Quantitative determination and toxicity evaluation of 2,4-dichlorophenol using poly(eosin Y)/hydroxylated multi-walled carbon nanotubes modified electrode

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This study aimed at developing simple, sensitive and rapid electrochemical approach to quantitatively determine and assess the toxicity of 2,4-dichlorophenol (2,4-DCP), a priority pollutant and has potential risk to public health through a novel poly(eosin Y, EY)/hydroxylated multi-walled carbon nanotubes composite modified electrode (PEY/MWNTs-OH/GCE). The distinct feature of this easy-fabricated electrode was the synergistic coupling effect between EY and MWNTs-OH that enabled a high electrocatalytic activity to 2,4-DCP. Under optimum conditions, the oxidation peak current enhanced linearly with concentration increasing from 0.005 to 0.1 μM and 0.2 to 40.0 μM, and revealed the detection limit of 1.5 nM. Moreover, the PEY/MWNTs-OH/GCE exhibited excellent electrocatalytic activity toward intracellular electroactive species. Two sensitive electrochemical signals ascribed to guanine/xanthine and adenine/hypoxanthine in human hepatoma (HepG2) cells were detected simultaneously. The sensor was successfully applied to evaluate the toxicity of 2,4-DCP to HepG2 cells. The IC50 values based on the two electrochemical signals are 201.07 and 252.83 μM, respectively. This study established a sensitive platform for the comprehensive evaluation of 2,4-DCP and posed a great potential to simplify environmental toxicity monitoring.

2,4-dichlorophenol (2,4-DCP) is a typical chlorophenol compound widely used in herbicides, fungicides, and insecticides. As a priority pollutant listed by the US Environment Protection Agency (EPA), 2,4-DCP seriously threatens environmental quality and public health. Despite the maximum permissible concentration of 2,4-DCP in drinking water is set as 0.5 mg L−1, higher concentrations are usually found in polluted environments. Until now, there has been no quantitative determination and toxicity evaluation method for early detection of 2,4-DCP. Due to its low allowable concentration and coexistence with interfering substances (e.g. phenols and metal ions), 2,4-DCP has been measured using UV spectrophotometry, gas chromatography, high-performance liquid chromatography, and capillary electrophoresis. However, most of these methods are complex, low sensitive, costly, and incapable of real-time monitoring. The electrochemical method newly developed is preferable owing to the simplicity, high sensitivity, low-cost, and continuous on-line detection.

As for the in vitro toxicity evaluation of pollutants, some techniques such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, flow cytometry experiment, and lactate dehydrogenase (LDH) release assay have been developed. Recently, cell-based electrochemical method that is simple, sensitive, rapid, label-free, and non-toxic has gained attention for toxicity assessment. An in-situ electrochemical method was successfully developed and applied in drug screening and toxicity evaluation of heavy metals in our previous studies.

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study\textsuperscript{22–24}, which was based on the electrical signals of the intermediates of cellular purine metabolism, and objectively revealed the cell viability at the molecular level\textsuperscript{25}. Graphene, carbon nanotubes, and threonine modified electrodes have been developed. Nevertheless, due to the insufficient sensitivity of these electrodes, only one electrochemical signal was detected at approximately $+0.65$ V corresponded to xanthine/guanine in human cancer cells. A carbon nanotubes and ionic liquid complex (MWNTs/IL) modified electrode captured two electrochemical signals ascribed to xanthine/guanine and hypoxanthine/adenine in MCF-7 cells at about $+0.70$ V and $+1.0$ V, respectively\textsuperscript{26}. However, the electrode was fabricated by coating a MWNTs/IL paste on the electrode surface, which is difficult to control the thickness of the film. Therefore, it is critical to develop simple and stable electrodes for the sensitive determination of purine bases.

Carbon nanotubes (CNTs) have been used as the sensor material owing to their unique thermal, excellent optical and mechanical properties\textsuperscript{27}. Although CNTs tend to entangle and hardly disperse in water because of their hydrophobic nature, they could be functionalized with different moieties to improve the solubility\textsuperscript{28,29}. Hydroxylated multi-walled carbon nanotubes (MWNTs-OH) with $\pi-\pi$ conjugating structure are a kind of functionalized CNTs that the structural defects can be obtained on the surface due to the $sp^3$ hybridization of C-C bonds formed in the functionalization process\textsuperscript{30–32}. These defects can not only improve the solubility but also significantly enhance the reactivity and electronic property. In the same time, Eosin Y (EY), as a xanthene dye containing bromine atoms, is widely applied as the biological stain, laser dye, fluorescent probe, and sensitizer due to its high stability, versatility, and light absorptivity\textsuperscript{33–38}. Recently, EY was successfully applied for the fabrication of electrodes to monitor catechol and hydroquinone\textsuperscript{39}, sodium dodecyl sulfate\textsuperscript{40}, and hydroquinone and catechol\textsuperscript{41}. EY acts as the excellent electron donor, and can generate stable redox active layers by the electrochemical polymerization. However, the development of EY as sensors is still at an early stage, and it has been rarely reported to interact with carbon nanomaterials. Given the compatible structure and physical properties of MWNTs-OH and EY, hybridization might be formed via $\pi-\pi$ interaction, through which the EY/MWNTs-OH composite would presumably produce synergic effects and exhibit higher electrocatalytic activity by improving their unique potential.

In this study, a sensitive electrode with high electrocatalytic activity toward not only 2,4-DCP but also cellular purine bases was developed, so that simple, rapid, sensitive and quantitative determination and toxicity assessment would be realized to simplify the detection and evaluation process of 2,4-DCP (Fig. 1). The poly(eosin Y)/hydroxylated multi-walled carbon nanotubes hybrid material modified glass carbon electrode (PEY/MWNTs-OH/GCE) was developed. The electrode was characterized using emission scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS), raman spectroscopy, and cyclic voltammetry (CV) technique. The electrochemical sensor was examined for the quantitative detection and toxicity assessment of 2,4-DCP on human hepatoma (HepG2) cells for the first time that have been proved as the sensitive and stable model organisms in environmental toxicology\textsuperscript{42}.

**Results and Discussion**

**Characterization of the PEY/MWNTs-OH/GCE.** The electrodeposition is a simple approach to immobilize organic molecules onto the electrode surface, which adjusts the surface properties including thickness, permeation, and charge transportation\textsuperscript{43}. The successive CV curves for the electrochemical modification of PEY/GCE and PEY/MWNTs-OH/GCE were conducted over the potential range from $-1.2$ to $+1.0$ V. On the GCE (Fig. 2A), an anodic peak attributed to the oxidation of EY (EY$^-$ $\rightarrow$ EY$^+$) appeared at $+0.75$ V and a
cathodic peak attributed to the reduction (\(EY^2^- + e^- \rightarrow EY^3^-\)) was observed at \(-0.87\) V in the 1st cycle. The peak current decreases with increase in number of cycles. On the MWNTs-OH/GCE (Fig. 2B), the background current was much larger than that of GCE, indicating the higher surface area of MWNTs-OH. Two oxidation and reduction peaks were found at the potential of \(+0.69\) V, \(+0.45\) V, \(-0.50\) V, and \(-0.87\) V, respectively. During the one-step electrodeposition process, the EY molecules acted as an excellent electron acceptor, which exist mainly in the form of \(EY^2^-\) and could be reduced to \(EY^3^-\) by the cleavage of the C=O bond on the benzene ring (Fig. 3). The obtained \(EY^3^-\) could rapidly combine with the electrode surface\(^{44}\). The peak current was virtually constant after 15 cycles, indicating that the polymerization reached saturation at the MWNTs-OH/GCE. The thickness of the film had a significant contribution to the property of the PEY/MWNTs-OH/GCE. Thus, the influences of the cycle number and scan rate of EY on the electrocatalytic performance of PEY/MWNTs-OH/GCE were investigated (the inset of Fig. 2B). The oxidation peak current of 2,4-DCP reached to the maximum after 15 scans at the scan rate of \(50\) mV s\(^{-1}\). The peak current began to drop when the polymerizing cycles were more than 15. Thick films may prevent the electron transfer process and the oxidation process. Hence, the optimal electropolymerization cycles are selected as 15 cycles.

The surface morphologies of each layer were characterized by SEM. Randomly oriented MWNTs-OH was detected with interconnected tubular structures (Fig. 4A), which was the characteristic of carbon nanotubes\(^{45}\). After the introduction of EY molecules, the EY polymer was uniformly distributed over the MWNTs-OH (Fig. 4B). The homogeneous and uniform film with three-dimensional network structure was produced, demonstrating that the EY could be modified effectively on the surface with the electrodeposition method. The highly conjugated PEY/MWNTs-OH composite exhibited high surface area, and thus providing more sites for the accumulation of the target molecule.

Then the CVs of different electrodes were studied in 5.0 mM [Fe(CN)\(_6\)]\(^{3-/4-}\) containing 0.1 M KCl (Fig. 4C). The electrochemically active surface area can be obtained according to the following equation\(^{46}\):

\[
\text{\(i_p = (2.99 \times 10^5)nACD^{1/2}v^{1/2}\)}
\]

where \(i_p\) is the peak current, \(n\) is the number of electrons involved in the redox reaction of Fe(CN)\(_6\)^{3-/4-} \((n = 1)\), \(A\) is the electroactive surface area, \(C\) is the reactant concentration, \(D\) is the diffusion coefficient \((6.30 \times 10^{-6} \text{ cm}^2 \text{s}^{-1})\),

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**Figure 2.** Electropolymerization curves of 500 \(\mu\)M EY in pH 5.0 PBS at (A) GCE and (B) MWNTs-OH/GCE. Inset: the influence of the cycle and scan rate on the oxidation peak current of 20 \(\mu\)M 2,4-DCP during the fabrication process of PEY/MWNTs-OH/GCE.

**Figure 3.** The structure and mechanism of electropolymerization process of EY.
and $\nu$ is the scan rate. The active surface areas for MWNTs-OH/GCE and PEY/MWNTs-OH/GCE were calculated as 0.079 cm$^2$ and 0.108 cm$^2$, respectively, which further confirmed that the PEY film increased the surface area of the MWNTs-OH/GCE.

EIS can provide details on the interfacial properties of the electrode interface during the fabrication process. The semicircular portion at higher frequencies represents the electron transfer-limited process, and the diameter corresponds to the electron transfer resistance ($R_{ct}$)\textsuperscript{47}. Figure 4D shows the typical Nyquist plots recorded at frequencies ranging from 0.01 to 10$^5$ Hz in 0.1 M KCl containing 5.0 mM $[\text{Fe(CN)}_6]^{3-}/4^-$ as the electrochemical redox probe. The inset shows the equivalent circuit model to fit the impedance data. $R_s$, $C$, and $W$ represented the solution resistance, pure capacitance, and Warburg impedance, respectively. The EIS at the bare GCE displayed a well-defined semicircle, with a huge interfacial $R_{ct}$ of 14.3 K$\Omega$. The increase in the $R_{ct}$ of PEY/GCE suggested a successful modification of EY on the GCE surface. The $R_{ct}$ decreased dramatically to 12.1 K$\Omega$ after the introduction of MWNTs-OH on the GCE, confirming an excellent electron conducting ability of MWNTs-OH. The lower $R_{ct}$ of PEY/MWNTs-OH/GCE (9.2 K$\Omega$) demonstrated the enhanced electron transfer reaction, which was mainly attributed to the synergistic effect between EY and MWNTs-OH.

Raman spectroscopy is a useful tool to obtain structural information of carbonaceous materials\textsuperscript{48}. The D band corresponds to the disordered structural defects and the G band is due to the first-order scattering of the E$_{2g}$ mode for sp$^2$ carbon lattice. The relative intensity ratio of D band to G band ($I_D/I_G$) can be used to examine the disorder and defects. The decrease of $I_D/I_G$ indicated the increase in the average size of sp$^2$ domains\textsuperscript{19}. PEY/MWNTs-OH (Fig. 5A, curve b) exhibits the D band at 1357 cm$^{-1}$ and the G band at 1580 cm$^{-1}$ with intensities lower than that of MWNTs-OH (Fig. 5A, curve a). The $I_D/I_G$ value of MWNTs-OH was estimated to be 1.09. In the case of PEY/MWNTs-OH, the $I_D/I_G$ value decreased to 0.78, which implied the successful formation of the PEY/MWNTs-OH hybrids and an efficient $\pi-\pi$ interaction between them.

**Voltammetric behavior of 2,4-DCP at the PEY/MWNTs-OH/GCE.** The CVs of 2,4-DCP at different electrodes were investigated (Fig. 5B). No peak was observed at the GCE (curve a), which was consistent with the previous study\textsuperscript{31}. A broad oxidation peak appeared at the PEY/GCE (curve b) but it was too weak to distinguish. In the case of MWNTs-OH/GCE, a peak at +0.78 V was observed (curve c), while a well-defined anodic peak was obtained at +0.77 on PEY/MWNTs-OH/GCE (curve d). There was no corresponding reduction peak in the inverse scan, which was the characteristic of an irreversible electrode process. The high background current of PEY/MWNTs-OH/GCE reflected its effective surface area. In addition, the peak current was 5.2 times that of
Figure 5. (A) Raman spectrum of MWNTs-OH (a) and PEY/MWNTs-OH nanocomposite (b); (B) CVs of GCE (a), PEY/GCE (b), MWNTs-OH/GCE (c), and PEY/MWNTs-OH/GCE (d) in 0.1 M pH 3.0 PBS containing 20μM 2,4-DCP.
the PEY/MWNTs-OH/GCE electrode exhibited relatively wide linear range and low detection limit (Table 1), and thus was a promising sensor for the sensitive determination of 2,4-DCP.

The selectivity of the PEY/MWNTs-OH/GCE was investigated by adding several metal ions and structurally similar phenols. 50-fold Mg\(^{2+}\), Al\(^{3+}\), Zn\(^{2+}\), Fe\(^{3+}\), Cu\(^{2+}\), and Ca\(^{2+}\) had negligible effects on the electrochemical oxidation of 2,4-DCP at PEY/MWNTs-OH/GCE except for Mn\(^{2+}\) (Fig. 7C). As shown in Fig. 7D, no peak was observed for hydroquinone (a) and catechol (b) between +0.3 and +0.9 V. Although PEY/MWNTs-OH/GCE showed the electrocatalytic activity to o-nitrophenol (c), p-nitrophenol (d), 2,4,6-trichlorophenol (g) and pentachlorophenol (h), the potential differences between these phenols and 2,4-DCP were larger than 60 mV. Thus they had no influence on the peak current of 2,4-DCP (f). However, a remarkable peak was observed for 2-chlorophenol (e), and its peak potential was similar to that of 2,4-DCP. Therefore, the PEY/MWNTs-OH/GCE is applicable for the detection of 2,4-DCP in the absence of Mn\(^{2+}\) and 2-CP.

The reproducibility was investigated by six measurements of 20.0 \(\mu\)M 2,4-DCP with the same PEY/MWNTs-OH/GCE. The relative standard deviation (RSD) was 3.74%. Additionally, six electrodes were examined at 20.0 \(\mu\)M 2,4-DCP and the RSD was 4.77%. These results proved that the sensor possessed excellent reproducibility and repeatability.

Furthermore, the analytical reliability and application potential of PEY/MWNTs-OH/GCE was investigated to determine 2,4-DCP in real water samples obtained from Yitong River (Changchun, China). The recoveries ranged from 93.2% to 105.6% (Table 2) indicating the excellent reliability and applicability of PEY/MWNTs-OH/GCE.

**Electrochemical behavior of HepG2 cells at the PEY/MWNTs-OH/GCE.** The potential application of the PEY/MWNTs-OH/GCE in HepG2 cell suspension (3.0 \(\times\) 10\(^6\) cells mL\(^{-1}\)) was explored (Fig. 8A). No peak appeared on the bare GCE (curve a). A broad oxidation peak was observed at about +0.65 V at the PEY/GCE (curve b). For MWNTs-OH/GCE (curve c), an oxidation peak was obtained at +0.60 V, and an oxidation peak appeared at +0.92 V, whereas, it was too weak to recognize. With the PEY/MWNTs-OH/GCE in HepG2 cell suspension (curve d), the background current was greater than other electrodes, suggesting the more effective surface area. Meanwhile, two well-defined oxidation peaks at +0.59 V and +0.90 V attributed to xanthine/adenine, and hypoxanthine/adenine\(^{22,23,26}\) were observed. The oxidation potentials shifted to less positive ones and the maximum peak currents were obtained. These results implied that the PEY/MWNTs-OH film possessed unique electrocatalytic activities toward the purine bases in HepG2 cells, and could promote electron transfer reactions.
The influence of accumulation condition on the electrochemical signal was investigated (Fig. 8B), and 420 s was chosen as the best accumulation time.

The two electrochemical signals on PEY/MWNTs-OH/GCE were applied to describe the growth of HepG2 cells (Fig. 9). The peak currents increased gradually with the culture time within 30 h owing to the proliferation of cells. Then the peak currents reduced substantially because of the cell death caused by the lack of nutrients. These results well corresponded with the cell counting method (inset of Fig. 9), implying that the PEY/MWNTs-OH/GCE can be applied to monitor cell growth in real-time mode.

**Table 1.** Comparison of different electrodes for detection of 2,4-DCP. HRP, horseradish peroxidase; MB-AG, myoglobin and agarose; PSS-GN-CTAB, poly(sodium-styrenesulfonate) functionalized graphene/cetyltrimethylammonium bromide; MIP, molecularly imprinted polymers; CS/CDs-CTAB, chitosan/carbon dots and hexadecyltrimethyl ammonium bromide.

| Electrode                  | Technique | Linear range (μM) | Detection Limit (μM) | Ref. |
|----------------------------|-----------|-------------------|----------------------|------|
| HRP/MWNTs/GCE             | Amperometry | 1.0–100          | 0.38                 | 10   |
| Graphene/HRP/GCE          | Amperometry | 0.01–13.0        | 0.005                | 11   |
| Nafion/MWNT/GCE           | Amperometry | 0.1–100          | 0.037                | 12   |
| MB-AG/GCE                 | Amperometry | 12.5–208         | 2.06                 | 13   |
| Nafion/PSS-GN-CTAB/GCE    | LSV       | 0.01–2           | 0.002                | 14   |
| MIP/GCE                   | DPV       | 5–100            | 1.6                  | 15   |
| CS/CDs-CTAB/GCE           | DPV       | 0.04–8           | 0.01                 | 5    |
| PEY/MWNT-OH/GCE           | DPV       | 0.005–0.1, 0.2–40 | 0.0015               | This work |

Figure 7. (A) DPVs of PEY/MWNTs-OH/GCE in pH 3.0 PBS with different 2,4-DCP concentrations (a–k: 0.005, 0.03, 0.05, 0.1, 0.2, 0.8, 3, 10, 20, 30, and 40 μM); (B) The relationship between peak current and the concentration of 2,4-DCP; (C) Column chart of the peak current of 20 μM 2,4-DCP containing 1 mM metal ions; (D) DPVs of 20 μM phenols.
Cytotoxicity assessment of 2,4-DCP on HepG2 cells. The electrochemical behaviors of HepG2 cells treated by 2,4-DCP at different times were investigated using PEY/MWNTs-OH/GCE (Fig. 10). Compared with

### Table 2. Recovery results for 2,4-DCP in real water samples.

| Sample                | Added (μM) | Found (μM) | Recovery (%) |
|-----------------------|------------|------------|--------------|
| Upstream water sample 1 | 0.1        | 0.099      | 99.0         |
| Upstream water sample 2 | 1          | 0.932      | 93.2         |
| Upstream water sample 3 | 5          | 5.28       | 105.6        |
| Upstream water sample 4 | 20         | 20.4       | 102.0        |
| Downstream water sample 1 | 0.1        | 0.095      | 95.0         |
| Downstream water sample 2 | 1          | 0.988      | 98.8         |
| Downstream water sample 3 | 5          | 4.88       | 97.6         |
| Downstream water sample 4 | 20         | 20.5       | 102.5        |

Figure 8. (A) CVs of HepG2 cell suspension at the (a) GCE, (b) PEY/GCE, (c) PEY/MWNTs-OH/GCE, and (d) PEY/MWNTs-OH/GCE. (B) Influence of accumulation time on the electrochemical signals of HepG2 cell suspension. Cell inoculation concentration: $4.0 \times 10^5$ cells mL$^{-1}$.

Figure 9. Growth curves of the HepG2 cells depicted by the PEY/MWNTs-OH/GCE and the cell counting method (inset).
control groups (curves a and b), the electrochemical signal I (curve c) and signal II (curve d) of 2,4-DCP treated groups increased slowly until 24 h and then decreased ascribed to the toxicity of 2,4-DCP. Meanwhile, the peak currents of 2,4-DCP were lower than those of the control group, indicating the inhibitory effects of 2,4-DCP on HepG2 cells. A notable decrease in the electrochemical response was observed after treated by 2,4-DCP for 30 h, and the cytotoxicity reached to the maximum (inset of Fig. 10). Therefore, 30 h was chosen as the best 2,4-DCP-treated duration.

The electrochemical behaviors of HepG2 cells after treated by 2,4-DCP with different concentrations for 30 h were studied using PEY/MWNTs-OH/GCE (Fig. 11). 2,4-DCP exhibited significant cytotoxicity with a concentration-dependent pattern (inset of Fig. 11). The cytotoxicity \( Y \) was linear to the logarithm of 2,4-DCP concentration \( X \) with the linear regression equations of \( Y = 46.35 \times - 56.76 (R = 0.990) \) for signal I and \( Y = 42.50 \times - 52.12 (R = 0.993) \) for signal II. The IC\(_{50}\) values based on the two electrochemical signals were 201.07 and 252.83 \( \mu \)M, respectively, reflecting that 2,4-DCP had greater impacts on xanthine/guanine than those on hypoxanthine/adenine. It also confirmed the toxicity differences of 2,4-DCP to purine metabolism, which had a significance for the study of the cellular physiological process. 2,4-DCP has been proved as a potential environmental endocrine disruptor and oxidative damage inducer\(^{55}\), and can change the antioxidant enzyme activities and induce oxidative stress, a mechanism responsible for DNA damage and inhibition of cell growth\(^{56,57}\). The lower level of cell viability reduced the signals on PEY/MWNTs-OH/GCE, which related to the cellular physiological changes.

Furthermore, the conventional MTT assay was applied to compare with the result of the PEY/MWNTs-OH/GCE. The linear regression equation obtained by the MTT assay was \( Y = 41.51 \times - 53.44 (R = 0.993) \) and the IC\(_{50}\) value was 309.03 \( \mu \)M. The result verified the sensitivity of the electrochemical method on the toxicity evaluation of 2,4-DCP.
Conclusion
A novel electrochemical sensing platform was constructed in a simple and green way. The PEY/MWNTs-OH/GCE significantly facilitated the electron transfer efficiency and possessed excellent electrocatalytic activity. Under the optimal condition, it exhibited a high sensitivity, wide linear range, excellent reproducibility and good stability toward 2,4-DCP. The easy-fabricated electrode developed was also employed in real water sample analysis and showed high accuracy. Moreover, the electrochemical behavior of HepG2 cells was investigated by the PEY/MWNTs-OH/GCE. The cytotoxicity of 2,4-DCP was successfully evaluated by this electrode. This study constituted a promising tool for 2,4-DCP analysis and toxicity evaluation, and revealed a simple sensitive electrochemical approach in the field of environmental monitoring and toxicology.

Experimental Section
Materials and chemicals. The MWNTs-OH (Nanjing XFNANO Materials TECH Co., Ltd.) was 20–40 nm in diameter with purity higher than 97%. The minimum essential medium (MEM), defined fetal bovine serum (FBS) (Gibco Co., USA), penicillin, streptomycin, and trypsin (Sigma, Co., USA) were used for cell culture. Dimethyl formamide and eosin Y (EY) (J&K Chemical Ltd., China) were applied for electrode fabrication. 2,4-DCP (J&K Chemical Ltd., China) was used as the target compound. Phosphate-buffered solutions (PBS) were prepared by mixing stock solutions of 0.1 M Na2HPO4 and 0.1 M KH2PO4.

Apparatus. The electrochemical measurements including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on the CHI 760E electrochemical workstation (Shanghai CH Instruments, apaparatus, USA). The Raman scattering spectra was obtained by the Jobin-Yvon HR 800 instrument (Sartorius Co., Germany).

Preparation of the PEY/MWNTs-OH/GCE. The glass carbon electrode (GCE) was sequentially polished using 1.0, 0.3 and 0.05μm alumina slurry and rinsed successively with double-distilled water and ethanol solution. After 10.0 mg MWNTs-OH was dispersed with 10.0 mL dimethyl formamide using ultrasonic agitation, 5.0μL MWNTs-OH dispersion was dropped onto GCE and dried under the infrared lamp to obtain the MWNTs-OH/GCE. For the toxicity investigation, the growth medium was replaced with the medium containing humidified 5% CO2. For the toxicity investigation, the growth medium was replaced with the medium containing humidified 5% CO2. The cytotoxicity of 2,4-DCP was successfully evaluated by this electrode. This study constituted a promising tool for 2,4-DCP analysis and toxicity evaluation, and revealed a simple sensitive electrochemical approach in the field of environmental monitoring and toxicology.

Cells culture and treatment. The HepG2 cells (COBIOER Biosciences Co., Ltd.) were cultured in 60 mm cell culture dish in the minimum essential medium (MEM) that was supplemented with 10% FBS, penicillin (100μg mL−1), streptomycin (100μg mL−1), 1% non-essential amino acids, and 1% sodium pyruvate at 37 °C in a humidified 5% CO2. For the toxicity investigation, the growth medium was replaced with the medium containing 2,4-DCP. The in-situ cell collection was conducted as our previous study23. Briefly, the PBS was added to cells after the medium was removed, and then the mixture was heated in the water bath at 50 °C for 30 min to obtain the HepG2 cell suspension.

Electrochemical determination. For the electrochemical detection of 2,4-DCP, the CV was employed between +0.1 and +1.0 V with the scan rate of 50 mV s−1. The DPV was performed with the following parameters: increment potential, 4 mV; pulse amplitude, 0.05 V; pulse width, 0.05 V; pulse period, 0.2 s; sample width, 50 ms. The in vitro toxicity of 2,4-DCP to HepG2 cells was investigated from 0.0 to +1.1 V with the scan rate of 50 mV s−1. After each measurement, the PEY/MWNTs-OH/GCE was scanned for five cycles between 0.0 and +1.1 V in PBS and rinsed thoroughly with double-distilled water.

The MTT assay. HepG2 cells (1.2 × 104 cells mL−1) in medium alone (200μL) or the medium containing 2,4-DCP (200μL) were added to the 96-well microtiter plates. 20μL 5 mg mL−1 MTT was added to each well after the incubation at 37 °C for 30 h. The medium containing MTT was removed after 4 hours, and 150μL sodium dodecyl sulfate was added. The measurement was registered on an ELX800 Microplate Reader (BioTek Instruments, Inc., USA) and determined by the absorbance values at 490 nm.

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**Author Contributions**

X.Y. and B.-k.L. designed and supervised the work, X.-l.Z. and K.-x.Z. fabricated the electrodes, X.-l.Z., K.-x.Z., and C.-z.W. performed the characterization, X.-l.Z. and K.-x.Z. tested the electrodes and discussed the results, X.-l.Z. wrote the manuscript, B.-k.L. and J.-n.G. revised the text, and all the authors reviewed and approved the manuscript.

**Additional Information**

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