

Research Article

Dichloroacetate Decreases Cell Health and Activates Oxidative Stress Defense Pathways in Rat Alveolar Type II Pneumocytes

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Dichloroacetate (DCA) is a water purification byproduct that is known to be hepatotoxic and hepatocarcinogenic and to induce peripheral neuropathy and damage macrophages. This study characterizes the effects of the haloacetate on lung cells by exposing rat alveolar type II (L2) cells to 0–24 mM DCA for 6–24 hours. Increasing DCA concentration and the combination of increasing DCA concentration plus longer exposures decrease measures of cellular health. Length of exposure has no effect on oxidative stress biomarkers, glutathione, SOD, or CAT. Increasing DCA concentration alone does not affect total glutathione or its redox ratio but does increase activity in the SOD/CAT oxidative stress defense pathway. These data suggest that alveolar type II cells rely on SOD and CAT more than glutathione to combat DCA-induced stress.

1. Introduction

Individuals may be exposed to haloacetic acids, and specifically dichloroacetate (DCA), via multiple sources. A byproduct of the chlorine disinfection process used in municipal water systems and the *in vivo* breakdown of the industrial solvent trichloroethylene, DCA is one of the most prevalent tap water contaminants in America with drinking water levels including up to 133 $\mu\text{g}/\text{L}$ DCA [1–3]. Due to its environmental presence, individuals are directly impacted by DCA through inhalation of aerosols, such as in showers and chlorinated pools [3, 4], and through the blood stream after ingestion [3]. For some individuals these environmental exposures are augmented by therapeutic doses of 10–50 mg/kg/day, as there are promising clinical applications for DCA in treatment of metabolic dysfunctions and antitumor activity [3, 5–14]. Keys to developing DCA's medicinal potential and properly regulating its environmental impacts lie in understanding DCA's toxicity in different cell types.

DCA's cellular impact is dependent upon duration of exposure and genetic background. Glutathione transferase 1 (GSTz1) converts DCA to the nontoxic metabolite, glyoxylate. However, GSTz1 is polymorphic with alleles showing variable inhibition due to repeated DCA exposure. As a result, in

a subset of individuals therapeutic doses of DCA would induce DCA accumulation to potentially toxic levels [15–20]. Environmental exposure to DCA, while at significantly lower than therapeutic levels, is likely to be chronic and, therefore, could manifest increased damage due to repeated exposure over time.

Because of the clinical potential and environmental presence of this compound it is important to characterize DCA's effects on cells where accumulation is likely to be the highest. Previous research shows that DCA exposure at and above clinical concentrations induces oxidative stress biomarkers and decreases viability in hepatocytes, is hepatocarcinogenic, and leads to peripheral neuropathy [21–27]. The liver, neurons, and astrocytes, however, are exposed to DCA only through circulation. In contrast, lung cells have two exposure routes, circulation, and inhalation, which could lead to increased effective doses in this tissue.

Lung alveoli walls contain type I and type II pneumocytes and macrophages. While DCA's effect on pneumocytes has not been characterized, J774.1 macrophage cells exposed to DCA revealed time-dependent decreases in viability and in the antioxidant, glutathione, and time-dependent increases in superoxide dismutase (SOD) activity [28–30]. Until this time, however, the effect of DCA on type I or type II

pneumocytes has not been reported. Type II cells secrete pulmonary surfactant, and, in response to lung damage, proliferate and differentiate into the type I pneumocytes that are responsible for gas exchange [31, 32]. Damage to type II pneumocytes is highly detrimental to lung function and, thus, understanding DCA's effects in type II pneumocytes is critical to the evaluation of its toxicological impact. To allow direct comparison between cell types, this study's design is modeled after previous work using 0–50 mM exposure to investigate peripheral neuropathy and liver and macrophage cell function [28–30, 33]. It extends our knowledge to include the effect of DCA on lung cells, using rat alveolar type II (L2) cells as a model.

2. Materials and Methods

2.1. Chemicals. Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville GA). Phenol-red-free, low glucose DMEM, and dichloroacetate (DCA) were purchased from Sigma-Aldrich (St. Louis, MO). 100x antibiotic-antimycotic was purchased from Gibco (Carlsbad, CA). Trypsin-EDTA 0.05% was obtained from VWR (Radnor, PA).

2.2. Cell Culture. Female rat noncancerous type II alveolar cells (L2, cc-149) were obtained from ATCC. L2 stocks were maintained in low glucose DMEM/10% FBS/1x antibiotic, antimycotic at 37°C + 6% CO₂. Cells were fed every 3–5 days and split when they reached 80–100% coverage. Cells were split via trypsinization, seeded into the tissue culture treated vessel indicated for that analysis, and prior to experimental treatments were allowed 16 hr to acclimate and attach. Replicates exposed to 0 mM, 8 mM, 16 mM, and 24 mM of DCA for 0, 6, 16, or 24 hours were used for analysis.

2.3. Determination of Cell Viability. Cells were seeded into clear bottom 96-well plates at 10⁵ cells/well (Corning, Corning, NY) and allowed to attach before the indicated DCA treatment. To determine relative viability, mitochondrial activity was assayed as per manufacturer's instructions (MTT Kit, Roche, Indianapolis, IN). The Abs₆₅₅ (background) and Abs₆₀₀ (formazan) were measured via Model 680 Microplate Reader (Bio-Rad). Prior to statistical analysis, values in each exposure time group were normalized to the control average (0 mM DCA) for that exposure time to control for slight variations in number amongst cells seeded $n = 6$.

2.4. Lysate Preparation for Antioxidant Enzyme Studies. Cells were seeded into 10 cm² tissue culture flask-tubes (TPP-US, St. Louis, MO) at 10⁶ cells per tube and treated with DCA as indicated in the text. Cells were harvested via scraping and centrifugation (2,000 g × 10 min, 4°C). Growth media were removed and cells resuspended in 1 mL of 50 mM potassium phosphate (pH 7.0) + 1 mM EDTA. Lysates were prepared via cycling three times between LiqN₂ and 37°C. Lysates were cleared via centrifugation (2,000 g × 5 minutes) and the supernatants collected and stored at –80°C. Assays were performed within 2 weeks of lysate preparation.

2.5. Determination of Superoxide Dismutase and Catalase Activity. Catalase (EC 1.11.1.6) and SOD (EC 1.15.1.1) activity were assayed from the same lysate sample and determined as per manufacturer's instructions (catalase kit and SOD kit, Cayman Chemical Company, Ann Arbor, MI). Absorbance values at 540 nm (catalase assay) and 450 nm (SOD assay) were determined via Model 680 Microplate Reader (Bio-Rad). For each time and concentration combination SOD $n = 10$ –12 and catalase $n = 5$ –6.

2.6. GSH/GSSG-Glo Assay. Cells were seeded in white bottom 96-well plates at 10⁴ cells/well (Corning, Corning, NY), allowed to attach, and treated with DCA. Prior to the assay growth media were removed and cells washed with PBS. Total glutathione and GSSG were each assayed in triplicate via GSH/GSSG Glo kit (Promega, Madison, WI) following manufacturers instructions and as in Chalfant and Bernd [34].

2.7. Statistical Analysis. Data obtained from independently prepared cultures were analyzed using Microsoft Excel and JMP software. Values from each exposure time sample were normalized to the mean 0 mM DCA samples value for that exposure time. Outliers were identified and removed using a Q-test with a 90% confidence interval. Data presented are the normalized mean ± SD. To determine the effects of independent variables DCA concentration and exposure length on the different biological markers, two-way ANOVA tests were performed followed by LSMean Differences Tukey HSD *post hoc* test ($p < 0.05$).

3. Results

To investigate the effects of DCA on rat alveolar type II (L2) cells, samples were treated with 0–24 mM DCA at the indicated time points and the MTT assay was used to determine percent viability. Both the duration of DCA exposure and the concentration of DCA in the treatment resulted in significant decreases in cell viability (Figure 1). While no length of exposure to 8 mM DCA compromised cell health, exposure to 16 mM or 24 mM DCA for 24 hours induced significant decreases in viability, 22 and 25%, respectively. In addition, statistical analysis revealed a significant interaction effect between the two variables, indicating that the combination of increased duration of exposure and higher DCA concentrations causes a more severe decline in cell health than changes in either variable alone ($p < 0.037$).

Because DCA significantly reduced cellular viability and the compound is known to induce oxidative stress in other cell types, the roles of different antioxidant pathways were characterized. Glutathione, a general antioxidant, exists in reduced and oxidized pools within the cytoplasm and mitochondria. A cell's exposure to oxidant can cause changes in the total amount of glutathione or may shift the ratio of glutathione in reduced versus oxidized forms. To examine the effect of DCA exposure, total glutathione in samples treated with the indicated concentration of DCA for 8, 16, or 24 hr was pooled and that average normalized to the

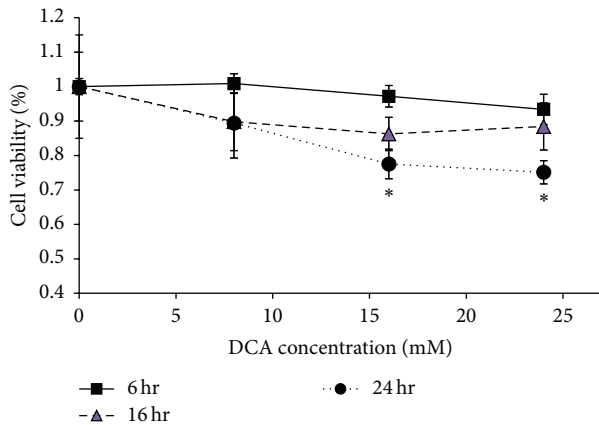


FIGURE 1: Effect of DCA treatment regimes on L2 viability. Cells were exposed to 0, 8, 16, and 24 mM DCA for 6, 16, and 24 hours. Viability was determined by MTT assay with the viability 0 mM DCA treatment for the corresponding exposure time defined as 100%. Bars represent mean \pm S.D., $n = 5-6$. * denotes a significant difference between data from that condition and those without * ($p < 0.037$).

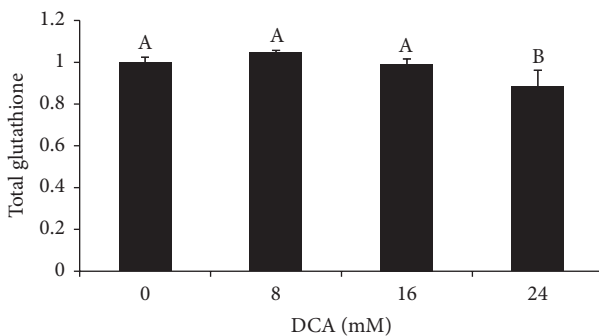


FIGURE 2: Effect of DCA on total glutathione. Cells were exposed to 0, 8, 16, and 24 mM DCA. Measurements of total glutathione at 8, 16, and 24 hr DCA exposure were pooled and that average value normalized to baseline glutathione levels (0 mM DCA) and the mean \pm S.D. is shown. $n = 9$. Bars with no shared superscripts are significantly different ($p < 0.0008$).

0 mM treatment. These data reveal a downward trend where increases in DCA concentration to 24 mM have a significant negative effect on total glutathione levels (Figure 2; $p < 0.0008$). The role of glutathione was further characterized by determining the effect of DCA on the antioxidant's oxidation state. Analysis of the amount of oxidized glutathione (GSSG) and the ratio of reduced to oxidized glutathione (GSH: GSSG) revealed that neither DCA concentration nor exposure time had a significant effect on levels of oxidized glutathione or on the ratio of reduced/oxidized glutathione (data not shown).

Superoxide dismutase (SOD) and catalase (CAT) function in an antioxidant pathway that detoxifies superoxide anion to molecular oxygen and water. The duration of exposure to DCA (0–24 hr) did not have an effect on this metric and no duration: concentration interaction effect was seen. However, SOD activity increases as the DCA treatment

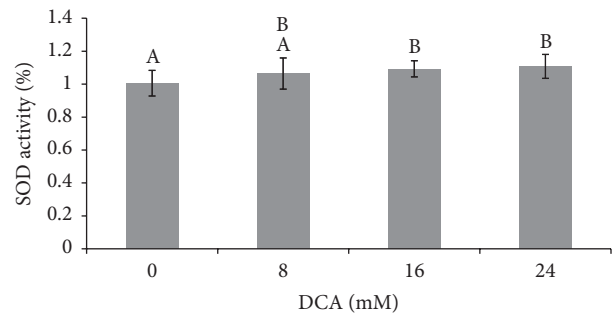


FIGURE 3: Effect of DCA on L2 cell SOD activity. Cells were exposed to DCA at concentrations of 0, 8, 16, and 24 mM. SOD activity at 8, 16, and 24 hr DCA exposure was pooled and that average value normalized to baseline SOD levels (0 mM DCA) and mean \pm S.D. is shown. $n = 33-36$. Columns with no shared superscripts are significantly different ($p < 0.003$).

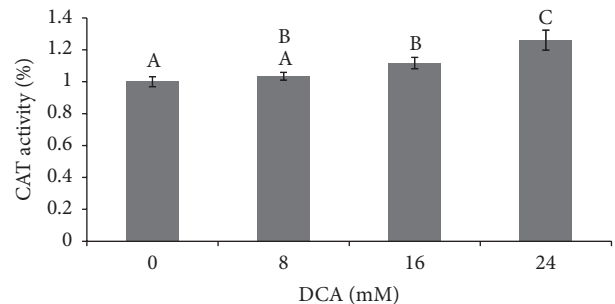


FIGURE 4: Effect of DCA on L2 cell CAT activity. Cells were exposed to DCA at concentrations of 0, 8, 16, and 24 mM. CAT activity at 8, 16, and 24 hr DCA exposure was pooled and that average value normalized to baseline CAT levels (0 mM DCA) and mean \pm S.D. is shown. $n = 20-24$. Columns with no shared superscripts are significantly different ($p < 0.0001$).

concentration rises (Figure 3). Treatment of L2 cells with DCA concentrations of 16 mM and 24 mM resulted in the increase of SOD activity by 9 and 11%, respectively, over the corresponding control ($p < 0.0003$).

CAT activity changes in response to DCA treatment are similar to those seen for SOD activity. Again, length of exposure did not affect the level of CAT activity and no significant interaction effect was seen between concentration and length of exposure. However, DCA concentration alone has a significant effect on CAT activity with 16 mM and 24 mM DCA treatments significantly increasing CAT activity (12 and 26%) as compared to the control treatments (Figure 4, $p < 0.0001$).

4. Discussion

Previous studies have found DCA to be hepatotoxic, hepatocarcinogenic, damaging to macrophage cells, and capable of inducing peripheral neuropathy *in vivo* [21–31, 33]. This study extends our understanding to a novel cell type, lung cells, and illustrates that DCA has a significant, negative effect on rat alveolar type II (L2) cells.

L2 cells show a distinct pattern of DCA sensitivity. Both the level and the duration of DCA exposure decrease L2 cell viability, with statistical analysis revealing a significant cross effect between the variables. This places L2 cell's 16 mM DCA threshold sensitivity between those reported for CHO cultures (2.0 mM DCA [35]) and J774A.1 macrophages (24 mM [31]).

Glutathione is known to be a component of lung, and particularly alveolar, antioxidant defense system [14, 36, 37]. Macrophages challenged with 24 mM DCA for 24–60 hr demonstrate time-dependent decreases in total glutathione [31]. By comparison, 0–24 hr DCA exposures caused no changes in L2 cell's total glutathione levels. Initially, statistical analysis revealed a significant glutathione decrease in L2 cells exposed to 16 mM or 24 mM DCA. However, the statistical correlation between decreases in cell viability and total glutathione accounts for 73% of the decrease in the antioxidant seen at exposures ≥ 8 mM. The small difference that remains is not significant. Therefore, while 24 mM DCA affects glutathione levels in macrophages, the conditions tested do not alter glutathione levels in a second component of alveoli, L2 cells.

Because DCA-induced decreases in glutathione have been reported in other culture and whole animal systems [23, 24, 29–31], the lack of a significant effect on total glutathione levels in L2 cells was unexpected and investigated further. In addition to altering total glutathione levels, oxidative stress can shift the GSH: GSSG ratio [38]. However, neither DCA concentration nor exposure length caused significant changes in oxidized glutathione levels or in the GSH: GSSG ratio. These data suggest that glutathione is not a major component of type II alveolar cell response to oxidative stress caused by acute DCA exposure.

DCA-induced superoxide anion production is expected to activate the SOD/CAT oxidative stress pathway. Consistent with our findings on DCA toxicity, L2 cells demonstrate a different and more sensitive SOD/CAT response than macrophage cells [28, 31]. In L2 cells, increases in SOD/CAT activity are dependent upon DCA concentration but not upon exposure duration. Treatment with 16 mM DCA causes similar increases in SOD and CAT activity (9% versus 12%). However, 24 mM DCA results in greater induction of CAT than SOD (26% versus 11%). The increases in catalase activity seen after exposure to DCA are consistent with the cell responding to H_2O_2 produced by SOD. However, in L2 cells, DCA's induction of CAT activity surpasses its induction of SOD activity. Reports show that DCA induces H_2O_2 production by hepatocyte peroxisomes [34]. Therefore the higher induction of CAT activity seen here could be due to 24 mM DCA exposure triggering similar peroxisome activity increases in L2 cells. However, the differences previously noted between CHO cells, macrophages, and L2 cells indicate that the complexity of the system supports future empirical testing of whether the catalase activity induction seen in L2 cells is due to peroxisome activity or a byproduct of increased SOD activity.

In conclusion, previous research has shown DCA has deleterious effects on liver and neuronal cells but these organs only experience the compound through the blood stream.

Because of their position at the interface between the internal and external environments, lung compartments experience exposure to this industrial solvent breakdown product/water purification byproduct through the blood stream and via inhalation. Lung alveoli are at this junction point in the body's defenses against oxidative damage. Previously only their macrophage component of lungs had been examined. We show that type II cells are more DCA-sensitive than macrophages and respond by differential activation of pathways that detoxify superoxide anion and H_2O_2 . Our results further highlight the need to investigate DCA's effects in multiple systems, as threshold sensitivity levels and responses vary among cell types. The fact that type II pneumocytes are negatively affected by exposure to lower concentrations of environmental DCA underscores the importance of multisystem studies when establishing exposure limits.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

- [1] R. J. Bull, "Mode of action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate," *Environmental Health Perspectives*, vol. 108, no. 2, pp. 241–259, 2000.
- [2] IARC (International Agency for Research on Cancer), "Dichloroacetic acid," *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 84, pp. 359–402, 2004.
- [3] P. W. Stacpoole, "The dichloroacetate dilemma: environmental hazard versus therapeutic Goldmine—both or neither?" *Environmental Health Perspectives*, vol. 119, no. 2, pp. 155–158, 2011.
- [4] X. Xu and C. P. Weisel, "Inhalation exposure to haloacetic acids and halo ketones during showering," *Environmental Science and Technology*, vol. 37, no. 3, pp. 569–576, 2003.
- [5] I. Papandreou, T. Goliaeva, and N. C. Denko, "Anticancer drugs that target metabolism: is dichloroacetate the new paradigm?" *International Journal of Cancer*, vol. 128, no. 5, pp. 1001–1008, 2011.
- [6] C. Granchi and F. Minutolo, "Anticancer agents that counteract tumor glycolysis," *ChemMedChem*, vol. 7, no. 8, pp. 1318–1350, 2012.
- [7] K. Berendzen, D. W. Theriaque, J. Shuster, and P. W. Stacpoole, "Therapeutic potential of dichloroacetate for pyruvate dehydrogenase complex deficiency," *Mitochondrion*, vol. 6, no. 3, pp. 126–135, 2006.
- [8] P. W. Stacpoole, L. R. Gilbert, R. E. Neiberger et al., "Evaluation of long-term treatment of children with congenital lactic acidosis with dichloroacetate," *Pediatrics*, vol. 121, no. 5, pp. e1223–e1228, 2008.
- [9] P. W. Stacpoole and Y. J. Greene, "Dichloroacetate," *Diabetes Care*, vol. 15, no. 6, pp. 785–791, 1992.

- [10] P. W. Stacpoole, D. S. Kerr, C. Barnes et al., "Controlled clinical trial of dichloroacetate for treatment of congenital lactic acidosis in children," *Pediatrics*, vol. 117, no. 5, pp. 1519–1531, 2006.
- [11] J. Y. Y. Wong, G. S. Huggins, M. Debidda, N. C. Munshi, and I. De Vivo, "Dichloroacetate induces apoptosis in endometrial cancer cells," *Gynecologic Oncology*, vol. 109, no. 3, pp. 394–402, 2008.
- [12] S. Bonnet, S. L. Archer, J. Allalunis-Turner et al., "A mitochondria-K⁺ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth," *Cancer Cell*, vol. 11, no. 1, pp. 37–51, 2007.
- [13] B. M. Madhok, S. Yeluri, S. L. Perry, T. A. Hughes, and D. G. Jayne, "Dichloroacetate induces apoptosis and cell-cycle arrest in colorectal cancer cells," *British Journal of Cancer*, vol. 102, no. 12, pp. 1746–1752, 2010.
- [14] P. W. Stacpoole, G. N. Henderson, Z. Yan, R. Cornett, and M. O. James, "Pharmacokinetics, metabolism, and toxicology of dichloroacetate," *Drug Metabolism Reviews*, vol. 30, no. 3, pp. 499–539, 1998.
- [15] A. Gonzalez-Leon, J. L. Merdink, R. J. Bull, and I. R. Schultz, "Effect of pre-treatment with dichloroacetic or trichloroacetic acid in drinking water on the pharmacokinetics of a subsequent challenge dose in B6C3F1 mice," *Chemico-Biological Interactions*, vol. 123, no. 3, pp. 239–253, 1999.
- [16] M. O. James, W. Li, Y. Gu, T. Langaee, and P. W. Stacpoole, "Dichloroacetate-dependent inactivation of GSTZ1 in human liver cytosol is modulated by physiologically important anions in a GSTZ1 haplotype-dependent manner," *Drug Metabolism Reviews*, vol. 43, p. 107, 2011.
- [17] W. Li, Y. Gu, M. O. James et al., "Prenatal and postnatal expression of glutathione transferase ζ 1 in human liver and the roles of haplotype and subject age in determining activity with dichloroacetate," *Drug Metabolism & Disposition*, vol. 40, no. 2, pp. 232–239, 2012.
- [18] W. Li, M. O. James, S. C. McKenzie, N. A. Calcutt, C. Liu, and P. W. Stacpoole, "Mitochondrion as a novel site of dichloroacetate biotransformation by glutathione transferase ζ 1," *Journal of Pharmacology and Experimental Therapeutics*, vol. 336, no. 1, pp. 87–94, 2011.
- [19] P. W. Stacpoole, E. M. Dunbar, A. L. Shroads, B. S. Coats, and T. Langaee, "Predictability of glutathione transferase zeta 1 haplotype on kinetics of dichloroacetate in human trials," *Drug Metabolism Reviews*, vol. 43, pp. 181–182, 2011.
- [20] A. L. Shroads, T. Langaee, B. S. Coats et al., "Human polymorphisms in the glutathione transferase zeta 1/maleylacetoacetate isomerase gene influence the toxicokinetics of dichloroacetate," *Journal of Clinical Pharmacology*, vol. 52, no. 6, pp. 837–849, 2012.
- [21] R. J. Bull, G. A. Orner, R. S. Cheng et al., "Contribution of dichloroacetate and trichloroacetate to liver tumor induction in mice by trichloroethylene," *Toxicology and Applied Pharmacology*, vol. 182, no. 1, pp. 55–65, 2002.
- [22] S. L. Herren-Freund, M. A. Pereira, M. D. Khoury, and G. Olson, "The carcinogenicity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid, in mouse liver," *Toxicology and Applied Pharmacology*, vol. 90, no. 2, pp. 183–189, 1987.
- [23] E. A. Hassoun, J. Cearfoss, and J. Spildener, "Dichloroacetate- and trichloroacetate-induced oxidative stress in the hepatic tissues of mice after long-term exposure," *Journal of Applied Toxicology*, vol. 30, no. 5, pp. 450–456, 2010.
- [24] E. A. Hassoun and J. Cearfoss, "Dichloroacetate- and trichloroacetate-induced modulation of superoxide dismutase, catalase, and glutathione peroxidase activities and glutathione level in the livers of mice after subacute and subchronic exposures," *Toxicological and Environmental Chemistry*, vol. 93, no. 2, pp. 332–344, 2011.
- [25] N. A. Calcutt, V. L. Lopez, A. D. Bautista et al., "Peripheral neuropathy in rats exposed to dichloroacetate," *Journal of Neuropathology and Experimental Neurology*, vol. 68, no. 9, pp. 985–993, 2009.
- [26] A. B. DeAngelo, F. B. Daniel, J. A. Stober, and G. R. Olson, "The carcinogenicity of dichloroacetic acid in the male B6C3F1 mouse," *Fundamental and Applied Toxicology*, vol. 16, no. 2, pp. 337–347, 1991.
- [27] V. C. Moser, P. M. Phillips, K. L. McDaniel, and R. C. MacPhail, "Behavioral evaluation of the neurotoxicity produced by dichloroacetic acid in rats," *Neurotoxicology and Teratology*, vol. 21, no. 6, pp. 719–731, 1999.
- [28] E. A. Hassoun and S. Ray, "The induction of oxidative stress and cellular death by the drinking water disinfection by-products, dichloroacetate and trichloroacetate in J774.A1 cells," *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, vol. 135, no. 2, pp. 119–128, 2003.
- [29] E. A. Hassoun, J. Spildener, and J. Cearfoss, "The induction of tumor necrosis factor- α , Superoxide anion, Myeloperoxidase, and superoxide dismutase in the peritoneal lavage cells of mice after prolonged exposure to dichloroacetate and trichloroacetate," *Journal of Biochemical and Molecular Toxicology*, vol. 24, no. 2, pp. 136–144, 2010.
- [30] E. A. Hassoun and S. Dey, "Dichloroacetate- and trichloroacetate-induced phagocytic activation and production of oxidative stress in the hepatic tissues of mice after acute exposure," *Journal of Biochemical and Molecular Toxicology*, vol. 22, no. 1, pp. 27–34, 2008.
- [31] E. A. Hassoun and J. Mehta, "Dichloroacetate-induced modulation of cellular antioxidant enzyme activities and glutathione level in the J774.A1 cells," *Journal of Applied Toxicology*, vol. 28, no. 8, pp. 931–937, 2008.
- [32] H. Fehrenbach, "Alveolar epithelial type II cell: defender of the alveolus revisited," *Respiratory Research*, vol. 2, no. 1, pp. 33–52, 2001.
- [33] N. A. Calcutt, V. L. Lopez, A. D. Bautista et al., "Peripheral neuropathy in rats exposed to dichloroacetate," *Journal of Neuropathology & Experimental Neurology*, vol. 68, no. 9, pp. 985–993, 2009.
- [34] M. Chalfant and K. K. Bernd, " 17β -estradiol alters rat type-II alveolar cell recovery from high levels of ozone," *PLoS ONE*, vol. 9, no. 3, Article ID e90530, 2014.
- [35] M. J. Plewa, J. E. Simmons, S. D. Richardson, and E. D. Wagner, "Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products," *Environmental and Molecular Mutagenesis*, vol. 51, no. 8–9, pp. 871–878, 2010.
- [36] I. Rahman, "Regulation of nuclear factor- κ B, activator protein-1, and glutathione levels by tumor necrosis factor- α and dexamethasone in alveolar epithelial cells," *Biochemical Pharmacology*, vol. 60, no. 8, pp. 1041–1049, 2000.

- [37] M. O. James, R. Cornett, Z. Yan, G. N. Henderson, and P. W. Stacpoole, "Glutathione-dependent conversion to glyoxylate, a major pathway of dichloroacetate biotransformation in hepatic cytosol from humans and rats, is reduced in dichloroacetate-treated rats," *Drug Metabolism and Disposition*, vol. 25, no. 11, pp. 1223–1227, 1997.
- [38] M. Chalfant and K. Bernd, "Detecting ozone-induced changes in cellular redox balance via GSH/GSSG-glo assay," Promega Corporation Website, 2012, http://worldwide.promega.com/resources/pubhub/tpub_059-detecting-ozone-induced-changes-in-cellular-redox-balance-via-gsh-gssg-glo-assay/.