Transcriptional Profile of Diuron-Induced Toxicity on the Urinary Bladder of Male Wistar Rats to Inform Mode of Action

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Received February 9, 2011; accepted April 27, 2011

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a substituted urea herbicide that induces rat urinary bladder urothelial tumors at high dietary levels (2500 ppm). The specific mode of action and molecular alterations triggered by diuron, however, have not been clarified. The present study evaluated the dose-dependent effects of mucosal alterations and transcriptional changes in the urinary bladder of rats exposed to diuron. Six-week-old male Wistar rats were treated with 0, 60, 125, 1250, and 2500 ppm of diuron in the diet for 20 weeks. Histologic examination showed urothelial hyperplasia present in rats treated with either 1250 or 2500 ppm of diuron but not 60 or 125 ppm. Comprehensive gene expression analyses of urothelial cell RNA were conducted using Affymetrix microarrays. The numbers of differentially expressed transcripts between each treatment group and control increased with diuron dose. Based on similar histology and gene expression responses, the treatment groups were regrouped into a high-dose (1250 and 2500 ppm) and low-dose group (60 and 125 ppm). These data suggest that persistent exposure to high dietary concentrations of diuron induces oxidative stress, increases cellular metabolism, and enhances cell death that is associated with sustained urothelial hyperplasia.

Key Words: diuron; urinary bladder; carcinogenesis; gene expression profiling; microarray analysis.

The mechanism of herbicidal action of diuron is inhibition of photosynthesis (Geoffroy et al., 2002). A previous study indicated that diuron generates reactive oxygen species (ROS) in plants, leading to peroxidative destruction of pigments, proteins, nucleic acids, and lipids (Geoffroy et al., 2002). Diuron does accumulate in the environment. It can be found in the soil, water and in fewer amounts in groundwater, because of its slow breakdown (a month to a year) (Cox, 2003; Giacomazzi and Cochet, 2004). The U.S. Department of Agriculture reported a half-life of 90 days for diuron in the soil (applied Cox, 2003). In rats and dogs fed diuron, the predominant metabolite in urine is 3,4-(dichlorophenyl) urea and only small amounts of DCA and others are found (Hodge et al., 1967). DCA was pointed as the most important pollutant resulting from the breakdown of diuron in the environment; however, there is no evidence to state that the DCA is the main compound in soil (Giacomazzi and Cochet, 2004). Because of the lack of epidemiological data, we are not aware of parental diuron or diuron metabolites in the urine of humans.

Diuron has been characterized as a “known/likely” human carcinogen by the U.S. Environmental Protection Agency (USEPA) based on a 2-year bioassay that demonstrated urinary bladder carcinomas in both sexes of the Wistar rat, renal pelvis carcinomas in the Wistar male rat, and mammary gland carcinomas in the female NMRI mouse after exposure to 2500 ppm diuron in the diet (Iyer, 2002; USEPA, 2003, 2004). A previous study from our laboratory observed an increased incidence of urothelial necrosis and hyperplasia associated with increased urothelial cell proliferation in male Wistar rats exposed to the carcinogenic dose of diuron for 20 weeks (Nascimento et al., 2006). As genotoxicity evaluations of diuron have generally been negative, the urothelial carcinogenicity of diuron is believed to occur through a nongenotoxic mode of action (MOA) (Gee, 1997; Iyer, 2002; Nascimento et al., 2006; Rocha et al., 2010; USEPA, 2003). Indirect effects of male Wistar Rats to Inform Mode of Action.
such as changes in urinary solids and pH have been shown not to be key events in diuron-induced carcinogenicity (Rocha et al., 2010). It is proposed that direct action of diuron and/or one of its urinary metabolites causes sustained cytotoxicity and necrosis followed by regenerative hyperplasia, which eventually leads to urothelial neoplastic development (Nascimento et al., 2006; Rocha et al., 2010).

The specific MOA and molecular alterations triggered by diuron, however, have not been been defined. The present study aimed to evaluate diuron-induced alterations and to assess the dose-response of diuron on urinary bladder urothelium in male Wistar rats. As the first genomic screening to determine the major altered pathways involved in diuron-induced urothelial toxicity in rats, genome-wide gene expression profiling of RNA combined with light microscopy was used to evaluate the urothelium. It was anticipated that data from the transcript profiling would inform the MOA. The microarray data were compared with the histological observations to relate the gene expression changes directly to observed toxic effects in the target tissue and determine if it is possible to distinguish toxic from nontoxic doses by gene expression studies.

**MATERIALS AND METHODS**

**Chemicals.** Diuron (3-(3,4-dichlorophenyl)-1,1-dimethyl-urea) was obtained from Sigma Chemical Co. (CAS no. 30-54-1, 97% purity, St Louis, MO). It was mixed with Nuvilab powdered commercial diet from Nuvital (Colombo, PR, Brazil) at final diuron concentrations of 60, 125, 1250, and 2500 ppm.

**Animal treatment and sample collection.** This study was approved by the Ethics Committee Animals Authors of the University of São Paulo (SP, Brazil). Six-week-old male Wistar rats were obtained from the Multidisciplinary Center for Biological Investigation—UNICAMP (Campinas, SP, Brazil). Following 2 weeks of acclimation, the rats were randomized into 5 groups of 15 animals each and received diuron in the pelleted feed at final concentrations of 0 (control), 60, 125, 1250, and 2500 ppm. Water and feed were provided ad libitum. Animals were monitored daily and cages, and bedding were changed three times per week. Water, feed consumption, and body weight were determined for each animal every other week of the experiment.

After 20 weeks, the rats were anesthetized with 3% sodium pentobarbital i.p. (30 mg/kg). Urinary bladders were removed from 10 rats in each group and processed by routine methods for histological analysis. Briefly, the urinary bladders were injected with Bouin’s fixative, removed, and immersed in the same fixative for 4 h. They were then sectioned mid-sagittally, washed in 70% alcohol, embedded in paraffin, and stained with hematoxylin and eosin. Immediately after removal of the bladder, the animals were euthanized by opening the abdominal cavity and sectioning the inferior vena cava.

Five animals per group were used for RNA isolation from the urothelium and subsequent gene expression analysis. The rats were anesthetized and the urinary bladder exteriorized, tied off adjacent to the trigone, and removed. Urine was withdrawn from the bladders which were then injected with 1 ml of cold RNase-free PBS (Applied Biosystems, Foster City, CA) to remove exfoliated cells. All steps were carried out rapidly to minimize autolysis. The bladder was everted on a glass rod to expose the epithelium. The cells were gently shaved and transferred to 1 ml of TRIzol solution (Invitrogen Corporation, Carlsbad, CA). The urinary bladders were also inserted into the TRIzol solution followed by brief vortexing and kept for 10 min at room temperature, then removed, and the TRIzol was transferred to liquid nitrogen followed by storage at −70°C until RNA extraction. The remaining urinary bladder was fixed in formalin and processed for histological examination as described above to evaluate the efficiency of removal of the urothelium. The efficiency of removal was graded according to the percentage (%) of epithelial cells remaining per bladder strip, being: < 5%—grade 0; 5 to 10%—grade 1; 11 to 50%—grade 2, and > 50%—grade 3. Only samples with grades 0 and 1 and with intact basal membrane were used in subsequent RNA isolation steps. The best three samples per group to proceed with the microarray experiments were chosen.

**Urinary pH determination.** The urine collection procedures were performed according to Cohen et al. (2007b). Briefly, for each rat, freshly voided urine was collected directly into a 1.5-ml microtube in the morning between 7:00 and 9:00 AM during the 6th and 14th weeks of the experiment. The urinary pH was measured immediately after collection of urine using a microelectrode (Analyser Comercio e Industria Ltda., Sao Paulo, SP).

**Statistical analyses.** Body weight, water and diet consumption, and urinary pH results were compared among the experimental groups using a one-way ANOVA (p < 0.05). Data demonstrating significant differences were then further evaluated with a Tukey’s test to determine significant differences among groups (p < 0.05). A chi-square test was applied to evaluate the incidence of urinary histological lesions. The significance level of 5% was adopted for all analyses and were performed using Prism 3 (San Diego, CA).

**RNA extraction.** Total RNA was isolated using TRIzol Reagent in accordance with the manufacturer’s protocol (Invitrogen). The total RNA pellet was resuspended in 100 µl of water and purified using the RNeasy Mini kit (Qiagen, Valencia, CA). Purified total RNA was eluted in dH2O and its concentration was measured using a NanoDrop ND-1000 spectrometer (Nanodrop, Wilmington, DE). The quality of RNA was assessed using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) and only samples having an RNA integrity number > 7.0 were used for microarray analyses. In the present study, one chip was used per animal and three chips were used per exposure group.

**Affymetrix microarray hybridization.** Gene expression analyses were conducted using Affymetrix GeneChip Rat Genome 230_2.0 Arrays (Affymetrix, Santa Clara, CA) which contain > 31,000 probe sets representing ~28,700 known rat genes. Total RNA (1 µg) for each sample was amplified and labeled by the BioArray RNA Amplification and Biotin labeling System (Enzo Life Science, Farmingdale, NY). For each array, 12 µg of amplified biotin-labeled RNA was fragmented and hybridized to the array for 16 h at 45°C in a rotating hybridization oven using the Affymetrix Eukaryotic Target Hybridization controls and protocol. The arrays were washed using the EukGE-WS2v5 protocol of the Affymetrix Fluidics Station FS450 and scanned using the Affymetrix Scanner 3000.

**Microarray data analysis.** Data from each microarray scan (.cel file) was imported into Rosetta Resolver version 7.1 (Rosetta Inpharmatics, Kirkland, WA) for data normalization (Weng et al., 2006) and for determination of transcripts that were differentially expressed between control and treated groups. To survey the data for within-group outliers and trends in data quality, unsupervised principal component analysis (PCA) was performed (Rosetta).

Transcripts that were differentially expressed among all treatment groups were identified using a one-way ANOVA with a false discovery rate (Benjami-Hochberg test) of p ≤ 0.05. A Tukey-Kramer post hoc test (p ≤ 0.05) was then used to identify significant changes in expression between each group. Expression values for each differentially expressed transcript (DET) were reported in terms of their relative abundance in each treatment versus control group. Venn diagrams using DETs were employed to identify unique and common sequences between treatment groups. Pathway analysis was performed using Ingenuity Pathways Analysis (IPA) (Ingenuity Systems, http://www.ingenuity.com). The most significant biological functions and altered canonical pathways associated with the DETs in each of the treatment groups were determined using IPA.
To evaluate genes related to cell proliferation, a series of genes have been identified that have cell cycle–dependent expression changes as observed in the HeLa cervical cancer cell line (Whitfield et al., 2002). The complete list of 1134 human genes was downloaded from http://genome-www.stanford.edu/Human-CellCycle/HeLa/data.shtml. The lists of DETs at 60, 125, 1250, and 2500 ppm were mapped to the 1134 human genes list to find the significant genes altered in a cell cycle phase-specific pattern (Fig. 5) (Nesnow et al., 2009).

RESULTS

Body Weight, Water and Diet Consumption, Urinary pH, and Histological Analyses

Table 1 summarizes the final body weigh and water and diet consumption. In general, all animals gained weight during the experiment. Mean body weight of the 2500-ppm group was less than control beginning from the third week of the experiment onward \( (p < 0.05) \). The 1250-ppm group also had significantly lower mean body weight gain compared with the control group. There was a reduction of food consumption by the 1250- and 2500-ppm groups \( (p < 0.05) \), and water consumption was significantly decreased in the 2500-ppm group (Table 1). The reduction in food consumption did not affect the relative diuron intake levels across the groups (Table 1).

Urinary pH values of each treatment group did not differ from the control (data not shown). Light microscopic examination of the urinary bladders identified an increased incidence of simple hyperplasia (SH) in rats treated with 1250- and 2500-ppm diuron (Table 2; Fig. 1).

Gene Expression Analysis

PCA was applied to the statistically significant DETs from all exposure groups (0, 60, 125, 1250, and 2500 ppm). PCA provides a 3D view of gene expression data, reducing its dimensionality and capturing the variability of the data set. Each chip is represented by a single point and points that cluster close together in 3D space have similar expression profiles. PCA therefore allows for the rapid and clear identification of outliers within treatment groups and similarities in expression trends among groups. Two clusters of separation were observed among the groups: the control, 60-ppm, and 125-ppm groups clustered together, whereas the 1250- and 2500-ppm groups clustered together separate from the control/low-dose cluster (Fig. 2).

### TABLE 1

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Final body weight (g)</th>
<th>Water consumption (ml/rat/day)</th>
<th>Food consumption (g/rat/day)</th>
<th>Diuron consumption (mg/rat/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>503.7 ± 45.7</td>
<td>33.3 ± 2.7</td>
<td>24.8 ± 1.5</td>
<td>—</td>
</tr>
<tr>
<td>60 ppm</td>
<td>477.2 ± 35.1</td>
<td>32.4 ± 2.0</td>
<td>22.4 ± 1.6</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>125 ppm</td>
<td>487.5 ± 50.7</td>
<td>34.2 ± 2.9</td>
<td>23.5 ± 1.6</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>1250 ppm</td>
<td>448.5 ± 54.9</td>
<td>32.8 ± 2.1</td>
<td>22.3 ± 1.8</td>
<td>22.9 ± 2.2</td>
</tr>
<tr>
<td>2500 ppm</td>
<td>390.5 ± 38.4†</td>
<td>28.0 ± 2.2†</td>
<td>19.0 ± 1.8†</td>
<td>47.8 ± 4.5</td>
</tr>
</tbody>
</table>

*The groups represent the amount of diuron added to the food.

**Note.** 1, Significantly different from the control; 2, significantly different from 60-, 125-, and 1250-ppm groups.

### TABLE 2

Incidence of Urinary Bladder Urothelial Lesions in Different Groups of Wistar Male Rats Fed for 20 Weeks with Diuron

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Normal</th>
<th>Simple hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>60 ppm</td>
<td>10</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>125 ppm</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>1250 ppm</td>
<td>10</td>
<td>2</td>
<td>8†</td>
</tr>
<tr>
<td>2500 ppm</td>
<td>10</td>
<td>3</td>
<td>7†</td>
</tr>
</tbody>
</table>

†Chi-square test, \( p < 0.05 \).

FIG. 1. Urinary bladder histology showing (A) normal epithelium in control and (B) simple hyperplasia in 2500 ppm diuron (hematoxylin and eosin ×40).
The number of DETs modulated by diuron treatment increased with the dietary concentrations of the herbicide. There were 257, 291, 532, and 997 DETs in cells from the 60-, 125-, 1250-, and 2500-ppm diuron-exposed groups, respectively (Table 3). The complete gene list is attached as a spreadsheet (.excel) as Supplementary data. More than 50% of DETs had reduced expression in the 60-, 1250-, and 2500-ppm groups, whereas ~21% of DETs had reduced expression in the 125-ppm group (Table 3).

Common and unique DETs across all groups were determined using Rosetta Resolver and are illustrated in Figure 3. This analysis identified that (1) the number of unique genes was 130, 219, 101, and 518 for 60-, 125-, 1250-, and 2500-ppm groups, respectively; (2) 78% (417) of the DETs in the 1250-ppm treatment group were also present in the 2500-ppm treatment group and 73% (308) of those sequences were exclusively shared between 1250- and 2500-ppm treatment groups; and (3) there were only 6 DETs in common among all treatment groups (AK3, PIK3R1, ATP1B1, HTATIP2, MSLN, PROM1), all of which had reduced transcript levels compared with controls.

Pathway Analysis

The lists of DETs were submitted for canonical pathway level analysis (IPA); only pathways statistically significant ($p < 0.05$) and containing at least three genes were retained for further evaluation (Hester et al., 2006). The most significant diseases and biological function pathways altered in high-dose animals included cancer, amino acid metabolism, small molecule biochemistry, and cell death (Supplementary table S1). The most significant pathways associated with the low-dose group were involved in drug metabolism, lipid metabolism, and small molecule biochemistry (data not shown).

Dose-Responsive Pathways

Transcripts that are associated with the higher level function of cell death were significantly altered for all doses with a dose-response relationship (Supplementary table S2). Several canonical pathways were also modulated in a dose-responsive manner, including those that participate in glutathione metabolism, xenobiotic metabolism, NRF2-mediated oxidative stress response, and tryptophan metabolism (Fig. 4). The number of significantly altered canonical pathways also had a dose-response; 60-, 125-, 1250-, and 2500-ppm diuron groups had 9, 33, 34, and 46 altered pathways, respectively. In general, the 1250- and 2500-ppm exposure groups had similar altered pathways, which were associated with oxidative stress, such as xenobiotic metabolism and antioxidant defense mediated by glutathione, with the magnitude of expression change of transcripts in these pathways increasing with dose.

Division of Treatment Doses into Two Major Groups

Patterns in the data of this study indicate that the four treatment groups could be combined into two larger groups, a low-dose group (60 and 125 ppm) and a high-dose group (1250 and 2500 ppm). This separation is supported by intragroup similarities and intergroup differences in several aspects of the data: PCA results (Fig. 2), Venn diagrams demonstrating common and unique genes (Fig. 3), pathway-level analyses (Fig. 4), and the pattern of increased SH (Table 2). Therefore, the samples were rearranged into three groups: control, low dose (LD; 60 and 125 ppm), and high dose (HD; 1250 and 2500 ppm). This analysis showed that compared with the control, the numbers of DETs were 93 and 832 for LD and HD, respectively. In the LD group, there were seven significantly altered pathways with three to four genes mapped to each significant pathway and a low ratio (number of altered genes/total of genes into the pathway) of the significantly altered pathways. The results showed predominantly CYP1A, CYP1B1, and UGT1A6 genes changed, reflecting metabolic effects. The HD group had 38 significantly altered pathways with an elevated ratio and high number of genes per pathway (3–24 genes/pathway). Because of redundancy of genes participating in more than one pathway, the pathways were grouped in major categories according to function. The categories included the genes that regulated amino acid metabolism, energy metabolism, lipid metabolism, oxidative stress response, signaling, immune system, etc.
adhesion, and cofactor/vitamin metabolism (Supplementary table S1).

**Mapping DETs to Cell Cycle Database**

To evaluate the relationship between changes in genes related to cell proliferation and the histological observation of simple hyperplasia, the DETs were mapped to cell cycle genes derived from Whitfield *et al.* (2002) and were organized by phases of the cell cycle (Fig. 5). There were only 6, 3, 5, and 13 genes changed at 60, 125, 1250, and 2500 ppm, respectively. Many of these genes function in G2/M phase of the cell cycle.

**DISCUSSION**

The most significant finding from the present study was a dose-response characterization of urothelial toxicity after 20 weeks of treatment with diuron. The urothelial gene expression profile exhibited a dose-response effect in terms of the number of significantly modulated genes increased with diuron at progressively higher concentrations. The microarray analysis showed a clear difference in gene expression at the higher doses (1250 and 2500 ppm) in comparison with the lower doses (60 and 125 ppm). Further, these findings were consistent with the phenotypic histological response. In

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**FIG. 3.** Venn diagram of significantly altered transcripts for different doses after a 20-week diuron exposure.

**FIG. 4.** Comparative analysis of significantly altered pathways presenting dose-response relationship using IPA. The y-axis depicts the probability that the genes within a data set are involved in a particular high-level biological function. The bars above the threshold line are statistically significant ($p < 0.05$).
addition, the results of this study suggest that 1250-ppm diuron induces the same gene expression changes and morphological response after 20 weeks as does the tumorigenic dose of 2500 ppm. The 1250-ppm dose has not been tested in a 2-year bioassay to evaluate whether it is carcinogenic.

Previously we reported that diuron increased urothelial necrosis identified by scanning electron microscopy after treatment with 2500-ppm diuron (Nascimento et al., 2006). Consistent with this finding, analyses using IPA showed a dose-dependent increase in the number of genes involved in cell death (Supplementary table S2). The specific biological pathways leading to diuron-induced necrosis is not known; however, the present study suggests several signaling pathways that could be involved, including stress-related genes such as those that control NRF2-mediated oxidative stress response, glutathione metabolism, and AHR signaling as well as those that control metabolism including amino acid, lipid, and nitrogen metabolism (Supplementary table S2). It has been proposed that diuron may act via ROS production (Geoffroy et al., 2002). Although the presence of ROS has not been determined directly in this study, oxidative stress can induce several of the stress pathways described above. ROS can play an important role in cancer development, not only by causing direct macromolecule (i.e., lipid, protein, nucleic acids) damage but by induction of signaling pathways that promote carcinogenesis (Klaunig et al., 2010). Therefore, sustained ROS production may contribute to necrosis and subsequent regenerative proliferation in animals exposed to high doses of diuron.

Diuron-induced urinary bladder alterations in the rat are similar to those produced by dimethylarsinic acid (DMA\textsuperscript{V}), the major urinary metabolite of inorganic arsenic in humans and rats, and a nongenotoxic rat bladder carcinogen (Sams et al., 2007). Like diuron, DMA\textsuperscript{V} is believed to exert its carcinogenic effect via direct exposure to urothelial cells rather than by causing indirect changes in urine composition as in the case of sodium saccharin (Chappel, 1992; Sams et al., 2007). Like most nongenotoxic urinary bladder carcinogens, DMA\textsuperscript{V} also causes necrosis followed by regenerative proliferation (Cohen et al., 2007a). The precise molecular mechanisms of DMA\textsuperscript{V} carcinogenesis are unknown. It is likely they are related to the production of one or more reactive metabolites that induce oxidative stress (Chung et al., 2008; Kitchin and Conolly, 2010; Wei et al., 2005). In contrast, antioxidants presented minimal inhibitory activity of various arsenicals induced urothelial toxicity in vitro or in vivo showing that it is possible that other pathways also can contribute to arsenic cytotoxicity (Cohen et al., 2007a; USEPA, 2005). It is unknown if DMA\textsuperscript{V} and diuron act similarly on a molecular level. In the present study, altered molecular pathways included some that were also altered by DMA\textsuperscript{V} exposure in the urothelium in vivo. This included lipid metabolism, protein degradation, cell death, and oxidative stress response (Supplementary table S1) (Sen et al., 2005, 2007).

**FIG. 5.** Alterations of cell cycle genes within the phases of the cell cycle by the different diuron treatments after 20 weeks of exposure. Genes in bold have increased expression, whereas genes in italic have decreased expression compared with controls.
Both 1250- and 2500-ppm diuron treatment increased incidence of simple urothelial hyperplasia after 20 weeks of exposure in the present study. A previous study reported increased proliferating cell nuclear antigen (PCNA) labeling in the urothelial cells of animals exposed to 2500-ppm diuron for 20 weeks (Nascimento et al., 2006). Analysis of diuron-responsive genes demonstrated individual differentially expressed genes that are associated with control of the cell cycle (Fig. 5) and could result in increased cell proliferation. As an example and based on IPA evaluations, ARHGDIB and MAP2K6 genes had decreased expression and have been associated with cell adhesion and cell cycle arrest, respectively, whereas, H1FO and LMO4 had increased expression and are associated with increased DNA synthesis. In this study, there was no clear modulation of cell cycle genes that promote proliferation after 20 weeks of exposure.

Some genes that have been reported to be involved in human bladder cancer, such as GSTM1 (La Vecchia & Airoldi, 1999; Murta-Nascimento et al., 2007), Cytokeratin K20 and NINJ1 (Sanches-Carbayo et al., 2003), CAVI, ZYX, and MSN (Sanches-Carbayo and Cordon-Cardo, 2003), had increased expression after diuron treatment in the present study, and ARHGDIB (Sanches-Carbayo and Cordon-Cardo, 2003) had decreased expression after diuron exposure. Alterations of lipid and protein metabolism pathways have been associated with progression in human bladder cancer (Sanches-Carbayo et al., 2003) and were also found to be altered in the present study. These data suggest concordance of some pathway-level alterations between human bladder cancer and rodent urothelial toxicity that lead to a tumor response.

Taking all the data together, we have summarized a proposed set of key events that describe a hypothesized MOA for diuron-induced tumorigenesis (Fig. 6). Diuron and/or its metabolites present in the urine directly cause urothelial cytotoxicity and consequent regenerative cell proliferation. Herein we demonstrated that these processes follow a dose-response pattern. At low doses, signs of cell toxicity as demonstrated by increased expression of transcripts related to metabolism of xenobiotics, such as CYP1A1, CYP1B1, and UGT1A, and dysfunction of genes that regulate mitochondrial function were observed. Mitochondrial dysfunction can occur when ROS are increased in relation to antioxidant systems (Tennant et al., 2009). However, the low number of transcripts, altered and involved in each molecular pathway, plus the absence of morphological lesions in the low-dose animals suggests that molecular alterations at these dose levels are involved in maintaining cellular homeostasis. Toxic doses of diuron (1250 and 2500 ppm) induced simple hyperplasia and were associated with alterations in gene expression which included effects on amino acid, lipid, phase I and phase II metabolism, and an enhanced stress response (Supplementary table S1). These data suggest that persistent exposure to high dietary concentrations of diuron induces oxidative stress, increases cellular metabolism, and enhances cell death that is associated with sustained urothelial hyperplasia. These persistent alterations have been shown to be associated with urinary bladder tumor development (Cohen, 1998).

In summary, we demonstrated that transcriptional profile analysis identified a dose-response after 20 weeks of exposure with clear differences in gene expressions at the higher doses.
(1250 and 2500 ppm) in comparison with the lower doses (60 and 125 ppm). The gene expression profile in the present study was supported by the histological response. The present study suggests that 125 ppm of diuron in the feed is a no effect level for histologic alterations, and transcriptional changes are related to the preservation of urothelial homeostasis. The present data also suggest that after 20 weeks of exposure to 1250 ppm, the response of the rat urothelium is the same as when treated with 2500 ppm. The cellular and molecular pathways involved in chronic toxicity from diuron treatment may also be associated with a tumor response.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

FUNDING

Sao Paulo Research Foundation (FAPESP 06/60506-1; 08/55644-1; 09/02754-7); Center for the Evaluation of the Environmental Impact on Human Health (TOXICAM); USEPA.

ACKNOWLEDGMENTS

The authors would like to thank Dr Merielen Nascimento and Mitscheli Sanches da Rocha for their expert technical assistance; Dr Mitchell Rosen for assistance in the molecular research; Dr William Ward for bioinformatics assistance; and Dr Chris Corton and Dr Jim Allen for reviewing the manuscript. This article was approved for publication by the National Health and Environmental Effects Research Laboratory of the Environmental Protection Agency, Sacramento, CA, p. 43.

The authors would like to thank Dr Merielen Nascimento and Mitscheli Sanches da Rocha for their expert technical assistance; Dr Mitchell Rosen for assistance in the molecular research; Dr William Ward for bioinformatics assistance; and Dr Chris Corton and Dr Jim Allen for reviewing the manuscript. This article was approved for publication by the National Health and Environmental Effects Research Laboratory of the Environmental Protection Agency (USEPA) but does not necessarily represent the opinions or policy of the USEPA.

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