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Toxicology 138 (1999) 43–53

TOXICOLOGY

www.elsevier.com/locate/toxicol

Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene

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Received 25 March 1999; accepted 16 June 1999

Abstract

Oxidative DNA damage is emerging as an biomarker of effect in studies assessing the health risks of occupational chemicals. Trichloroethylene (TCE) and perchloroethylene (PERC) are used in the dry cleaning industry and their metabolism can produce reactive oxygen compounds. The present study examined the potential for TCE and PERC to induce oxidative DNA damage in rats that was detectable as increased urinary excretion of 8-hydroxydeoxyguanosine (8OHdG). Thiobarbaturic acid reactive substances (TBARS) and 8-epi-prostaglandin $F_{2\alpha}$ (8epiPGF) were also measured as biomarkers of increased oxidative stress. Male Fischer rats were administered a single i.p. injection of 0, 100, 500, or 1000 mg/kg of PERC or TCE. Control rats received only vehicle (1:4 v/v of Alkamuls/water). A positive control group received 100 mg/kg 2-nitropropane (2NP). Rats were sacrificed 24 h after dosing. In rats receiving 2NP or TCE but not PERC, TBARS and the 8OHdG/dG ratios were significantly elevated in liver. Lymphocyte 8OHdG/dG was not affected significantly by 2NP, TCE or PERC. In rats receiving 2NP, urinary excretion of 8OHdG and 8epiPGF2 were significantly increased. In rats receiving TCE or PERC, significant increases in 8epiPGF2 or 8OHdG were not evident. Results indicate that a single high dose of TCE, but not PERC, can induce an increase in oxidative DNA damage in rat liver. However, the usefulness of 8OHdG as a biomarker of TCE-induced oxidative DNA damage is questionable.

Keywords: 8-Hydroxydeoxyguanosine; 8-Epi-prostaglandin $F_{2\alpha}$; 2-Nitropropane; Perchloroethylene; Tetrachloroethylene; TBARS

1. Introduction

Trichloroethylene (TCE) and perchloroethylene (PERC) are used in the dry cleaning industry where PERC is the solvent of choice for laundering clothes and TCE is used as a spot remover. TCE is also extensively used as a metal degreaser in other industries (IARC, 1995). The National Institute for Occupational Safety and Health (NIOSH) estimates that in the USA more than 500 000 workers are exposed to PERC and more than 400 000 workers are exposed to TCE (NIOSH, 1994). Both are rodent carcinogens and also produce a wide array of toxicological effects in animals and humans; most notable of which are hepatic-, renal-, and neuro-toxicity (NCI, 1976; NTP, 1990; ATSDR, 1997a,b).

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0300-483X/99/$ - see front matter
PII: S0300-483X(99)00083-9

Trichloroacetic acid (TCA) and dichloracetic acid (DCA) are metabolites of TCE and PERC in both man and experimental animals, and are also rodent heptocarcinogens (Bull et al., 1990; DeAngelo et al., 1991; ATSDR, 1997a,b; Volkel et al., 1998). Because TCA and DCA induce lipid peroxidation and oxidative DNA damage, oxidative stress has been implicated in their carcinogenesis (Larson and Bull, 1992; Austin et al., 1996). TCE has recently been reported to induce hepatic lipid peroxidation and elevate oxidative DNA damage in mouse liver (Cojocel et al., 1989; Channel et al., 1998). Although there is no direct evidence that PERC causes oxidative damage, the ability of Vitamin E to protect against PERC toxicity in mice provides indirect evidence (Ebrahim et al., 1996).

Presently, biomonitoring for TCE or PERC exposure in the workplace is accomplished by breath or urine analysis of the parent compound or metabolites (IARC, 1995). These represent biomarkers of exposure, but provide little information on potential activation of these compounds or interactions with target tissues. If TCE and PERC induce oxidative stress, then urinary biomarkers of oxidative damage could serve as biomarkers of biologically effective dose for workers exposed to these compounds in the dry cleaning industry.

DNA is subject to continuous oxidative damage from oxygen radicals generated during normal cellular respiration (Loft et al., 1992, 1994). Unrepaired DNA damage can lead to mutation and possibly cancer (Ames, 1989; Guyton and Kensler, 1993; Hemminki, 1993). Environmental exposures that increase the rate of damage above background levels increase the potential for unrepaired lesions to become permanent mutations (Kuchino et al., 1987; Feig et al., 1994). This concept has led to the use of oxidative DNA damage in the assessment of occupational and environmental exposures to chemicals that are capable of inducing an oxidative stress. Oxygen-based radicals can produce a variety of DNA lesions (Breen and Murphy, 1995), but the 8-hydroxydeoxyguanosine (8OHdG) adduct has been used most extensively as a biomarker of effect (Loft et al., 1993; Tagesson et al., 1993; Lagorio et al., 1994; Schins et al., 1995; Liu et al., 1996; Loft and Poulsen, 1998; Toraason, 1999).

The objective of the present study was to determine if a single exposure to PERC or TCE could induce an oxidative stress in rats that is detectable in urine using a rapid and relatively inexpensive immunoassay for 8OHdG. Malondialdehyde assessed by the Thiobarbituric Acid-Reactive Substances (TBARS) assay is the most widely used marker of lipid peroxidation resulting from oxidative stress. Although the rapid metabolism of malondialdehyde makes it unreliable as a urinary biomarker (DeZwart et al., 1998), it is applicable for assessing oxidative damage in target tissues of experimental animals. In the present study, the TBARS assay was used to determine if TCE or PERC exposures induced oxidative damage in liver or kidney. 8-Epi-prostaglandin $F_{2\alpha}$ (8epiPGF) is a free radical-catalyzed isomer of arachidonic acid and has proven useful in exploring the role of free radicals in disease processes because it does not undergo metabolism in the body and is stable in excreted urine (Morrow and Roberts, 1997). 8epiPGF was used as a urinary marker of oxidative damage to cell membranes.

2. Materials and methods

2.1. Chemicals

TCE (99.9% pure, HPLC grade), PERC (99.5% pure, spectrophotometric grade) and 2-nitropropane (2NP) (97% pure, laboratory grade) were purchased from Aldrich (Milwaukee, WI). Alkamuls EL-620 was a gift from Rhone-Poulenc (Cranbury, NJ). Nuclease P16 and alkaline phosphatase were purchased from Boehringer Mannheim. All other reagents were purchased from Sigma (St Louis, MO) or Fisher (Cincinnati, OH).

2.2. Animals

Forty-eight male Fischer rats (150–200 g) were purchased from Charles River Laboratories (Cambridge, MA). Prior to experimental treatment, rats were housed individually and provided

food and water ad libitum in the animal quarters of NIOSH, which is accredited by the American Association for Accreditation of Laboratory Animal Care. Rats were identified, weighed and randomized into eight groups of six rats per group.

2.3. Experimental design

Twenty-four hours prior to treatment, rats were housed in metabolic cages with free access to food and water. One 12-h urine collection was made just prior to experimental treatment. On the morning of treatment, control rats were injected i.p. with 2 ml/kg of vehicle (1:4 v/v of Alkamuls/water). Positive-controls received 100 mg/kg 2-nitropropane (2NP) in the vehicle. Test rats received 100, 500, or 1000 mg/kg of TCE or PERC. Two sequential 12-h urine samples were collected after treatment. Twenty four hours after treatment, rats were anesthetized with pentobarbital and necropsied to obtain blood, liver, kidney and brain. Blood was obtained by cardiac puncture and drawn into syringes containing citrate to prevent coagulation.

2.4. Extraction of DNA from blood and tissue

Blood was layered into 15-ml two-chamber tubes containing Histopaque-1077 (Sigma, St Louis, MO). The Histopaque-1077 kit procedure was used to obtain mononuclear blood cells, which were frozen at −80°C for subsequent DNA extraction. Livers and kidney were excised, trimmed, weighed and rinsed in ice-cold 0.15 M NaCl–0.015 M trisodium citrate. Tissues were frozen in liquid nitrogen prior to being stored at −80°C. DNA isolation from liver and kidney were by the procedure of Fiala et al. (1989). DNA was isolated from lymphocytes with Puregene DNA isolation kits (Gentra Systems, Minneapolis, MN).

2.5. ELISA-based measurement in urine of 8OHdG and 8epiPGF

8OHdG and 8epiPGF in all urine samples were analyzed by Genox (Baltimore, MD) using ELISA-based kits. Standard procedures were followed for both kits.

2.6. HPLC-EC measurement of 8OHdG in urine, tissue and lymphocytes

DNA extracted from lymphocytes and liver was hydrolyzed by incubating 40–200 μg DNA in 200 μl 20 mM sodium acetate (pH 5.1) containing 5 μl of 1 U/μl nuclease P1 at 65°C. After 10 min, 10 μl of 1 M Tris–HCl (pH to 8.5) and 5 μl of 1 U/μl of alkaline phosphatase were added and the solution was incubated for 1 h at 37°C. The pH was adjusted to 5.1 with 10 μl of 3 M sodium acetate buffer and the solution was filtered through a 5 k Millipore concentrator tube by centrifuging $5000 \times g$ for 10 min. Samples were assayed for 8OHdG using high pressure liquid chromatography and electrochemical detection (HPLC-EC) by ESA Laboratories (Chelmsford, MA).

2.7. Creatinine

Creatinine in urine was assayed by the Stanibo Creatinine Procedure No. 400 (Stanibo Laboratory, San Antonio, TX).

2.8. Liver and kidney TBARS

TBARS were measured in liver and kidney according to Tirmenstein et al. (1995) with the following modification: 1 cm^3 tissues pieces were weighed and homogenized in nine parts of 1.15% KCl. The homogenate was centrifuged for 10 min at $1000 \times g$. A 1-ml aliquot was combined with an equal volume of 12% trichloroacetic acid and centrifuged for 10 min at $1000 \times g$ to remove precipitated protein. One milliliter of supernatant was added to 1 ml thiobarbituric acid reagent (0.6% thiobarbituric acid, 0.01% BHT, 1.0 mm EDTA), and the mixture was heated at 100°C for 20 min prior to extraction of TBARS with 3 ml of 1-butanol.

2.9. Statistical analysis

All statistical procedures were performed using Statgraphics statistical package (STSC, Rockville, MD). Data were compared using ANOVA. Data are presented as the mean ± S.D. $P < 0.05$ was considered statistically significant.

Table 1
Body and organ weights of rats following single i.p. injection of 2NP, PERC, or TCE

Treatment	Dose (mg/kg)	Body wt (g)	Liver wt (g)	Kidney wt (g)	Brain wt (g)
Control	0	262 ± 14	10.8 ± 0.9	1.9 ± 0.1	1.8 ± 0.1
2-Nitropropane	100	235 ± 13[a]	10.5 ± 1.2[b]	2.0 ± 0.2	1.8 ± 0.1
Trichloroethylene	100	262 ± 25	10.6 ± 1.4	1.9 ± 0.2	1.8 ± 0.1
Trichloroethylene	500	240 ± 6[a]	7.5 ± 0.5[a,b]	1.8 ± 0.2	1.8 ± 0.1
Trichloroethylene	1000	242 ± 9[a]	7.5 ± 0.6[a,b]	1.9 ± 0.1	1.8 ± 0.1
Perchloroethylene	100	254 ± 12	9.7 ± 0.9[b]	1.9 ± 0.2	1.8 ± 0.1
Perchloroethylene	500	243 ± 20[a]	8.1 ± 0.9[a,b]	1.8 ± 0.1	1.8 ± 0.1
Perchloroethylene	1000	238 ± 11[a]	7.5 ± 0.8[a,b]	1.9 ± 0.1	1.8 ± 0.1

[a] Body or organ weight significantly different than control, $P<0.05$.
[b] Liver/body weight ratio significantly different than control, $P<0.05$.

3. Results

3.1. Morbidity and mortality

A single exposure to 100 mg/kg 2NP, TCE or PERC was without apparent effect on rats at the time of dosing. TCE and PERC at 500 mg/kg anesthetized rats to stage II: loss of righting reflex but maintained reflex response. At 1000 mg/kg PERC or TCE, rats were at level III or IV anesthesia: absence of reflex response. Five of six rats treated with 1000 mg/kg TCE, and two of six rats treated with 1000 mg/kg PERC produced burgundy colored urine during the first 12 h of urine collection. At the time of sacrifice (24 h post dosing), two rats treated with 1000 mg/kg TCE were comatose and hypothermic. None of the rats died from the exposures, although some rats in the 1000 mg/kg TCE and PERC dose groups may not have survived another 24 h.

3.2. Body and organ weights (Table 1)

A single exposure to 2NP, TCE or PERC significantly reduced body weights relative to controls, and the effect was dose-dependent for TCE and PERC. TCE and PERC also decreased liver weights and liver/body weight ratios in a dose-dependent fashion. 2NP had a significant effect only on liver/body weight ratio at the single dose tested. Kidney and brain weights were not affected by acute exposure to TCE, PERC or 2NP.

3.3. Urine volume (Fig. 1)

Urine volume declined significantly during the first 12 h following treatment of rats with 1000 mg/kg TCE or PERC, but recovered to the extent that 24-h post-exposure urine volumes were decreased, but not significantly from control values. Urine volume was significantly increased for 12 and 24 h in rats dosed with 2NP. Water consumption was not measured, but may have decreased significantly due to moribundity in rats treated with TCE or PERC.

3.4. Creatinine excretion (Fig. 2)

Creatinine excretion decreased significantly in rats dosed with 2NP, TCE or PERC. The de-

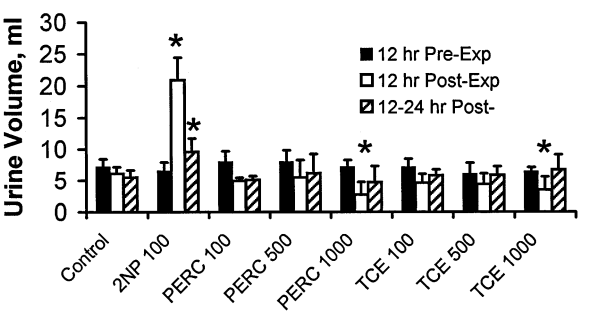

Fig. 1. Twelve-hour urine volumes pre- and post-i.p. injection of rats with control vehicle, 2NP, PERC or TCE. Doses were 100, 500, or 1000 mg/kg. Bars are mean ± S.D. *Indicates values are statistically significantly different than corresponding control values ($P<0.05$).

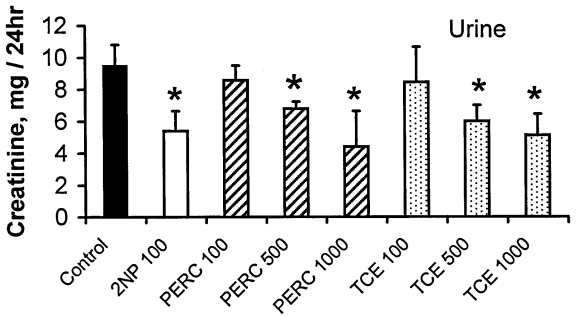

Fig. 2. Twenty-four hour creatinine excretion following i.p. injection of rats with control vehicle, 2NP, PERC or TCE. Doses were 100, 500, or 1000 mg/kg. Bars are mean ± S.D. *Indicates values are statistically significantly different than corresponding control values ($P < 0.05$).

crease in creatinine could be attributed to either a decrease in plasma creatinine or a decrease in glomerular clearance. Without a measurement of plasma creatinine it is not possible to determine this.

3.5. 8epiPGF excretion (Fig. 3)

In control rats, urinary excretion of 8epiPGF remained constant over the 36 h. In 2NP-exposed rats, urinary 8epiPGF increased significantly during the 24 h after dosing, and was significantly elevated above control values regardless of mode of expression. Excretion 8epiPGF expressed per mg creatinine (12–24 h post exposure) in rats exposed to PERC or TCE was not different from controls. However, total excretion of 8epiPGF 24 h post exposure to 500 or 1000 mg/kg TCE or PERC was significantly reduced from the control value.

3.6. ELISA analysis of 8OHdG excretion (Fig. 4)

Urinary excretion of 8OHdG was measured by ELISA in urine excreted 12 h prior to exposure to 2NP, TCE or PERC and 12 and 24 h post exposure. This provided the opportunity to express urinary output of 8OHdG on the basis of 12- or 24-h total output, creatinine excretion, pre- versus post-exposure levels, and combinations of the aforementioned modes of expression. Because both urine volume and creatinine excretion were affected by experimental treatment, urinary excretion of 8OHdG changed significantly based on the mode of data expression. In control rats, urinary excretion of 8OHdG remained constant over the 36 h it was measured.

Urinary excretion of 8OHdG in 2NP-exposed rats was not significantly increased for the 24 h post exposure. However, when only the 12- to 24-h period was examined, 8OHdG excretion was significantly increased regardless if expressed as total output (data not shown) or per mg creatinine.

TCE nor PCE exposure at any concentration tested did not result in elevated 8OHdG regardless of the method of expressing excretion. In fact, when data were expressed on the basis of 24-h excretion, TCE and PERC at 500 and 1000 mg/kg resulted in a significant reduction in 8OHdG excretion.

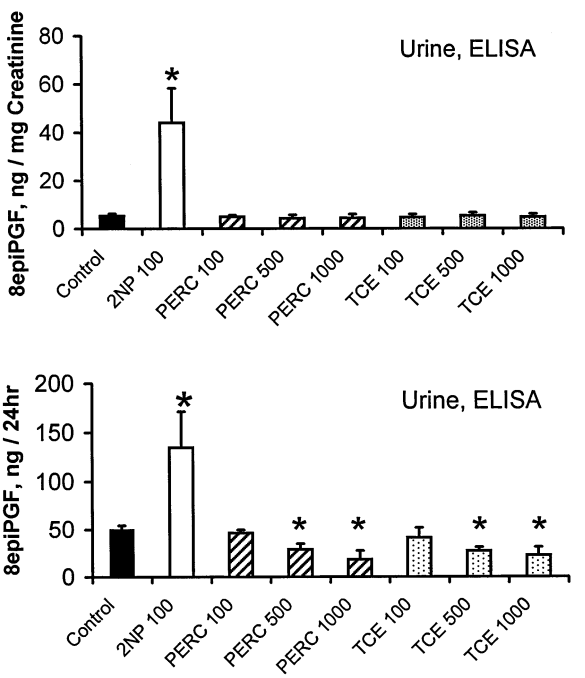

Fig. 3. Urinary 8epiPGF excretion following i.p. injection of rats with control vehicle, 2NP, PERC or TCE. Doses were 100, 500, or 1000 mg/kg. Bars are mean ± S.D. *Indicates values are statistically significantly different than corresponding control values ($P < 0.05$).

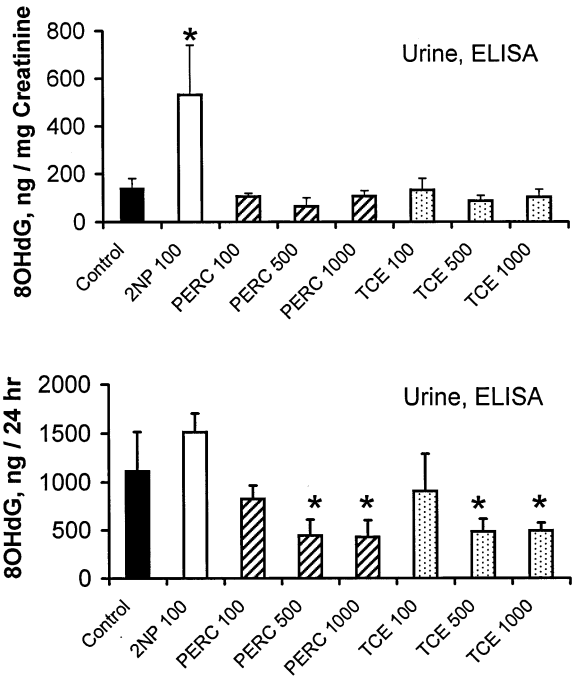

Fig. 4. Urinary 8OHdG excretion (determined by ELISA) following i.p. injection of rats with control vehicle, 2NP, PERC or TCE. Doses were 100, 500, or 1000 mg/kg. Bars are mean ± S.D. *Indicates values are statistically significantly different than corresponding control values ($P < 0.05$).

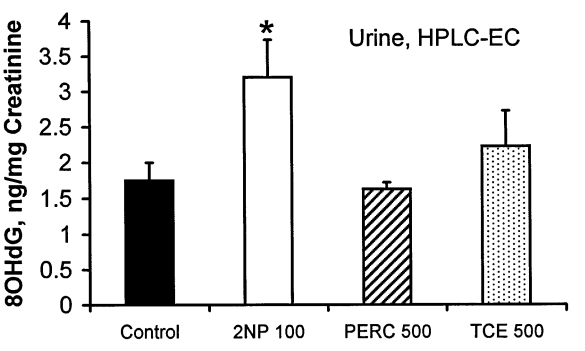

Fig. 5. Urinary 8OHdG excretion (determined by HPLC-EC) following i.p. injection of rats with control vehicle, 100 mg/kg 2NP, 500 mg/kg PERC or 500 mg/kg TCE. Bars are mean ± S.D.

3.7. HPLC-EC analysis of 8OHdG excretion (Fig. 5)

Urine samples from controls and rats dosed with 100 mg/kg 2NP, and 500 mg/kg TCE or PERC were analyzed by HPLC-EC. Subsequent to analysis of the subset of urine samples noted above, additional urine samples were analyzed by HPLC-EC. Unfortunately, internal controls revealed subset variation in results that were independent of the experimental design. As a consequence, it was not possible to present additional data on urine samples analyzed by HPLC-EC without the potential of introducing analysis bias. HPLC-EC is the method of choice for 8OHdG (Loft and Poulsen, 1998) and the results reported here for control animals are similar to those reported previously. Fraga et al. (1990) using HPLC-EC, reported that the 8OHdG urinary output for the 4-month-old Fischer 344 rat was about (bar graph) 370 pmol/kg per day. In the present study, 12-h urine specimens were analyzed by HPLC-EC. Extrapolation from 12 to 24 h for 3-month-old control rats resulted in 8OHdG urinary output of 383 pmol/kg per day. This supports the conclusion that HPLC-EC analysis of urine for 8OHdG can be measured consistently in different laboratories.

3.8. 8OHdG analysis; HPLC-EC vs ELISA

Despite the fact that values obtained with HPLC-EC analysis were more than two orders of magnitude lower than values obtained with the ELSA analysis, the experimental outcome was comparable. The correlation coefficient for HPLC-EC vs ELISA was 0.6604 with a P-value of 0.0049, $N = 16$. We are not aware of other studies comparing urinary ELISA-8OHdG results to HPLC-EC results. Yin et al. (1995) measured 8OHdG in placental DNA and reported a correlation coefficient for HPLC-EC vs ELISA of 0.86 with a P-value of 0.001, $N = 23$. ELISA values were within one order of magnitude of HPLC-EC values. The higher correlation and decreased difference between ELISA and HPLC-EC values reported by Yin et al. (1995) could be due to the measurement of tissue DNA and/or their use of

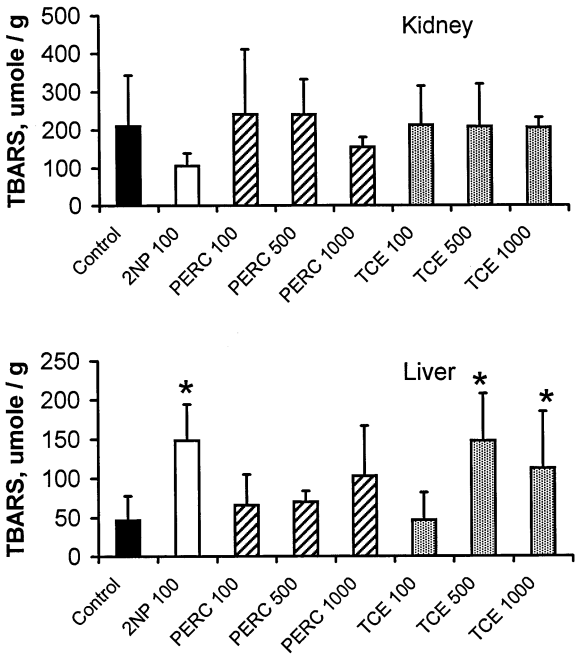

Fig. 6. Liver and kidney TBARS following i.p. injection of rats with control vehicle, 2NP, PERC or TCE. Doses were 100, 500, or 1000 mg/kg. Bars are mean ± S.D. *Indicates values are statistically significantly different than corresponding control values ($P < 0.05$).

an immunoaffinity purification step prior to performing the ELISAs. Regardless, the present results support the use of ELISA-based 8OHdG methods as a screen to detect relative changes within a study (2NP vs control), but do not provide an indication that they are suitable for inter-study comparisons.

3.9. Liver and kidney TBARS (Fig. 6)

In the kidney, there were no treatment-related differences in TBARS levels among the groups. In liver, treatment with 100 mg/kg 2NP, 500 mg/kg TCE or 1000 mg/kg TCE significantly increased TBARS above control values. PERC exposure did not significantly affect liver TBARS.

3.10. Liver and lymphocyte 8OHdG (Fig. 7)

8OHdG/dG × 10^6 levels were measured in DNA extracted from liver and lymphocytes from a subset of rats exhibiting the highest TBARS levels. Cost prohibited analysis of all tissue samples for 8OHdG/dG. 2NP and TCE, but not PCE, significantly increased 8OHdG/dG ratios in liver DNA relative to controls. There were small increases in 8OHdG/dG levels in lymphocytes from rats exposed to 2NP, PCE or TCE, but the increases were not statistically significant.

4. Discussion

Previous studies (Channel et al., 1998) and present results demonstrate the capacity of TCE exposure to induce oxidative stress and oxidative DNA damage in rodent liver. The objective of the present investigation was to test the hypothesis that TCE or PERC increased urinary excretion of oxidative stress biomarkers in experimental animals. Positive results would serve as a foundation for using 8OHdG and

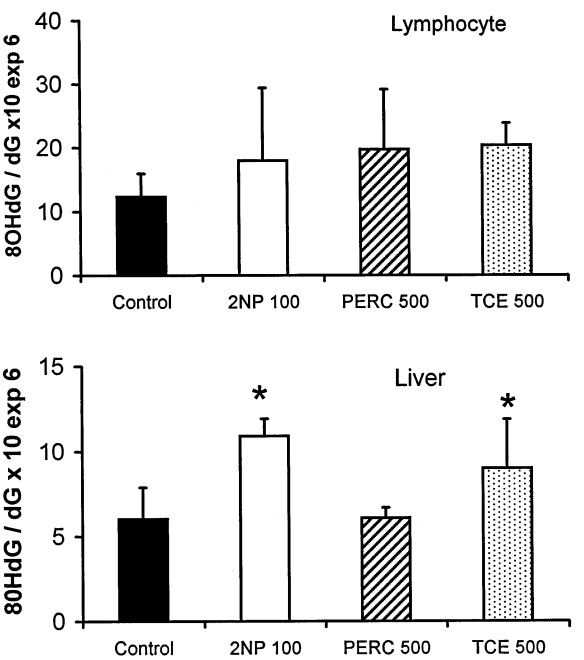

Fig. 7. Liver and lymphocyte 8OHdG/dG following i.p. injection of rats with control vehicle, 100 mg/kg 2NP, 500 mg/kg PERC, or 500 mg/kg TCE. Bars are mean ± S.D.

8epiPGF as markers of a biologically-effective dose in studies of workers exposed to TCE and/or PERC. Although 8-OHdG is a nonspecific DNA adduct, specific TCE- or PERC-DNA adducts have not been identified (ATSDR, 1997a,b). In the absence of specific adducts, a nonspecific adduct such as 8OHdG may serve as an index of a genotoxic effect of TCE or PERC workplace exposures. 8epiPGF was used as a corollary biomarker of increased oxidative stress in TCE- and PERC-exposed rats. Because there was only circumstantial evidence that PERC could induce oxidative damage, this study also tested the more basic hypothesis that acute exposure to PERC induced oxidative stress in rats. The present data do not support this hypothesis.

4.1. Positive control

2NP is a potent hepato-carcinogen that has previously been demonstrated to increase liver but not kidney 8OHdG and TBARS, and urinary excretion of 8OHdG (Fiala et al., 1987, 1989; Adachi et al., 1993; Suzuki et al., 1995). The experimental design of the present study was based on designs that successfully demonstrated these effects of 2NP. In the present study, a single dose of 2NP significantly increased 8OHdG and 8epiPGF. 2NP also increased liver TBARS levels, but not those in the kidney. Elevated TBARS were accompanied by increased 8OHdG/dG ratios in liver DNA. Increased oxidative damage was evident in urinary biomarkers despite the fact that urine volume and creatinine excretion were also affected by 2NP exposure. Results also indicated that lymphocytes do not serve as a useful surrogate (Kiyosawa et al., 1990; Takeuchi et al., 1994; Nakajima et al., 1996) for tissue oxidative DNA damage induced by 2NP.

4.2. Morbidity

The TCE and PERC doses of 1000 mg/kg were excessively toxic, and would have little application to the development of useful biological markers for TCE- or PERC-exposed humans. Rats in this group excreted burgundy colored urine, were comatose and hypothermic at sacrifice. In fact, two rats were so cold and stiff that they were presumed dead, but at necropsy faintly beating hearts were evident. The severe toxicity resulting from 1000 mg/kg TCE or PERC exposure undermined the relevance of these doses for biomarker assessment. Therefore, complex and expensive HPLC analysis of samples for 8OHdG/dG was limited to the tissues from animals dosed with 500 mg/kg TCE or PERC.

4.3. Urine volume and creatinine excretion

Exposure of rats to 2NP, TCE or PERC significantly altered urine output. In 2NP-exposed rats, urine volume increased dramatically during the first 12 h following exposure, indicating a likely reduction in tubular reabsorption. Within 24 h, however, 2NP-exposed rats appeared to recover from this effect. Twelve-hour creatinine excretion was also significantly decreased, but in the absence of a plasma creatinine measurement it is not possible to determine the source of this effect. Despite the changes in urine volume and creatinine excretion, there was no indication of renal damage in 2NP-exposed rats based on gross examination at necropsy, kidney weights, or TBARS levels. TCE and PERC exposure significantly decreased urine volume only at the dose of 1000 mg/kg and only during the first 12 h following dosing. Although water consumption and duration of anesthesia were not recorded, it is reasonable to conclude that a lack of water consumption and anesthetic state contributed to reduced urinary output. Creatinine is a by-product of muscle metabolism and urinary excretion is a reflection of serum creatinine levels. As with urine volume, reduced creatinine could be secondary to the possible decreased food and water consumption and reduce respiration associated with anesthesia.

4.4. Liver and kidney TBARS and 8OHdG/dG

The absence of increased TBARS or 8OHdG/dg in liver or kidney of PERC-exposed rats indicates an absence of oxidative stress in these

organs. The fact that TCA and DCA are metabolites of PERC in rodent and humans would lead one to expect that PERC would have produced some oxidative damage. However, this was not the case. Although producing the same metabolites, the metabolism kinetics of PERC and TCE are dissimilar and the toxicity of each is dependent upon its metabolism (Buben and O'Flaherty, 1985). This would likely contribute to the distinction between TCE and PERC in inducing oxidative damage. Only in TCE-exposed rats were TBARS and 8OHdG/dG significantly increased. This suggests that the number of free radical intermediates is greater for TCE. The increase in oxidative stress and DNA damage produced by TCE is consistent with previous reports on TCE and its metabolites TCA or DCA (Cojocel et al., 1989; Larson and Bull, 1992; Austin et al., 1996; Channel et al., 1998). Comparable to 2NP-exposed rats, evidence for oxidative damage was found only in the liver and not the kidney of TCE-exposed rats. Although TCE is a hepatic carcinogen in mice, it induces a low incidence of renal tumors in rats (NTP, 1990). The present results do not provide support for the involvement of a free radical-mediated mechanism in the induction of renal tumors in rats.

4.5. Urinary excretion of 8OHdG and 8epiPGF

Exposure of mice to 1200 mg/kg per day TCE significantly increases nuclear liver DNA 8OHdG (Channel et al., 1998). The present results demonstrating elevated liver 8OHdG in rats dosed with 500 mg/kg TCE are consistent with this finding. Despite the clear evidence for the occurrence of oxidative stress in rat liver, there was no evidence for this damage in urine. However, urine excretion was reduced in TCE-exposed rats and this may have masked the increased output of oxidative stress biomarkers.

The high doses of TCE used in the present study were severely toxic based on morbidity. It may be that elevated liver 8OHdG and TBARS were secondary to this toxicity. However, this is not supported by the results from PERC-exposed rats that exhibited comparable morbidity. It could be argued that an absence of 8OHdG in urine was the result of reduced DNA repair. But this would not explain the elevated TBARS and absence of a corresponding increase in urinary 8epiPGF in TCE-exposed rats. The consistent findings in 2NP-exposed rats indicates the experimental design and analytical methods employed in the present study can detect chemically induced oxidative damage in tissues and the by-products of damage in urine. The lack of a comparable effect in TCE-exposed rats leads to the conclusion that evidence for oxidative damage is equivocal. In contrast, the present results provide no evidence for oxidative damage in PERC-exposed rats.

4.6. 8OHdG/dg in lymphocyte DNA

Lymphocytes have been used successfully as a surrogate tissue for assessing oxidative DNA damage in humans. The present finding that liver 8OHdG is reflected in urine but not lymphocytes of 2NP-exposed rats indicates it is not a useful surrogate for 2NP exposure. The same is true of TCE. These findings demonstrate the essentiality of verifying that surrogates such as lymphocytes do reflect exposures, before they are used in field studies.

5. Conclusions (Table 2)

Assessment of urinary 8OHdG and 8epiPGF are useful biological markers for detecting oxidative damage produced by acute exposure of rats to 2NP. For unexplained reasons, these two urinary biomarkers did not reflect the oxidative damage that was evident in the liver of rats exposed to a single injection of 500 mg/kg TCE. Nonetheless, the present results confirm previous reports that TCE can elevate liver oxidative DNA damage. The present results provide no evidence for single exposures to PERC to induce oxidative stress in rats, even at near-lethal doses.

Table 2
Observations and summary of statistical analysis in rats following single i.p. injection of 2NP, PERC or TCE[a]

	2NP (mg/kg)	PERC (mg/kg)			TCE (mg/kg)		
	100	100	500	1000	100	500	1000
Morbidity							
Moribund	−	−	−	+	−	−	+
Burgundy urine	−	−	−	+	−	−	+
Body weight	↓	−	↓	↓	−	↓	↓
Liver weight	−	−	↓	↓	−	↓	↓
Urine volume	↑	−	−	↓	−	−	↓
Urine creatinine excretion	↓	−	↓	↓	−	↓	↓
Oxidative damage							
Liver TBARS	↑	−	−	−	−	↑	↑
Kidney TBARS	−	−	−	−	−	−	−
Liver 8OHdG	↑		−			↑	
Biological markers							
Lymphocyte 8OHdG	−		−			−	
Urine 8epiPGF	↑	−	−/↓	−/↓	−	−/↓	−/↓
Urine 8OHdG	↑	−	−/↓	−/↓	−	−/↓	−/↓

[a] For PERC and TCE groups 1, 2, and 3 represent doses of 100, 500 or 1000 mg/kg respectively. Dose for 2NP was 100 mg/kg. Controls received vehicle only. Empty cell indicates analysis was not performed; +, Evident in one or more rats in experimental group; −, no significant change from control; ↓, statistically significant decrease relative to control; ↑, statistically significant increase relative to control.

References

Adachi, S., Takemoto, K., Hirosue, T., Hosogai, Y., 1993. Spontaneous and 2-nitropropane levels of 8-hydroxy-2′-deoxyguanosine in liver DNA of rats fed iron-deficient or manganese- and copper-deficient diets. Carcinogenesis 14, 265–268.

Agency for Toxic Substances and Disease Registry (ATSDR), 1997a. Toxicological Profile for Trichloroethylene (Update). U.S. DHHS Publication.

Agency for Toxic Substances and Disease Registry (ATSDR), 1997b. Toxicological Profile for Tetrachloroethylene (Update). U.S. DHHS Publication.

Ames, B.N., 1989. Endogenous DNA damage as related to cancer and aging. Mutat. Res. 214, 41–46.

Austin, E.W., Parrish, J.M., Kinder, D.H., Bull, R.J., 1996. Lipid peroxidation and formation of 8-hydroxydeoxyguanosine from acute doses of halogenated acetic acids. Fundam. Appl. Toxicol. 31, 77–82.

Breen, A.P., Murphy, J.A., 1995. Reactions of oxyl radicals with DNA. Free Radic. Biol. Med. 18, 1033–1077.

Buben, J.A., O'Flaherty, E.J., 1985. Delineation of the role in metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: Dose-effect study. Toxicol. Appl. Pharmacol. 78, 105–122.

Bull, R.J., Sanchez, I.M., Nelson, M.A., Larson, J.L., Lansing, A.J., 1990. Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate. Toxicology 63, 341–359.

Channel, S.R., Latendresse, J.R., Kidney, J.K., Grabau, J.H., Lane, J.W., Steel-Goodwin, L., Gothaus, M.C., 1998. A subchronic exposure to trichloroethylene causes lipid peroxidation and heptocellular proliferation in male B6C3F1 mouse liver. Toxicol. Sci. 43, 145–154.

Cojocel, C., Beuter, W., Muller, W., Mayer, D., 1989. Lipid peroxidation: a possible mechanism of trichloroethylene-induced nephrotoxicity. Toxicology 55, 131–141.

DeAngelo, A.B., Daniel, F.B., Stober, J.A., Olson, G.R., 1991. The carcinogenicity of dichloroacetic acid in the male B63CF1 mouse. Fundam. Appl. Toxicol. 16, 337–347.

DeZwart, L.L., Hermanns, R.C.A., Meerman, J.H.N., Commandeur, J.N.M., Salemink, P.J.M., Vermeulen, N.P.E., 1998. Excretion of multiple urinary biomarkers for radical-induced damage in rats treated with three different nephrotoxic compounds. Biomarkers 3, 347–365.

Ebrahim, A.S., Babakrishnan, K., Sakisekaren, D., 1996. Perchloroethylene-induced alterations in glucose metabolism and their prevention by 2-deoxy-D-glucose and vitamin E in mice. J. Appl. Toxicol. 16, 339–348.

Feig, D.I., Reid, T.M., Loeb, L.A., 1994. Reactive oxygen species in tumorgenesis. Cancer Res. 54, 1890s–1894s.

Fiala, E.S., Czerniak, R., Castonguay, A., Conaway, C.C., Rivenson, A., 1987. Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane, and 2-azoxypropane for car-

cinogenicity by gavage in Sprague–Dawley rats. Carcinogenesis 8, 1947–1949.

Fiala, E.S., Conaway, C.C., Mathis, J.F., 1989. Oxidative DNA and RNA damage in the livers of Sprague–Dawley rats treated with the hepatocarcinogen 2-nitropropane. Cancer Res. 49, 5518–5522.

Fraga, C.G., Shigenaga, M.K., Park, J.-W., Degan, P., Ames, B.N., 1990. Oxidative damage to DNA during aging: 8-hydroxy-2′-deoxyguanosine in rat organ DNA and urine. Proc. Natl. Acad. Sci. USA 87, 4533–4537.

Guyton, K.Z., Kensler, T.W., 1993. Oxidative mechanisms in carcinogenesis. Br. Med. Bull. 49, 523–544.

Hemminki, K., 1993. DNA adducts, mutations and cancer. Carcinogenesis 14, 2007–2012.

International Agency for Research on Cancer (IARC), 1995. Dry cleaning, some chlorinated solvents used in dry cleaning and other industrial chemicals. IARC Monographs on the Carcinogenic Risks to Humans 63, 33–223.

Kiyosawa, H., Suko, M., Okudaira, H., Murata, K., Miyamoto, T., Chung, M.H., Kasai, H., Nishimura, S., 1990. Cigarette smoking induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages in human peripheral leukocytes. Free Radic. Res. Commun. 11, 23–27.

Kuchino, Y., Mori, F., Kasai, H., 1987. Misreading of DNA templates containing 8-hydroxyguanosine at the modified base and at adjacent residues. Nature 327, 77–79.

Lagorio, S., Tagesson, C., Forastiere, F., Iavarone, I., Axelson, O., Carere, A., 1994. Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. Occup. Environ. Med. 51, 739–743.

Larson, J.L., Bull, R.J., 1992. Metabolism and lipoperoxidation activity of trichloracetate and dichloroacetate in rats and mice. Toxicol. Appl. Pharmacol. 115, 268–277.

Liu, L., Zhang, Q., Feng, J., Deng, L., Zeng, N., Yang, A., Zhang, W., 1996. The study of DNA oxidative damage in benzene-exposed workers. Mutat. Res. 370, 145–150.

Loft, S., Poulsen, H.E., 1998. Estimation of oxidative DNA damage in man from urinary excretion of repair products. Acta Biochim. Pol. 45, 133–144.

Loft, S., Vistisen, K., Ewertz, M., Tjnneland, A., Overvad, K., Poulsen, H.E., 1992. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 13, 2241–2247.

Loft, S., Fischer-Nielson, A., Jeding, I.B., Vistisen, K., Poulsen, H.E., 1993. 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. J. Toxicol. Environ. Health 40, 391–404.

Loft, S., Astrup, A., Buemann, B., Poulsen, H.E., 1994. Oxidative damage correlates with oxygen consumption in humans. FASEB J. 8, 534–537.

Morrow, J.D., Roberts, L.J., 1997. The isoprostanes: unique bioactive products of lipid peroxidation. Prog. Lipid Res. 36, 1–21.

Nakajima, M., Takeuchi, T., Takeshita, T., Morimoto, K., 1996. 8-Hydroxydeoxyguanosine in human leukocyte DNA and daily health practice factors: Effects of individual alcohol sensitivity. Environ. Health Perspect. 104, 1336–1338.

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

National Institute for Occupational Safety and Health (NIOSH), 1994. National Occupational Exposure Survey, 1981–1983. NIOSH, Cincinnati, OH.

National Toxicology Program (NTP), 1990. Carcinogenesis studies of trichloroethylene (CAS No. 79-0106) in F344/N rats and B6C3F1 mice (gavage study). DHHS, NTP Technical Report 243.

Schins, R.P., Schilderman, P.A., Borm, P.J., 1995. Oxidative DNA damage in peripheral blood lymphocytes of coal workers. Int. Arch. Occup. Environ. Health 67, 153–157.

Suzuki, J., Inoue, Y., Suzuki, S., 1995. Changes in the urinary excretion level of 8-hydroxyguanine by exposure to reactive oxygen-generating substances. Free Radic. Biol. Med. 18, 431–436.

Tagesson, C., Chabiuk, D., Axelson, O., Baranski, B., Palus, J., Wyszynska, K., 1993. Increased urinary excretion of the oxidative DNA adduct, 8-hydroxyguanosine, as a possible early indicator of occupational cancer hazards in the asbestos, rubber, and azo-dye industries. Pol. J. Occup. Med. Environ. Health 6, 357–368.

Takeuchi, T., Nakajima, M., Ohta, Y., Mure, K., Takeshita, T., Morimoto, K., 1994. Evaluation of 8-hydroxyguanosine, a typical oxidative DNA damage, in human leukocytes. Carcinogenesis 15, 1519–1523.

Tirmenstein, M.A., Plews, P.I., Walker, C.V., Woolery, M.D., Wey, H.E., Toraason, M.A., 1995. Antimony-induced oxidative stress and toxicity in cultured cardiac myocytes. Toxicol. Appl. Pharmacol. 130, 41–47.

Toraason, M., 1999. 8-Hydroxydeoxyguanosine as a biomarker of workplace exposures. Biomarkers 4, 3–26.

Volkel, W., Friedwald, M., Ledrer, E., Pahler, A., Parker, J., Dekant, W., 1998. Biotransformation of perchloroethene: Dose-dependent excretion of trichloroacetic acid, *N*-acetyl-*S*-(trichlorovinyl)-L-cysteine in rats and humans after inhalation. Toxicol. Appl. Pharmacol. 153, 20–27.

Yin, B., Whyatt, R.M., Perera, F.P., Randall, M.C., Cooper, T.B., Santella, R.M., 1995. Determination of 8-hydroxydeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA. Free Radic. Biol. Med. 18, 1023–1032.