Trichloroethylene induced cutaneous irritation in BALB/c hairless mice: Histopathological changes and oxidative damage

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Abstract

Trichloroethylene (TCE), a colorless and volatile organic solvent, has long been a major chemical hazard during occupational exposure because of its extensive use in industry. Exposure to TCE can cause significant skin lesions, but the effect of TCE on skin irritation has received little attention. We therefore investigated the effect of TCE on skin irritation and oxidative stress using hairless mice. BALB/c hairless mice were subjected to acute and cumulative topical TCE treatment. Skin reactions were evaluated by visual inspection, histopathology examined by microscopy and oxidative stress assessed by measurement of malondialdehyde (MDA) levels, superoxide dismutase (SOD) activities and nitric oxide (NO) production. Under acute and cumulative TCE irritation, the skin developed erythema and edema, and the predominant histopathological features were hyperkeratosis, spongiosis and inflammatory cell infiltrates. In parallel to these morphological changes, acute TCE irritation also concentration-dependently increased MDA levels and inhibited SOD activities of the skin. However, in cumulative irritation, the MDA levels and SOD activities were initially elevated with increased TCE concentrations, but thereafter reduced with further concentration increments; the linear dose–response relationship was not preserved. TCE also concentration-dependently increased NO production both in acute and cumulative irritation. These results suggest that TCE is capable of producing skin irritation effect in vivo, with histopathological changes characterized by hyperkeratosis, spongiosis and inflammatory cell infiltrates. Moreover, oxidative stress may be associated with the clinical manifestations and histopathological abnormalities in TCE-induced skin irritation.

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1. Introduction

Trichloroethylene (TCE), a colorless and volatile organic solvent, is widely used in industry as metal degreasing and dry cleaning agent for various cleaning operations, such as computer chips and electronic product, and has been a major chemical hazard during occupational exposure (ATSDR, 1995; IARC, 1995). Under current technological and economical conditions, direct skin contact with TCE still often occurs and is inevitable during the process of various operations. TCE has been shown to produce a broad spectrum of toxicological effects in the skin. With the development of industry, an increasing number of reports on skin injuries have been implicated with TCE in recent years. TCE-caused dermatitis by occupational exposure has become a new and urgent problem to be solved in the field of public health in fast-developing countries including China (Huang et al., 2002; Nakajima et al., 2003; Chiu et al., 2006).

Evidence suggests that TCE-elicited dermatitis involves complex immune response and is regarded as a delayed-type hypersensitivity (DTH) response (Griffin et al., 2000; Kaneko et al., 2000; Chen et al., 2006). As an allergen, the skin sensitization potential of TCE has been studied in the past (Tang et al., 2002). As the patch test was negative in TCE-caused dermatitis in some of the patients, the DTH did not fully explain the mechanisms underlying TCE-induced skin lesions. Skin inflammatory reactions are under the control of a network of cytokines (Corsini and Galli, 2000; Effendy et al., 2000).
The primary irritation caused by irritants is believed to condition the development and severity of allergic contact dermatitis, due to the initiation of local inflammatory reaction by the release of primary cytokines. The release of cytokines leads to the attraction of inflammatory cells and to the destruction of keratinocytes by cell-mediated cytotoxicity (Levin and Maibach, 2002; Bonneville et al., 2007). Previous studies in our laboratory have demonstrated that TCE could cause cytotoxicity and induce apoptosis associated with oxidative stress in vitro using cultured normal human epidermal keratinocytes (NHEK), and determined the NR50 values (used to predict the toxicity in vivo) to be 4.53 mM, a concentration encountered in the workplace, for TCE from NHEK by neutral red uptake (NRU) assay (Zhu et al., 2005a,b; Shen et al., 2007). These results indicated that TCE was not only an allergen, but also an irritant. However, the effect of TCE on skin irritation has received little attention.

The mechanisms underlying skin irritation are complex. In recent years, there has been considerable interest in oxidative stress as a potential mechanism in the pathogenesis of skin disease. It has been observed that oxidative stress has been implicated in the pathogenesis of various conditions including some inflammatory skin diseases such as atopic dermatitis, psoriasis vulgaris, and vitiligo (Fuchs et al., 2001; Okayama, 2005; Bickers and Athar, 2006). Oxidative stress resulting from the formation of excessive reactive oxygen species (ROS) and nitric oxide (NO) species, may damage cell membranes through production of lipid peroxides (LPO), as well as molecules such as nucleic acids, proteins and carbohydrates (Briganti and Piccolo, 2003; Kuchel et al., 2003; Nishigori et al., 2004; Sezer et al., 2007). Whether TCE would exert a potent irritant effect by topical application in vivo is yet to be answered: if so, could this be mediated by increased oxidative stress? The aim of the present study was therefore to test the above hypothesis by evaluating the effect of TCE on skin irritation and histopathological alterations in hairless mice, and in parallel experiments, oxidative stress was determined by measuring malondialdehyde (MDA) levels, superoxide dismutase (SOD) activities and NO production.

2. Materials and methods

2.1. Chemicals

All agents including TCE (99.5% purity, analytical grade or the highest commercial grade available) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Concentrations of TCE used in this study were: 20%, 40%, 80%, and 100% (v/v dissolved in olive oil). Olive oil and distilled water were used as vehicle and blank control.

2.2. Animals

Eight- to ten-week-old female BALB/c hairless mice, were purchased from Shanghai Shilaike Experimental Animal Co. Ltd., China, and housed in groups of 5 in stainless steel wire-mesh cages, and had free access to diet and tap water. The animal room was maintained on a 12-h light/12-h dark cycle, temperature and relative humidity were kept at 20−25 °C and 55 ± 5%, respectively. The animals were acclimated for 1 week prior to the treatment, those healthy and weighing 20–30 g were selected for experiments. The mice were randomly assigned to treatment or control groups of 5 animals each. All animal study protocols were conducted in accordance with the guidelines for humane treatment set by the Animal Care and Use Committee of Anhui Medical University.

2.3. Induction of acute and cumulative irritation

In order to enhance adherence, the dorsal skin of the animals about 2.5 cm² size area were de-epidermized with ethanol-wetted cotton pads. After air-drying, 50 μl of TCE dissolved in olive oil or control agents were painted topically on de-epidermized site, and then covered with sterile plastic film, which was fixed with non-irritant adhesive tape. The film was removed 4h post-application (as an occupational hazard, 4h of application represent a maximum continuous exposure in a working session at a workplace) and the treated area was gently wiped with normal solution to remove any residual liquid from the skin surface. One hour later, skin reactions were observed visually. This treatment was given twice daily (at 3 h interval) for 1 or 14 days in acute or cumulative irritation, respectively.

2.4. Skin reaction inspection and histopathological examination

Skin response to TCE treatment at each application site was visually assessed after each application. Reactions were graded as negative, mild (erythema alone), moderate (erythema with edema), or severe (erythema, edema, and vesiculation).

At the end of treatment, the animals were anesthetized and sacrificed by cervical dislocation. Skin biopsies from treated sites were taken and fixed in 10% buffered formaldehyde solution for at least 48 h, embedded into paraffin blocks and processed according to routine protocols. Sections of 5-μm thickness were cut from each sample, stained with hematoxylin-eosin (H&E). All sections were examined with a light microscope (BH-2, Olympus, Japan), by a pathologist unaware of the treatment of the samples. Histological parameters of the skin were evaluated using PAS-9000 pathological image analysis system (Logene Biological & Medical Engineering Co. Ltd.), and included hyperkeratosis (thickening of the stratum corneum), parakeratosis, spongiosis, exocytosis and dermal infiltration; these were scored as follows: (−) absent, (±) equivocal, (+) mild, (+++) moderate and (++++) severe.

2.5. Homogenate preparation of skin tissue and quantification of protein content

The skin samples were excised promptly and rinsed in ice-cold saline, then homogenized in 4 ml phosphate buffered saline (PBS, pH 7.8) using a Polytron homogenizer. Homogenates were centrifuged at 1000 rpm for 10 min at 4°C and the supernatant was collected and used for assay. The protein content in the homogenate was determined according to the method of Lowry et al. (1951). Bovine serum albumin served as standard.

2.6. LPO and SOD activities measurement

The levels of MDA, served as an indicator of LPO, were determined using the thiobarbituric acid-reactive substances (TBARS) method and performed according to the procedures described by Heath and Packer (1968) with slight modifications. 0.1 ml of the homogenate supernatant was transferred into a test tube containing 0.2 ml of 8% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA) and vortexed. The mixture was incubated for 40 min in boiling water. After cooling, an n-butanol and pyridine mixture (15:1, v/v) was added and centrifuged at 1000 × g for 10 min. Absorbance was determined at 532 nm, 1,1,3,3-tetra-methoxypropane was used as a standard. The results were expressed as mmol/mg protein.

The anti-oxidative enzyme-SOD activities was determined according to the method of Nishigori et al. (1989), which is based on inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine oxidase system as a superoxide generator. 50μl of the homogenate supernatant was added in the reaction mixture containing 985 μl of 100 mM PBS (pH 7.4), 0.3 mM K2H2-EDTA, 0.5 mM NBT, and 0.1 mM xanthine. The mixture was preincubated for 3 min at 25 °C. Then 10 μl of 0.02 U/ml xanthine oxidase was added and centrifuged. Absorbance was recorded at 550 nm. SOD activities was calibrated from a standard curve of percentage inhibition of NBT reduction and expressed as units/mg protein. One unit of SOD was defined as the amount causing 50% inhibition in the NBT reduction rate.

2.7. Determination of NO production

Since NO rapidly degrades to nitrate and nitrite in aqueous solution, the total nitrate and nitrite levels were estimated as an index of NO production. This test used a spectrophotometric method based on the Griess reaction (Green et al., 1982). To measure nitrite plus nitrate levels, 0.1 ml supernatant was mixed with Griess reagent (consisting of one part 1% sulfanilamide in 5% orthophosphoric acid) at room temperature for 10 min, and the absorbance was then measured at 550 nm. The concentration of nitrite plus nitrate was calculated according to a NaNO2 standard linear curve. Results were expressed as μmol/mg protein.

2.8. Statistical analysis

Throughout the text, data were expressed as mean ± standard deviation (S.D.). All statistical analysis was performed by ANOVA followed by Student–Newman–Keuls test, with SPSS 12.0 software package. p < 0.05 was considered statistically significant.

3. Results

3.1. TCE induced skin irritation and histopathological changes in hairless mice

In acute irritation test, TCE treatment for 1 day resulted in mild to moderate irritation, and the skin developed erythema and edema
Fig. 1. Histological changes of skin biopsies taken from BABL/c hairless mice in acute irritation induced by different concentrations of TCE (H&E, 100 ×). (A) Treated with water, the mouse skin had a thin epidermis with a thin and wavy stratum corneum and distinct boundary between epidermis and dermis. (B) Treated with olive oil, the skin showed no significant change compared to untreated mouse dorsal skin. (C) Treated with 20% TCE, the specimen exhibited slight hyperkeratosis and inflammatory cell infiltrates of the dermis. (D) Treated with 40% TCE, the sample displayed mild hyperkeratosis and spongiosis, moderate inflammatory cell infiltrates of the dermis. (E) Treated with 80% TCE, the tissue presented with mild hyperkeratosis and mild acanthosis with severe infiltration into the dermis. (F) Treated with 100% TCE, the skin biopsy showed moderate hyperkeratosis with parakeratosis and spongiosis, as well as severe infiltration into the dermis.

Table 1
Histological features of the hairless mice skin by different doses of TCE in acute irritation

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The histological parameters were evaluated semi-quantitatively (−, absent; ±, equivocal; +, mild; ++, moderate; ++++, severe). The values were those evaluated in the most dominant areas of the histological sections.
by visual observation. The irritation by TCE was concentration-dependent. Epidermal necrosis appeared in a few mouse at 100% TCE group. There were no visible alterations to any mouse in vehicle and blank controls. Fig. 1 and Table 1 show the histological features in acute irritation. In blank and vehicle controls, skin biopsies had a thin epidermis with a thin and wavy stratum corneum (SC), and showed no detectable histopathological changes (Fig. 1A and B, Table 1). In TCE treatment groups, however, various degrees of hyperkeratosis (thickening of SC), spongiosis (intercellular edema) and inflammatory cell infiltrates of the dermis (mainly composed of neutrophils) were presented (Fig. 1C–F, Table 1). Parakeratosis (persistence of nuclei in SC) was present in the majority of the biopsies from 80% to 100% TCE groups (Fig. 1E and F, Table 1). Equivocal exocytosis appeared in 100% TCE group (Fig. 1E, Table 1).

In cumulative irritation test, the skin was treated with TCE for 14 days. In the first week, the irritation reaction escalated each day and produced exudation and fissures on the skin surface in some of the mice, and reached a peak level by day 7. In the second week, the irritation response was reduced slightly and the skin exhibited flaking and thickening. Treatment with olive oil alone did not result in any visible changes to any mouse throughout the experiment. As shown in Fig. 2 and Table 2, in the cumulative irritation test, the predominant histopathological feature in majority of the biopsies was epidermal hyperplasia, hyperkeratosis, spongiosis and inflammatory cell infiltration of dermis. Exocytosis appeared in 80% and 100% TCE group (Fig. 2E and F, Table 2). Compared with acute irritation, hyperkeratosis and spongiosis were more marked, while inflammatory cell infiltration of dermis was not conspicuous in cumulative irritation.

3.2. Effect of TCE irritation on MDA content and SOD activities in the hairless mice skin

To obtain evidence for the involvement of oxidative damage in TCE-mediated skin injury, we studied the effect of TCE on oxidative stress in the skin from the same experimental model. For this purpose, LPO and anti-oxidative capacity in the skin homogenates were evaluated by MDA level and SOD activities after TCE treatment. TCE concentration-dependently increased MDA levels and inhibited SOD activities in acute irritation. A significant difference compared with blank control was observed at a dose greater than 40% (p < 0.05, Figs. 3 and 4). However, in cumulative irritation, there was no evident alteration of MDA levels and SOD activities in comparison to their respective blank control. Compared to acute irritation, the MDA levels with cumulative irritation were significantly lower at the concentrations greater than 80% (p < 0.05, Fig. 3), and the SOD activities were significantly higher with doses above 40% (p < 0.05, Fig. 4). In cumulative irritation, the MDA levels and SOD activities were firstly elevated then decreased, reaching a maximum elevation at the TCE concentration of 40% (Figs. 3 and 4). These results indicate that TCE can produce oxidative damage in hairless mice skin.

3.3. Effect of TCE irritation on NO production in the hairless mice skin

It is known that oxidative stress was associated with the production of free radical species including NO (Wang et al., 2007). To test whether the skin oxidative damage induced by TCE could also involve NO formation, we next measured the accumulation of nitrite and nitrate, the stable end products of NO oxidation, in hairless mice skin. As evident from Fig. 5, the levels of nitrite plus nitrate in hairless mice treated with TCE for both acute and cumulative irritation were significantly higher in comparison to their respective controls. The minimum effective concentration for nitrite/nitrate content elevation was 80% TCE at both time points (p < 0.05, Fig. 5). Moreover, the increase of NO production in acute irritation tended to be greater than in cumulative irritation, although the difference did not reach statistical significance. These results show that TCE irritation causes an increase in NO production in hairless mice skin.

4. Discussion

Skin lesions resulting from contact with TCE are characterized by irritant reactions, profound erythema, local inflammation, even desquamative dermatitis and toxic epidermal necrosis (Bauer and Rabens, 1974; Phoon et al., 1984; Goh and Nq, 1988; Kaneko et al., 1997; Goon et al., 2001; Pantuchaoensiri et al., 2004; Kamijima et al., 2007). There have been several reports on the mechanism of action of TCE mediated dermatoxity. Chen et al. (2006) has found that the dermatoxity by TCE involved complex immune response and inflammatory reactions (Chen et al., 2006). Wang et al. (2007) illustrated that TCE exposure not only led to oxidative/nitrosative stress, but also was associated with induction/exacerbation of autoimmune response in MRL-1 +/+ mice (Wang et al., 2007). However, to date, the irritation effects of TCE on skin have not been examined. Such effects have important implications in occupational health.

Skin irritation is defined as a non-immunological, local inflammatory reaction which is usually reversible and is characterized by erythema and edema, following a single or repeated application of a chemical to the same cutaneous site (Chew and Maibach, 2003). This reaction has a complex mechanism involving epidermal and dermal cells interacting with each other and with blood cells. Following the application of a chemical irritant or exposure to a physical insult, the epidermal barrier is perturbed and the local inflammatory reaction can appear within minutes to hours after the insult, initiated by the release of primary cytokines mainly from the epidermal keratinocytes. This usually leads to cutaneous vasodilatation and is manifested by erythema and local swelling (Corsini and Galli, 2000). In this study, we investigated skin irritation caused by topical application of TCE in hairless mice using the method of visual inspection of erythema and edema (a subjective method that is rapid, convenient, and widely used). Our results demonstrated that TCE was capable of causing erythema and edema in a dose dependent manner in hairless mice, under both acute and cumulative irritation. This has therefore provided experimental evidence that TCE has the effect of irritation on skin in vivo.

Histological assessment was made according to the changes observed for a number of histological parameters, which allowed us to quantify the damage caused to the skin tissue, such as swelling of the individual cells (intracellular oedema) (Lashmar et al., 1989). Our assessment demonstrated that hyperkeratosis, spongiosis and inflammatory cell infiltrates of dermis mainly composed of neutrophils were the predominant histopathological feature in acute irritation. The presence of neutrophils, precursors to an inflammatory response, was expected in that TCE would produce some kind of inflammatory response in hairless mice in acute irritation. In cumulative irritation, however, the predominant histopathological feature in majority of biopsies was epidermal hyperplasia, hyperkeratosis, spongiosis and neutrophil infiltration. Compared with the acute irritation, hyperkeratosis and spongiosis were more marked and inflammatory cell infiltration was less pronounced in cumulative irritation. This suggests that repetitive TCE irritation may evoke different histological changes from acute irritation, and characterized by epidermal hyperplasia with minimal inflammatory infiltration. This was in accordance with the report by Wigger-Alberti et al. (2000) and also supported by skin reactions by visual observation (Wigger-Alberti et al., 2000).
Oxidative stress has been defined as a disturbance in the pro-oxidant–antioxidant balance in favour of the former. Several lines of evidence indicate that TCE could generate free radicals and induce oxidative stress increase in other tissue, such as liver, lung and also lead to hepatitis and lung lesion (Channel et al., 1998; Mark et al., 1999; Griffin et al., 2000; Chen et al., 2002). In the present study, the hypothesis that TCE exerts an effect on skin via oxidative stress-mediated mechanism was also tested.

LPO has been considered a major presentation of oxidative stress and results from the oxidation of membrane-associated polyunsaturated fatty acids of phospholipids. The increased membrane LPO is considered to evoke immune and inflammatory responses, and to activate gene expression of cytokines and cell proliferation. MDA has been extensively utilized as the biomarker of LPO. Increased formation and subsequent accumulation of MDA have been found in various pathological conditions including skin disease (Briganti...
and Picardo, 2003; Sezer et al., 2007). In this study, our results demonstrated that TCE exposure led to an increase in MDA levels in a concentration-dependent manner in acute irritation. Interestingly, MDA levels of cumulative irritation were lower than those of acute irritation at the same TCE concentration, and initially elevated then decreased. These changes may be due to a reduced MDA formation from the body which initiates stress mechanism to eliminate the excessive free radicals, in addition to vast amount of MDA being metabolized or covalently bound with endogenous macromolecules to form the adduct, following prolonged exposure to high concentration TCE in cumulative irritation. All these indicate that TCE may cause LPO elevation in the skin of hairless mice both under acute and cumulative irritation.

SOD is an endogenous enzymatic scavenger and constitutes the first line of defense against oxygen-derived free radicals, converting the superoxide anion (O$_2^•$−) into H$_2$O$_2$ (Briganti and Picardo, 2003; Sezer et al., 2007). In the present study, TCE induced reduction of SOD activities was mirrored by an increase of LPO in acute irritation. This parallel reduction in SOD activities could represent significant enzyme depletion due to clearance of the free radicals inside the skin and thus indicate a high degree of free radical production and LPO occurrence. The anti-oxidative capacity is apparently damaged in the skin by TCE treatment, which would exacerbate toxic effects due to LPO. On the other hand, in cumulative irritation, SOD activities were higher than in acute irritation at the same TCE concentration, and also their activities firstly elevated then decreased with the increased concentration, and reached a maximum elevation at TCE concentration of 40%. This may be explained by a compensatory activation of SOD caused by long exposure to high concentrations of TCE. While an imbalance in the antioxidant status may result in accumulation of free radical, the raised SOD activities could also be a consequence of prolonged production of free radicals, suggesting a complex in vivo pathogenesis under sub-chronic intoxication, involving the interplay between pro- and anti-oxidative systems.

NO is a gaseous free radical and is generated by NO synthase (NOS) mediated biotransformation. In general, low level of NO constitutively released by the skin cell could play an important role in keratinocyte proliferation, regulating the blood flow and directly influence the wound healing process (Boissel et al., 2004; Brown et al., 2006). However excessive NO production may not merely serve as a mediator for normal physiological activities, but is also

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Fig. 3. Effect of TCE on MDA levels of the BALB/c hairless mice skin in acute and cumulative irritation. BALB/c hairless mice skin was treated with various doses of TCE for 1 or 14 days. Homogenates of skin tissue were obtained from dorsal skin at the end of irritation. The MDA levels were determined as described in Section 2. TCE increased MDA levels in a concentration-dependent manner in acute irritation. However there was no evident alteration of MDA in cumulative irritation. The MDA levels in cumulative irritation were lower than those of acute irritation. Data are presented as mean ± S.D. (n = 5). *p < 0.05 versus control, **p < 0.01 versus control, #p < 0.05 versus acute irritation, ##p < 0.01 versus acute irritation.


responsible for nonspecific immune defense mechanisms and have pathological consequences, such as hyperplaserative, immunemediated skin inflammatory diseases and tissue injury (Ormerod et al., 1999; Kuchel et al., 2003; Paunel et al., 2005; Russo and Halliday, 2006). As a vasodilator, NO is clearly involved in a number of inflammatory reactions of the skin, causing enhanced vascular permeability, edema, erythema and enhanced cell infiltration by increasing local blood flow in skin microcirculation (Rhods et al., 2001). In this study, we found that NO generation was significantly increased in TCE treated mice skin both with acute and cumulative irritation. The NO production was higher in acute irritation than in cumulative irritation and consistent with the conspicuous histopathological feature of hyperkeratosis and spongiosis in cumulative irritation. The increase in TCE-evoked NO production may serve as a mediator and contribute to the development of erythema and inflammation, as well be responsible for nonspecific immune defense mechanisms in TCE induced skin injuries.

The enhanced MDA levels and NO production in conjunction with suppressed SOD activities indicate oxidative stress elevation in the skin. Increased oxidative stress results in up-regulation of the expression of cyclo-oxygenase-2, and cell adhesion molecules, such as E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1. The regulated expression of ICAM-1 is important in facilitating recruitment of inflammatory cell at the site of inflammation and interaction, which may result in the dermal perivascular inflammatory cell infiltration and lymphocyte exocytosis observed in histopathology (Fuchs et al., 2001; Bickers and Athar, 2006). These findings not only provide experimental evidence that TCE is capable of increasing oxidative stress by cutaneous irritation, but also support our hypothesis that increased

**Fig. 4.** Effect of TCE on SOD activity of the BALB/c hairless mice skin in acute and cumulative irritation. BALB/c hairless mice skin was treated with various doses of TCE for 1 or 14 days. Homogenates of skin tissue were obtained from dorsal skin at the end of irritation. The SOD activity was determined as described as Section 2. TCE inhibited SOD activity in a concentration-dependent manner in acute irritation. There was no evident alteration to SOD activity in cumulative irritation. The SOD activity was higher than that of acute irritation. Data are mean ± S.D. (n = 5). * p < 0.05 versus control, # p < 0.05 versus acute irritation.

**Fig. 5.** Effect of TCE on NO production of the BALB/c hairless mice skin in acute and cumulative irritation. Homogenates of skin tissue were obtained from dorsal skin at the end of irritation. The nitrate plus nitrite levels as an index of NO production were determined using the Griess reaction as described in Section 2. TCE concentration-dependently increased nitrate plus nitrite levels of the BALB/c hairless mice skin in acute and cumulative irritation. Data are mean ± S.D. (n = 5). * p < 0.05 versus control, ** p < 0.01 versus control.
and inflammatory cell infiltrates. There was an association between oxidative stress and TCE induced irritation. To our knowledge, this is the first report on irritation of TCE in vivo. It is important to note that this study did not investigate skin sensitization; it was particularly focused on irritation, but not allergic reactions. However, one must bear in mind that allergic reactions to TCE do occur in some susceptible individuals and contact irritation could modulate allergic response.

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