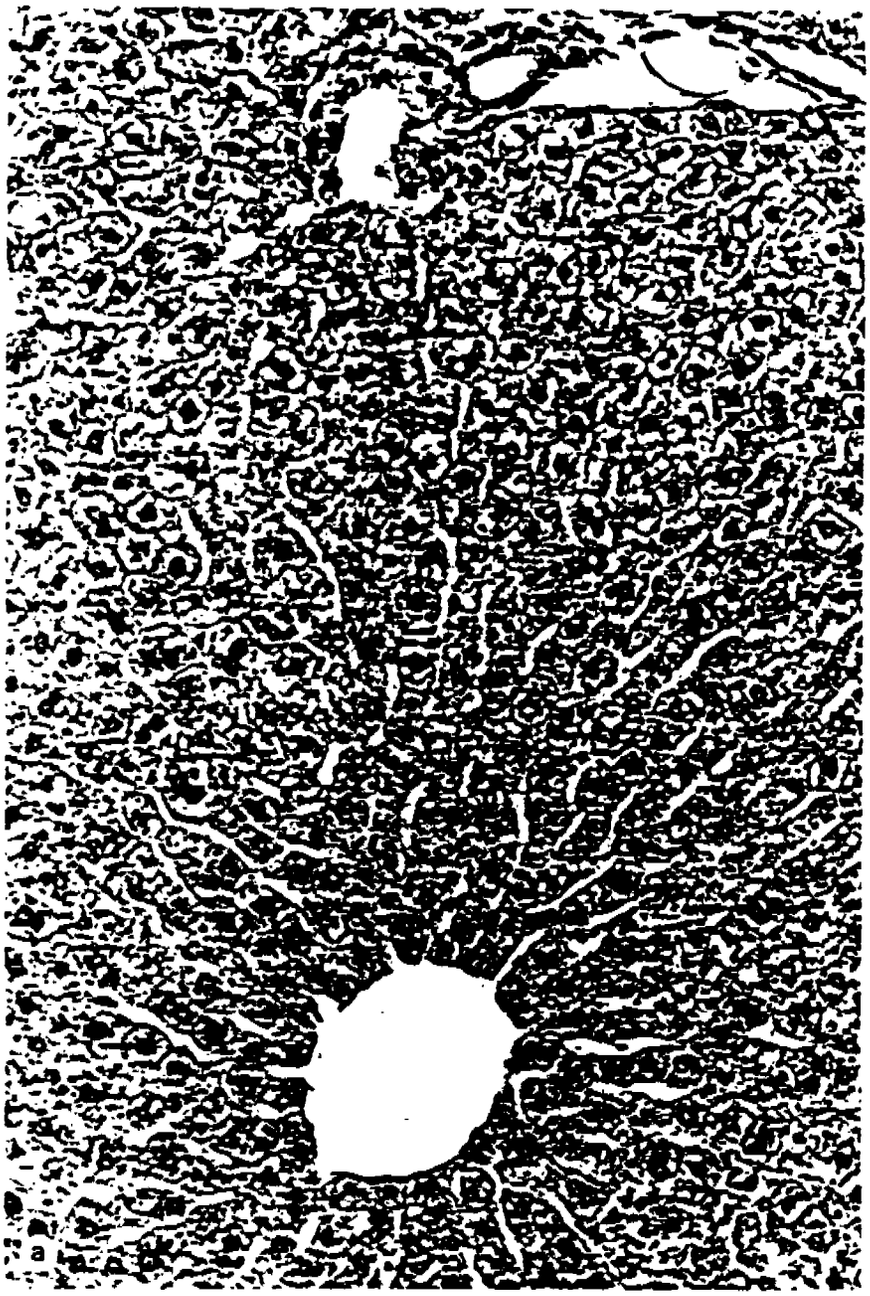

FIG. 1. Photomicrographs of hepatic tissues from (a) control mouse and (b) a mouse dosed with 2400 mg/kg/day for 3 weeks (5 days/week). Note the presence of dystrophic mineralized cells in the centrilobular region shown in the latter micrograph.

weight gains were not significantly affected by exposure to TRI. Overall, the Osborne-Mendel rats response to administered TRI was considerably less than that of the B6C3F1 mouse.

In Vivo DNA Alkylation

Investigation of the ability of TRI to alkylate hepatic DNA *in vivo* in mice indicated that TRI-alkylated DNA to only a

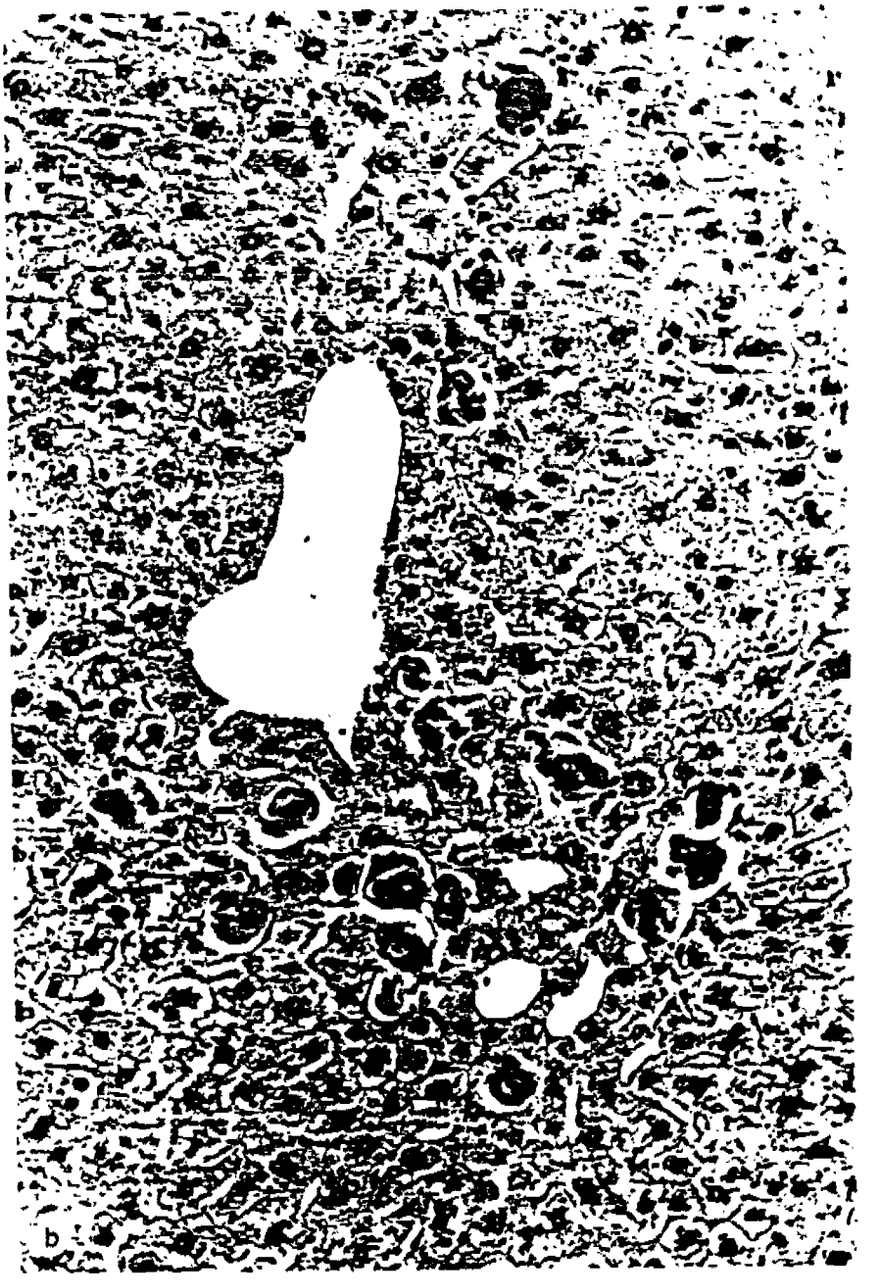

FIG. 1.—*Continued.*

very small degree (Table 7). In mice dosed with 1200 mg/kg of relatively high-specific-activity [^{14}C]TRI (2.4 mCi/mmol), a maximum estimate of the average DNA alkylation level of 0.62 ± 0.42 alkylations/10^6 nucleotides was observed in three of four treated mice. The possible erroneous contribution of trace contamination of the DNA isolate with alkylated proteins or of metabolic incorporation (C-1 pool) or TRI-de-

TABLE 6

ORGAN-TO-BODY WEIGHT RATIOS, DNA CONTENT, DNA SYNTHESIS, AND HISTOPATHOLOGY DATA FROM MALE OSBORNE-MENDEL RATS ADMINISTERED 1,1,2-TRICHLOROETHYLENE BY GAVAGE FOR 3 DAYS OR 3 WEEKS (5 DAYS/WEEK)[a]

Experiment (dose level)	Percentage organ weight/body weight	μg DNA/g tissue	DNA synthesis: dpm [³H]dT/μg DNA	DNA synthesis: Treated/control	Histopathology[b]
3-Day repeated gavage					
Control (corn oil)					
Liver	4.00 (0.19)	2.94 (0.26)	35.4 (13.5)		–
Treated (1100 mg/kg)					
Liver	4.40 (0.27)	2.65 (0.29)	42.8 (6.78)	1.21	–
3-Week repeated gavage (5 days/week)					
Control (corn oil)					
Liver	3.77 (0.21)	3.27 (0.40)	1.21 (0.61)		–
Kidney	0.79 (0.04)	3.65 (0.81)	5.50 (2.34)		–
Treated (1100 mg/kg)					
Liver	4.43 (0.21)*	3.27 (0.26)	2.12 (0.96)*	1.75	–
Kidney	0.82 (0.04)	3.53 (0.59)	6.31 (1.72)	1.15	–

[a] Average of four animals (±SD).
[b] –, No observable histopathology.
* $p < 0.01$.

rived radioactivity into normal DNA bases to the alkylation value was eliminated to a large extent by HPLC analysis of isolated DNA digests. This procedure is particularly necessary for compounds such as TRI which undergo considerable catabolism and protein binding *in vivo*. As shown in Fig. 2, a majority of the radioactivity (> counting error) eluted from the column with this particular DNA isolate was present in the void volume or coeluted with normal purines. Void volume radioactivity was attributed to trace

TABLE 7

IN VIVO ALKYLATION OF HEPATIC DNA BY 1,1,2[UL-^{14}C]TRICHLOROETHYLENE IN MALE B6C3F1 MICE DOSED WITH 1200 mg/kg [^{14}C]TRI BY GAVAGE

Animal	Detection limit (alk/10^6)	Non-C_1 dpm[a] >2 Sigma error	Maximum alkylations 10^6 nucleotides[b]	Maximum CBI[c]
1	0.12	18.0	0.50	0.055
2	0.52	ND[d]	—	—
3	0.15	7.1	0.27	0.030
4	0.18	26.6	1.1	0.120

[a] dpm not associated on HPLC analysis with normal DNA bases from normal metabolic incorporation and not attributable to counting error (i.e., mean background dpm + 2 SD).
[b] "Maximum" alkylations possible due to the relatively large amount of dpm associated with protein binding and metabolic incorporation into normal bases.
[c] Covalent binding index (Lutz, 1979). Comparative hepatic CBI for dimethylnitrosamine, diethylnitrosamine, methylnitrosourea, and aflatoxin B-1 are approximately 5500, 125, 640, and 17,000 respectively. CBI = (μmol adduct/mol dN)/(mmol/kg dose).
[d] None detected.

protein contamination in the isolated DNA and unhydrolyzed nucleotide oligomers since alkylated DNA bases routinely elute considerably beyond the solvent front and often beyond the normal DNA bases with the SCX column. A relatively large amount of void volume radioactivity also has been reported by Parchman and Magee (1980) in DNA isolated from [^{14}C]TRI-exposed rats and mice and by Laib *et al.* (1979) in *in vitro* microsome–RNA preparations. Radioactivity was associated with purine bases in all samples analyzed either as bases (acid hydrolysis) or as nucleosides (enzyme hydrolysis). This normal purine associated radioactivity was identical to that observed in experiments (data not shown) utilizing [^{14}C]formic acid to label the C-1 pool in mice and subsequently label purines (and eventually thymine also) within 5 hr of dosing. The remaining radioactivity (> counting error) not associated with normal bases or the void volume was assumed to represent true alkylated bases. However, the possibility of "spill over" of some [^{14}C]-labeled peptides from the void volume, the short retention times of these peaks (less than that expected for most alkylated bases), and the fact that no chemical identification of possible adducts was undertaken dictate that these alkylation values be viewed with caution. Thus, it is important that the base alkylation values obtained from these data should be regarded as a maximum possible alkylation which may have occurred.

DISCUSSION

The data presented provide a possible explanation of the observed differences in the tumorigenic response of male B6C3F1 mice and Osborne–Mendel rats to TRI (NCI, 1976) based upon their ability to metabolize more TRI to a reactive compound and subsequent hepatotoxicity. Mice metabolize more inhaled TRI than rats at both low (10 ppm)- and high (600 ppm)-exposure levels (123 and 260%, respectively). Further, the amount of total *in vivo* hepatic and renal macromolecular binding of TRI in these animals was observed to be as much as three-

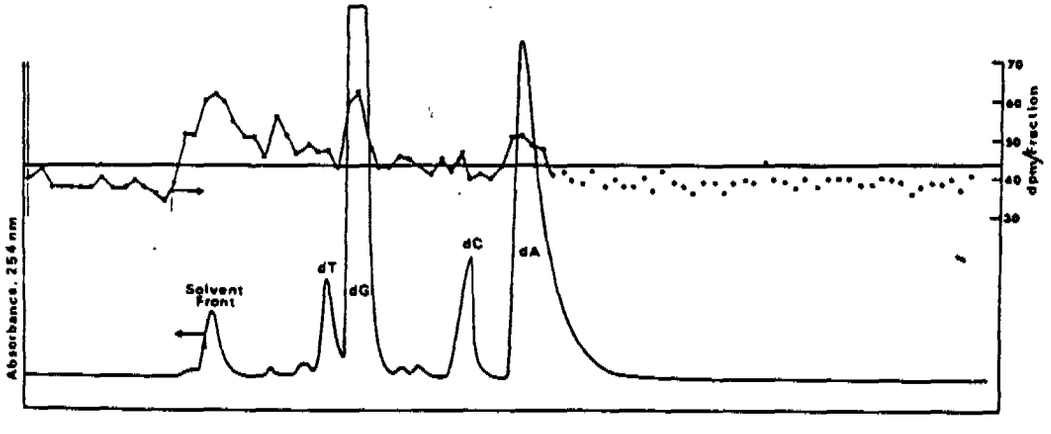

FIG. 2. HPLC strong cation-exchange chromatogram of enzymatic digest of hepatic DNA isolated from a B6C3F1 mouse 6 hr after dosing with 1200 mg/kg [^{14}C]TRI. Normal nucleosides shown are thymidine (dT), deoxyguanosine (dG), deoxycytidine (dC), and deoxyadenosine (dA). Radioactivity in collected fractions are also plotted (dots). The solid line at 43 dpm/fraction represents the mean background dpm/fraction plus counting error (2σ).

fold higher in mice than in rats (600-ppm exposure). Thus B6C3F1 mice appear to be capable of metabolizing more inhaled TRI to a toxic, reactive intermediate on a weight basis than Osborne-Mendel rats, especially at relatively high-exposure levels.

Humans metabolize TRI (inhaled) similar to rodents and excrete trichloroethanol and trichloroacetic acid in the urine (Ertle *et al.*, 1972; Ikeda and Imamura, 1973; Kimmerle and Eben, 1973; Monster *et al.*, 1976; Müller *et al.*, 1974). Based upon the kinetics of TRI clearance in humans (Monster *et al.*, 1976) (approximately 3.7 liters/hr·kg), humans metabolize approximately 20 times less TRI on a weight basis than rats (77 liters/hr·kg) at similar exposure levels of 70 and 65 ppm, respectively (Filser and Bolt, 1979). Thus any risk extrapolation made from rodent data to humans should take into account this fact. As demonstrated by Reitz *et al.* (1978, 1980b) with chloroform, chemicals which are metabolically activated may be less toxic in man than rodents if man metabolically activates less when expressed on a per kilogram basis.

The mechanism by which a compound may cause tumors has important implications in terms of the degree of carcinogenic hazard it may pose to humans. This subject has been extensively reviewed by several authors (Reitz *et al.*, 1980a; Schumann *et al.*, 1980; Stott *et al.*, 1981; Watanabe *et al.*, 1980; Weisburger and Williams, 1980). Briefly, the genetic mechanism of carcinogenesis is characterized by a direct interaction (alkylation or intercalation) of the chemical with DNA while epigenetic mechanisms of carcinogenesis do not involve a direct interaction of the chemical with genetic material. An epigenetic mechanism that reportedly may characterize the tumorigenic action of several chlorinated ethylenes in the B6C3F1 mouse has been that of recurrent cytotoxicity (Reitz *et al.*, 1980a; Schumann *et al.*, 1980). As reviewed by Stott *et al.* (1981) and Watanabe *et al.* (1980), this mechanism of tumorigenicity involves the enhancement of the normal cellular mutation rate during regenerative activity (increased DNA synthesis) in response to a cytotoxic challenge by a chemical. Little alkylation of DNA occurs and the toxicological impact of the chemical (i.e., cytotoxicity) displays characteristics of reversibility and threshold. Thus, a tumorigenic response of animals to chemicals of this type is observed only after prolonged exposure to toxic dose levels.

Consistent with a recurrent cytotoxicity mechanism of tumorigenicity, administration to mice of a dose of TRI which was tumorigenic upon chronic administration (2400 mg/kg/day for 3 days) caused a localized cell necrosis, an enhanced DNA synthesis, and a minimal degree of centrilobular hepatocellular swelling. Upon a more prolonged exposure (3 weeks), however, the primary response observed in mice was a dose-related centrilobular hepatocellular swelling and the occurrence of mineralized cells, presumably a result of hepatocellular necrosis in early stages of TRI dosing. Treatment-related hepatic effects were seen in male Osborne–Mendel rats only after prolonged (3 weeks) dosing of a maximum tolerated dose of TRI (1100 mg/kg/day). Increased liver weights and elevated DNA synthesis levels were not coupled with observable histopathology or decreased DNA content per gram of tissue suggesting a simple increase in the number of normal hepatocytes rather than hepatic degenerative/regenerative changes as seen in the mouse. Similar differences in the responses of Wistar rats and B6C3F1 mice to repeated administration of TRI have been reported by Elcombe *et al.* (1981).

Despite reports of weak mutagenic activity in *in vitro* assays (Bronzetti *et al.*, 1978; Greim *et al.*, 1975), TRI (nonepoxide inhibited) did not readily bind hepatic DNA of B6C3F1 mice *in vivo*. The observed maximum of only 0.62 ± 0.42 alkylated bases/

10^6 nucleotides contrasts sharply with the hundreds of alkylations observed when mice were dosed with powerful genotoxins such as dimethylnitrosamine or methylnitrosourea (Lawley, 1976). While qualitative considerations are of obvious importance in the assessment of the significance of DNA binding, strictly quantitative data relative to dose (convalent binding index) have been useful in the ranking of the carcinogenic potential of many tumorigenic compounds (Lutz, 1979). Thus the genotoxic potential of TRI based upon *in vivo* DNA binding data and numerous negative bacterial mutagenicity assays appears to be very slight.

The observed weak genotoxic potential and hepatotoxicity of TRI in B6C3F1 mice dosed with a high level of TRI are consistent with an epigenetic recurrent cytotoxic mechanism of tumorigenic action. However, the observed hepatic hypertrophic response and reported induction of the hepatic mixed-function oxygenase enzyme system (Norpoth *et al.*, 1974; Pessayre *et al.*, 1979; Savolainen *et al.*, 1977) of animals treated with TRI suggest another possible epigenetic mechanism of action. As suggested by Tennekes (1979) for several other enzyme-inducing agents (e.g., phenobarbital), this mechanism may simply involve the enhancement of preexisting oncogenic factors present in this mouse strain. Indeed, the B6C3F1 mouse reportedly has a very high spontaneous liver tumor rate (5 to 45%) (Elashoff *et al.*, 1979). In either case, the B6C3F1 mouse appears to be predisposed metabolically to produce more reactive TRI metabolite than Osborne–Mendel rats and to respond to subsequent tissue damage by the production of tumors.

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