SUPPORTING INFORMATION

Development and Evaluation Of An Immunoassay For Biological Monitoring Chlorophenols In Urine As Potential Indicators Of Occupational Exposure

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Spectral Data:

General Methods and Instruments. ^{1}H and ^{13}C NMR spectra were obtained with a Varian Unity-300 (Varian Inc., Palo Alto, CA, USA) spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) or on a Gemini 200 (199.975 MHz for ^{1}H and 50.289 for ^{13}C). Infrared spectra were measured on a Bomen MB120 FTIR spectrophotometer (Hartmann & Braun, Québec, Canada). Gas chromatography-mass spectrometry (GC-MS) was performed on a MD-800 capillary gas chromatograph with MS quadrupole detector (Fisons Instruments, VG, Manchester, U.K.) with a electronic impact (EI) source of 70 eV provided of a capillary column (HP-5, 25 mm). The positive ions were recorded on SCAN mode. The spectroscopic and spectrometric data is given as supplementary material.

2-Hydroxy-3,5,6-tichlorobenzaldehyde **1.** ^{1}H NMR (300MHz, $CDCl_3$); ^{1}H NMR (300MHz, $CDCl_3$); δ: 7.72 (s, $1H_{Ar}$ *para*), 10.42 (s, 1H, -CHO), 12.50 (s, 1H, -OH). ^{13}C NMR (75MHz, $CDCl_3$); δ: 118.0 (C-3'), 122.2 (C-1'), 123.9 (C-5'), 134.3 (C-6'), 137.2 (C-4'), 158.2 (C-2'), 195.2 (C-1). EM, m/z (%); 224 (M^+, 94), 223 (100), 206 (32), 195 (4), 178 (27), 160 (14). IR, ν (KBr, cm^{-1}); 1658, 1465, 1184, 773.

3-(2-Hydroxy-3,5,6-trichlorophenyl)-2-propenoic acid (hapten **2**). ^{1}H NMR (300MHz, DMSO-d_6); δ: 6.78 (d, J=16Hz, 1H, =CHCOO-), 7.72 (d, J=16Hz, 1H, PhCH=), 7.81 (s, $1H_{Ar}$ *para*). ^{13}C NMR (75MHz, DMSO-d_6);δ: 121.2 (C-2), 122.8 (C-3'), 123.8 (C-1'), 126.5 (C-5'), 130.0 (C-4'), 130.7 (C-6'), 135.9 (C-3), 151.8 (C-2'), 167.2 (C-1). IR, ν (KBr, cm^{-1}); 3368, 1681, 1448, 1182, 763. Anal. Calcd. for $C_{10}H_7Cl_3O_3$; C: 42.87 H: 2.52 Cl: 37.47; Determined C: 42.74 H: 2.52 Cl: 37.76

Methyl 3-(2-hydroxy-3,5,6-trichlorophenol)-2-propenoate: ^{1}H NMR (300MHz, $CDCl_3$); δ: 3.84 (s, 3H, $-OCH_3$), 6.17 (s, 1H, -OH), 6.92 (d, J=16.2Hz, 1H, =CHCOO-), 7.48 (s, $1H_{Ar}$ *para*), 7.95 (d, J=16.2Hz, 1H, PhCH=). EM, m/z (%); 280 (M^+, 18), 249 (37), 248 (48), 245 (83), 220 (100), 193 (26), 157 (72). IR, ν (KBr, cm^{-1}); 3174, 1695, 1261,798.

Methyl 3-(2-hydroxy-3,5,6-trichlorophenol)propanoate: ^{1}H NMR (200MHz, $CDCl_3$); δ: 2.74 (t, J=6.8Hz, 2H, $-CH_2COO-$), 3.14 (t, J=6.8Hz, 2H, $PhCH_2-$), 3.73 (s, 3H, $-OCH_3$),

7.38 (s, $1H_{Ar}$ *meta*), 7.67 (s, 1H, -OH). ^{13}C NMR (75MHz, $CDCl_3$); δ: 23.9 (C-2), 32.4 (C-3), 52.4 (OCH_3), 120.8 (C-3'), 124.5 (C-1'), 128.3 (C-4'), 128.4 (C-5'), 131.8 (C-6'), 149.8 (C-2'), 175.3 (C-1). EM, m/z (%); 282 (M^+, 15), 251 (17), 250 (56), 247 (32), 223 (32), 222 (100), 209 (29), 208 (25), 205 (16).

3-(2-Hydroxy-3,5,6-trichlorophenyl)propanoic acid (hapten **3**): ^1H NMR (300MHz, DMSO-d_6); δ: 2.38 (t, J=8.1Hz, 2H, -CH_2COO-), 3.01 (t, J=8.1Hz, 2H, PhCH_2-), 7.61 (s, $1H_{Ar}$ *meta*). ^{13}C NMR (75MHz, DMSO-d_6); δ: 24.4 (C-3), 32.1 (C-2), 120.4 (C-3'), 122.0 (C-1'), 127.7 (C-4'), 130.0 (C-5'), 130.3 (C-6'), 150.8 (C-2'), 173.3 (C-1).

Methyl 3-(2-hydroxy-3,6-dichlorophenyl)-propanoate : ^1H NMR (300MHz, $CDCl_3$); δ: 2.66 (t, J=7.2Hz, 2H, -CH_2COO-), 2.93 (t, J=7.2Hz, 2H, PhCH_2), 3.68 (s, 3H, -OCH_3), 6.38 (s, 1H, -OH), 7.03 (d, J=2.5Hz, $1H_{Ar}$, *meta*), 7.20 (d, J=2.5Hz, $1H_{Ar}$ *para*). ^{13}C NMR (75MHz, $CDCl_3$); δ: 25.8 (C-2), 33.7 (C-3), 51.8 (OCH_3), 121.0 (C-3'), 125.1 (C-1'), 126.9 (C-5'), 129.0 (C-4'), 129.8 (C-6'), 148.6 (C-2'), 173.8 (C-1). EM, m/z (%); 248 (M^+, 13), 217 (16), 216 (57), 189 (27), 188 (100), 175 (26), 174 (20).

3-(2-Hydroxy-3,6-dichlorophenyl)propanoic acid (hapten **4**): ^1H NMR (300MHz, DMSO-d_6); δ: 2.49 (t, J=7.5Hz, 2H, -CH_2COO-), 2.81 (t, J=7.5Hz, 2H, PhCH_2-), 7.13 (d, J=2.7Hz, $1H_{Ar}$ *meta*), 7.30 (d, J=2.7Hz, $1H_{Ar}$ *para*). ^{13}C NMR (75MHz, DMSO-d_6); δ: 25.4 (C-3), 33.0 (C-2), 121.5 (C-3'), 122.9 (C-1'), 126.5 (C-5'), 128.1 (C-4'), 131.9 (C-6'), 149.6 (C-2'), 173.4 (C-1).

3-(4-Hydroxyphenyl)-2-propanoic acid ^1H NMR (200MHz, DMSO-d_6); δ: 2.46 (t, J=7.4Hz, 2H, -CH_2COO-), 2.71 (t, J=7.4Hz, 2H, PhCH_2-), 6.67 (d, J=8.4Hz, $2H_{Ar}$ *meta*), 7.02 (d, J=8.4Hz, $2H_{Ar}$ *ortho*), 9.20 (s, 1H, -COOH).

Methyl 3-(4-hydroxyphenyl)-propanoate (92% yield): ^1H NMR (200MHz, $CDCl_3$); δ: 2.61 (t, J=7.6Hz, 2H, -CH_2COO-), 2.88 (t, J=7.6Hz, 2H, PhCH_2-), 3.68 (s, 3H, -OCH_3), 5.85 (s, 1H, -OH), 6.76 (d, J=8.4Hz, $2H_{Ar}$ *meta*), 7.05 (d, J=8.4Hz, $2H_{Ar}$ *ortho*).

Methyl 3-(4-hydroxy-3,5-dichlorophenyl)-propanoate. ^{1}H NMR (200MHz, $CDCl_3$); δ: 2.59 (t, J=7.4Hz, 2H, $-CH_2COO-$), 2.85 (t, J=7.4Hz, 2H, $PhCH_2-$), 3.68 (s, 3H, $-OCH_3$), 5.89 (s, 1H, -OH), 7.11 (s, $2H_{Ar}$ *ortho*). ^{13}C NMR (75MHz, $CDCl_3$); δ: 29.6 (C-2), 35.3 (C-3), 51.7 (OCH_3), 120.9 (C-3', C-5'), 128.0 (C-2', C-6'), 133.8 (C-1'), 146.3 (C-4'), 172.8 (C-1). EM, m/z (%); 248 (M^+, 23), 188 (81), 175 (100).

3-(4-Hydroxy-3,5-dichlorophenyl)propanoic acid (hapten **6**): ^{1}H NMR (300MHz, DMSO-d_6); δ: 2.49 (t, J=7.5Hz, 2H, $-CH_2COO-$), 2.71 (t, J=7.5Hz, 2H, $PhCH_2-$), 7.20 (s, $2H_{Ar}$ *ortho*), 9.78 (s, 1H, -COOH). ^{13}C NMR (75MHz, DMSO-d_6); δ: 28.8 (C-2), 34.8 (C-3), 121.9 (C-3', C-5'), 128.2 (C-2', C-6'), 133.9 (C-1'), 147.0 (C-4'), 173.3 (C-1).