ABSTRACT:

Induction of the endogenous human NAD(P)H:quinone oxidoreductase (HQOR₁) gene in the human hepatoma cell line HepG2 was measured at both the enzyme activity and RNA levels after exposure to a variety of industrial compounds. An RNA probe was designed that was complementary to both portions of the coding region and the 3'-nontranslated region unique to the largest (2.7-kilobase) HQOR₁ transcript. Induction by three strong inducers of HQOR₁ verified the utility of the antisense RNA probe. Ten industrial chemicals were evaluated as potential inducers, i.e. acrylonitrile, Sb₂O₃, BaO, CdCl₂, CuCl₂, ethyl acrylate, methyl acrylate, MoO₃, phenol, and toluene. Induction at the RNA level was about 2-fold higher than at the enzyme activity level except in the case of acrylonitrile, for which induction at the enzyme activity and RNA levels was similar. There was no preferential induction of the 2.7-kilobase transcript for any chemical tested, including 2,3,7,8-tetrachlorodibenzo-p-dioxin, which had previously been reported to preferentially induce this transcript. Six of the 10 industrial chemicals, including four previously untested chemicals (phenol, Sb₂O₃, CuCl₂, and MoO₃), were found to induce the HQOR₁ gene. By comparison, previous studies in rodent systems failed to accurately predict the human HQOR₁ gene response. Two chemicals previously shown to be inducers in rodent systems (methyl acrylate and CdCl₂) failed to induce the HQOR₁ gene. These results emphasize the importance of analyzing induction of the endogenous human gene, rather than simply extrapolating from rodent systems or gene fusion experiments.

Numerous xenobiotics elevate expression of QOR₁ (EC 1.6.99.2). With few exceptions, these chemicals are electrophiles or become electrophiles during metabolism (1–3). Induction of QOR leads to detoxification of electrophiles and has been proposed to protect cells from the toxic and neoplastic effects of further xenobiotic assaults (2–7). QOR is a homodimeric flavoprotein that catalyzes a two-electron reduction of quinones and redox dyes, as well as the reduction of chromium(IV) to chromium(III) (2, 3, 8, 9). Thus, this enzyme protects cells against free radicals and toxic oxygen metabolites generated by the one-electron reductions catalyzed by cytochromes P450 and other enzymes, as well as exogenous free radicals, metals, and other electrophiles.

To date, most studies on QOR induction have been performed in rodent systems, primarily mouse Hepa 1c1c7 cells (1–6, 10–12) and rats (9, 13–16). In these systems the QOR gene is induced by a wide variety of chemicals, which fall into the general categories of phenols, quinones, Michael reaction acceptors, isothiocyanates, hydroperoxides, mercaptans, arsenicals, and heavy metals (2, 3, 7). The majority of inducible QOR enzyme activity in human tissues has been attributed to the product of the HQOR₁ gene (3, 7, 17).

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1 Abbreviations used are: QOR, NAD(P)H:quinone oxidoreductase; HQOR₁, human NAD(P)H:quinone oxidoreductase 1; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; HQOR₂, human NAD(P)H:quinone oxidoreductase 2; ARE, antioxidant response element; nt, nucleotide(s); PBS, phosphate-buffered saline; GAPDH, glyceraldehyde phosphate dehydrogenase; 3'-NTR, 3'-nontranslated region; PCR, polymerase chain reaction; kb, kilobase; BA, 1,2-benzanthracene; tBHQ, t-butylhydroquinone; ACN, acrylonitrile.

Send reprint requests to: Mark D. Brennan, Department of Biochemistry, School of Medicine, University of Louisville, Louisville, KY 40292.
quantification of QOR activity or RNA could be used as a biomarker of exposure to that chemical. To assess the feasibility of this, we used an RNAse protection assay to monitor induction of the endogenous HQOR<sub>1</sub> gene. Additionally, the probe allowed us to measure the 2.7-kb transcript as a fraction of the total HQOR<sub>1</sub> transcripts. We tested a series of nine important, and in some cases potentially carcinogenic, industrial chemicals for their ability to induce the endogenous HQOR<sub>1</sub> gene in the human hepatoma cell line HepG2. In contrast to expectations based on results from rodent systems, the human gene showed only modest induction in response to five of these chemicals. Moreover, preferential induction of the 2.7-kb RNA was not generally observed.

**Materials and Methods**

Cuprous chloride was from Baker Chemical Co. (Phillipsburg, NJ). Molybdenum trioxide and BA were obtained from Sigma Chemical Co. (St. Louis, MO). ACN, antimony(III) oxide, barium oxide, dimethylsulfoxide, ethyl and methyl acrylates, and tBHQ were all purchased from Aldrich Chemical Co. (Milwaukee, WI). TCDD was provided by Steve Safe (Texas A&M University). All chemical reagents were of the highest purity available commercially. Restriction endonucleases and other DNA-modifying enzymes were obtained from New England Biolabs (Beverly, MA) and used according to the supplier’s recommendations.

HepG2 cells (hepatocellular carcinoma, HB865; American Type Culture Collection) were grown in Dulbecco’s modified Eagle medium (JRH Biosciences) containing 10% fetal calf serum (Gibco BRL), 50 units/ml penicillin, and 50 units/ml streptomycin (JRH Biosciences), at 37°C and 5% CO<sub>2</sub>. Cells were subcloned at approximately 72-hr intervals to maintain logarithmic growth. Twenty-four hours after subcloning, cells were exposed to target chemicals in graded doses, with control cultures receiving solvent (dimethylsulfoxide) only (29). Unless otherwise specified, cell viability was ≥85% for all control and experimental cultures, as determined by trypan blue exclusion and/or intracellular lactate dehydrogenase measurements.

After removal of the medium, the cell monolayer (1–2 × 10<sup>5</sup> cells/T-25 flask) was washed twice with cold PBS. The cells were exposed to 1 ml of trypsin solution (trypsin-EDTA; JRH Biosciences) for 2 min. Trypsin solution was removed by aspiration, and the cells were washed from the surface of the flask with PBS. The cells, suspended in PBS, were divided into two tubes and sedimented in a microcentrifuge for 1 min. The PBS was then removed by aspiration. Cells used in enzyme activity assays were resuspended in 500 μl of PBS and frozen at −80°C. The cells from which RNA was to be isolated were disrupted by the addition of 600 μl of RTL lysis buffer (Qiagen catalogue number 74106) and then frozen at −80°C.

QOR activity was measured as the NADPH-dependent reduction of cytochrome c in the presence of 10 μM menadione (8). Cellular lactate dehydrogenase was measured according to the method of Hogberg et al. (30). Total protein was determined by the method of Lowry et al. (31).

Whole-cell RNA was isolated according to product specifications using the Qiagen reagents and RNeasy spin-column chromatography. Total RNA was determined by absorbance at 260 nm. RNA levels were determined by RNAse protection assays, as previously described (32, 33). Briefly, the total RNA from 0.5–1 × 10<sup>5</sup> cells (typically 1–10 μg) was hybridized to 50,000 cpm (≈0.1 pmol) of each of two probes. The GAPDH probe was a 225-nt product derived from the template pTRI-GAPDH-Human (Ambion), which had been digested with SstI (34). This probe is complementary to 133 nt of the GAPDH RNA. The HQOR<sub>1</sub> probe was a 417-nt product produced by *in vitro* transcription of pBSK<sup>+</sup>+53 (described below). Protected products were resolved by electrophoresis on 8% polyacrylamide-urea (DNA-sequencing) gels (32, 33). The protected probe was quantified by exposing dried gels to Molecular Dynamics Phosphorlmager.

The plasmid pBSK<sup>+</sup> +53 was constructed in two steps. First, a *Pst*I to * Bam*HI fragment from HMNOR1d (coordinates 364 to 529) (17) was inserted into the corresponding sites in pBSK<sup>+</sup> (Stratagene) to produce pBSK<sup>+</sup> +50. Next, PCR was used to produce a fragment of the 3'-NTR of the 2.7-kb HQOR<sub>1</sub> transcript from HepG2 cells. Two primers, i.e. 5'-aatacgaggtgctcggagaaagagttg-3', containing an added XbaI site on the 5'-end, and primer 5'-aagacgtctgagcagaa-3', were used to amplify the region corresponding to nt 2201 to 2388 (17). PCR conditions included 2 mM MgCl<sub>2</sub>, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 1 min. PCR products were separated on a 1.5% agarose gel, and the band representing the desired product was excised, purified using an NACS column (Gibco BRL), and digested with XbaI and SacI. The PCR product, 3'-NTR, was cloned into the XbaI and SacI sites of pBSK<sup>+</sup> +50 (see above) to produce pBSK<sup>+</sup> +53 (fig. 1). The sequence of the Xba-Sacl insert corresponded to that previously reported (17), as determined by the dideoxynucleotide method (35) using Sequenase T7 DNA polymerase (United States Biochemicals, Cleveland, OH). This construction encodes an RNA complementary to both 169 nt of the HQOR<sub>1</sub> coding region and 188 nt of the 3'-NTR of the 2.7-kb transcript.

**Results**

Previous work has shown that the endogenous HQOR<sub>1</sub> gene in HepG2 cells is induced by TCDD (17) and that preferential induction of the largest (2.7-kb) transcript occurs in HepG2 cells treated with 100 nM TCDD (17, 19, 36). To quantify induction of HQOR<sub>1</sub> and to determine whether the 2.7-kb transcript is preferentially induced in response to TCDD and other chemicals, we constructed a plasmid for the production of an antisense probe for use in RNAase protection analysis (fig. 1). The resulting antisense RNA is complementary to both a 169-nt portion of the coding region and a 188-nt portion of the 3'-NTR unique to the 2.7-kb transcript.

To assess the utility of the antisense RNA probe, it was used for RNAase protection of RNA from cells singly dosed with chemicals that are known or predicted to be strong inducers of the endogenous HQOR<sub>1</sub> gene. Three chemicals, TCDD, BA, and tBHQ, were used for this analysis. TCDD has been shown to be an inducer in HepG2 cells, whereas BA and tBHQ have been shown to be inducers in rodent systems (17, 36, 37). Typically, induction in these systems is assessed after 48–72 hr (10, 15, 29, 38). Therefore, we used a similar time course. As can be seen in fig. 2, a single dose of BA, tBHQ, or TCDD resulted in induction of HQOR<sub>1</sub>, relative to control, as measured by RNAase protection.

![Fig. 1. Construction of the plasmid for synthesis of antisense RNA complementary to the HQOR<sub>1</sub> coding region and to the 3'-NTR of the 2.7-kb transcript.](image-url)
It has been reported that the 2.7-kb HQOR1 RNA increases as a percentage of all HQOR1 transcripts in the presence of TCDD (17). To determine the magnitude of this effect for TCDD and the other strong inducers, RNA was obtained from cells singly dosed with the known inducers for 72 hr and analyzed by RNAse protection. Generally, despite overall induction in the level of HQOR1 RNA, there were only modest changes in the relative amounts of the 2.7-kb transcript (fig. 2). In independent experiments this transcript varied between 30 and 60% of all HQOR1 transcripts, but the variation appeared to be unrelated to chemical exposure (data not shown).

Fig. 3 shows HQOR1 RNAse protection, compared with enzyme activity assays, as a measure of gene induction for BA, tBHQ, and TCDD. A single dose of the test chemicals increased enzyme activity by 72 hr. Whereas previously published results indicated an approximately 5-fold increase in QOR enzyme activity in HepG2 cells in response to 100 nM TCDD after 72-hr treatment (17), our results indicated only a 2.1-fold increase in enzyme activity. However, the enzyme specific activities after induction were very similar in the two cases (between 1200 and 1500 nmol/min/mg of protein). The difference in fold induction arises from an approximately 2-fold higher basal HQOR1 enzyme activity in our study. The final enzyme specific activities after induction are similar to those in cultured adult rat hepatocytes, where BA, tBHQ, and TCDD increase QOR enzyme activity by 72 hr (37).

As can be seen in fig. 3, RNA levels, as measured by RNAse protection, parallel the results at the level of enzyme activity. The RNA values shown in fig. 3 represent the RNAse protection signals for the HQOR1 coding region plus those for the 3'-NTR of the 2.7-kb transcript divided by that for GAPDH. Because the expression of the 3'-NTR of the 2.7-kb transcript was not altered preferentially in response to the chemicals tested, the RNAse protection values for the coding region and the 3'-NTR were combined. Maximum steady-state RNA levels in TCDD-dosed cells were reached at 48 hr, with a 4.6-fold induction. TCDD was previously tested as an inducer of the endogenous gene in HepG2 cells (17). Both nuclear run-on and slot blot experiments (48 hr, 100 nM TCDD) indicated a 3-fold induction of HQOR1 gene expression (19, 36). BA and tBHQ maximally induced HQOR1 RNA at 72 hr, with BA inducing levels 3.5-fold and tBHQ inducing levels 2-fold, relative to control. These two chemicals have not been previously tested in a human system for
HQOR₁ induction at the RNA level.

After the utility of the antisense probe was established, the probe was used, in conjunction with QOR activity assays, to monitor the induction of the HQOR₁ gene in response to selected industrial chemicals. These results are summarized in table 1. Five of the chemicals tested showed no reproducible induction. Three of the chemicals that failed to show induction (barium oxide, ethyl acrylate, and toluene) had not been previously tested as inducers of QOR. However, the other two, CdCl₂ and methyl acrylate, are known inducers of QOR in mouse Hepa 1c1c7 cells (2, 3, 12). Unexpectedly, in HepG2 cells methyl acrylate caused a slight repression to 0.73 of control at 40 μM (p ≤ 0.001, N = 9).

Of the tested chemicals that induced QOR, only ACN was previously reported to be an inducer. A single dose of ACN (50 μM) doubles QOR enzyme activity in mouse 1c1c7 cells (12). Fig. 4 shows a 72-hr dose-response curve over a wide range of ACN concentrations. HQOR₁ RNA levels were increased 2-fold by treatment with 38 μM ACN. Enzyme activity followed a similar dose-response curve, having a slightly lower fold induction at a given ACN concentration. The ACN dose sufficient to cause statistically significant induction was 9 μM for both enzyme activity and RNA level.

Interestingly, whereas enzyme activities closely paralleled RNA levels for induction by ACN, the other chemical inducers tested showed significantly stronger induction at the RNA level (fig. 5). In fact, only the strongest of these inducers, CuCl₂, resulted in statistically significant induction in enzyme activity within the dose range tested. Sb₂O₃ and phenol showed weaker induction (about 2-fold at the RNA levels). Of the tested chemicals that induced QOR, only ACN was previously reported to be an inducer. A single dose of ACN (50 μM) doubles QOR enzyme activity in mouse 1c1c7 cells (12). Fig. 4 shows a 72-hr dose-response curve over a wide range of ACN concentrations. HQOR₁ RNA levels were increased 2-fold by treatment with 38 μM ACN. Enzyme activity followed a similar dose-response curve, having a slightly lower fold induction at a given ACN concentration. The ACN dose sufficient to cause statistically significant induction was 9 μM for both enzyme activity and RNA level.

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### TABLE 1

<table>
<thead>
<tr>
<th>Chemical (Range, μM)</th>
<th>Minimum Concentration Required</th>
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<tbody>
<tr>
<td></td>
<td>Activity</td>
</tr>
<tr>
<td>ACN (0.75–500)</td>
<td>4 μM</td>
</tr>
<tr>
<td>Antimony(III) oxide (3–300)</td>
<td>&gt;40 μM³</td>
</tr>
<tr>
<td>Barium oxide (30–500)</td>
<td>Not tested</td>
</tr>
<tr>
<td>Cadmium chloride (0.5–22)</td>
<td>No induction to 22 μM²</td>
</tr>
<tr>
<td>Cuprous chloride (24–750)</td>
<td>&gt;750 μM²</td>
</tr>
<tr>
<td>Ethyl acrylate (10–400)</td>
<td>Not tested</td>
</tr>
<tr>
<td>Methyl acrylate (10–160)</td>
<td>Not tested</td>
</tr>
<tr>
<td>Molybdenum trioxide (32–1000)</td>
<td>&gt;250 μM³</td>
</tr>
<tr>
<td>Phenol (1.5–200)</td>
<td>&gt;200 μM³</td>
</tr>
<tr>
<td>Toluene (1.5–100)⁴</td>
<td>&gt;100 μM³</td>
</tr>
</tbody>
</table>

¹ The highest tested concentration is given and resulted in an induction of <1.5-fold.
² CdCl₂ at 22 μM was toxic to the cells, as indicated by a 50% decrease in intracellular lactate dehydrogenase levels.
³ Methyl acrylate caused a slight repression to 0.73 of control at 40 μM (p ≤ 0.001, N = 9).
⁴ Data were variable for both activity and mRNA.

RNA and protein values were determined as in fig. 3. Cells were given a single dose of specified chemical. The treatment period was 72 hr, because pilot experiments indicated maximal induction within this time period.

**Fig. 4. Parallel induction of QOR enzyme and mRNA in response to ACN.**

Enzyme activity (○) and RNA (□) values are as described in the legend to fig. 3. Inset, dose range from 0.75 to 20 μM. Values are means ± SEs (N = 7 except 0.75, 300, and 500 μM, where N = 3). Values at all dose levels of ≥9 μM are significantly different from their matched controls for both the protein and RNA measurements (p < 0.01), and all values at dose levels of ≥75 μM differ from control values (p < 0.005).
Induction by MoO₃ was unusual, in that it was biphasic, with maximal induction below 200 μM.

**Discussion**

In the current study, we have described the induction of the endogenous HQOR₁ gene after exposure to a variety of widely used industrial chemicals. HQOR gene induction has been extensively studied in murine Hepa 1c1c7 cells. Using this system, it has been shown that the HQOR gene responds with increased transcriptional activity when exposed to a variety of chemicals (2, 3, 7, 11). What is less well understood is the extent to which these and other data on rodent systems predict the response of the HQOR₁ gene to the same or similar compounds. Our results indicate that there are clearly species-specific responses to some xenobiotics, thus emphasizing the need to examine the HQOR₁ system directly.

The RNAse protection method described here allows one to specifically follow HQOR₁ gene induction at the RNA level. This method is more specific than enzyme activity assays, in that it quantifies only those RNAs that are complementary to the antisense RNA probe. Based on the known QOR sequences, only HQOR₁ RNA would hybridize to this probe (17). The enzyme activity assay, on the other hand, measures the reduction of cytochrome c in the presence of menadione (8). Other enzymes, such as nonspecific diaphorases, could potentially contribute to this activity (5, 39). We attribute the greater relative response at the RNA level, for some inducers, to the higher signal-to-noise ratio made possible by the specificity of the RNAse protection assay. For example, the strong inducers BA and tBHQ maximally induced HQOR₁ RNA at 72 hr. BA (50 μM) induced levels 3.5-fold and tBHQ (50 μM) induced levels 2-fold, relative to control, whereas the corresponding enzyme activities were induced 1.9- and 1.2-fold. TCDD (100 nM) increased HQOR₁ RNA 4.6-fold by 48 hr, compared with a corresponding enzyme activity increase of 1.5-fold. This increased specificity and resulting greater fold response for the RNAse protection assay permit detection of subtle changes in HQOR₁ expression that may otherwise go undetected.

The maximal enzyme activities we observed in the presence of BA, tBHQ, and TCDD are quantitatively similar to those reported by others (17, 37). Specifically, the value of 1260 ± 84 mlU/mg of protein observed by us in HepG2 cells after 72 hr of exposure to TCDD is comparable to the value previously reported for the same cell line under similar conditions (17).

Similarly, our estimate of HQOR₁ gene induction at the RNA level is of the same magnitude as that reported by others. Both nuclear run-on and slot blot experiments with HepG2 cells treated with 100 nM TCDD for 48 hr indicated a 3-fold increase in gene induction at the RNA level (19, 36). Under similar conditions, our RNAse protection assay indicated a 4.6-fold increase. However, unlike the results of others (17), we did not find preferential induction of the 2.7-kb transcript in response to TCDD. We did observe an approximately 2-fold variation in the fraction of this transcript from experiment to experiment but not between treated and control groups within experiments, suggesting that culture conditions may be a factor.

Although the results described above show that some chemicals that are strong inducers in the rodent system also induce the endogenous HQOR₁ gene in HepG2 cells, other chemicals that induce QOR in the rodent system do not induce it in the human system. Methyl acrylate is one of the stronger inducers of QOR in the Hepa 1c1c7 cell line (12), with a concentration of 20 μM being sufficient to cause a 2-fold induction. However, in HepG2 cells, methyl acrylate was not an inducer of HQOR, and, if anything, may slightly repress HQOR₁ RNA levels and QOR activity. Likewise, a dose of 10.5 μM CdCl₂

![Fig. 5. Dose-response curves for four industrial chemicals that increase QOR enzyme and RNA levels.](https://www.aspetjournals.org/dmd/article-pdf/doi/10.2133/dmdcj20000289/179QOR.INDUCTION-IN.HepG2.CELLS.pdf/71cf7b7a0d80e63cf875224f02c9d82f1853773e)

Enzyme activity (○) and RNA (□) values are as displayed in fig. 4. The abscissa shows chemical concentration (micromolar). Insets, low dose ranges. Values shown are means ± SEs (N = 6 except CuCl at 750 μM and MoO₃ at 500 and 1000 μM, where N = 3). *, Significant differences between treatment groups and controls (p < 0.01).
causes a 2-fold induction of QOR in Hepa 1c1c7 cells (2, 3), but in HepG2 cells it failed to induce HQOR1 at doses up 22 μM, a level toxic to the cells. The failure of these two chemicals to induce QOR in HepG2 cells, while inducing levels in the murine cell line, clearly shows the importance of examining the endogenous human gene.

Three widely used industrial chemicals not previously tested in the murine system (barium oxide, ethyl acrylate, and toluene) failed to induce HQOR1. Water-soluble barium salts produce a variety of toxic effects in humans (40, 41). Nevertheless, Ba<sup>2+</sup> was not expected to be an inducer of QOR, because it is apparently not involved in redox cycling (42). Similarly, ethyl acrylate, which is a major industrial acrylic acid ester used for the production of polymers and copolymers contained in paints, textiles, and speciality dental and medical devices (43), did not cause HQOR1 gene induction. Because ethyl acrylate contains an electron-withdrawing group next to a double bond, like the murine QOR inducer methyl acrylate, one might have predicted that ethyl acrylate would be an HQOR1 inducer. Toluene, which was tested because of its extensive industrial use as a solvent, cleaner, and fuel additive (44), likewise was not an inducer.

In contrast to the findings described above, four other previously untested industrial chemicals did induce the HQOR1 gene. For each of these, induction was considerably higher at the RNA level than at the level of activity. Of these, phenol was the only organic compound. The moderate induction by phenol may result from the products of its metabolism. Phenol is hydroxylated in vivo to produce catechol and hydroquinone (45), which are classic QOR inducers that act through ARE promoter elements (1, 22).

To our knowledge the three important industrial chemicals Sb<sub>2</sub>O<sub>3</sub>, CuCl, and MoO<sub>3</sub> have not been previously tested for QOR gene induction in any system. However, a number of metal salts, such as HgCl<sub>2</sub>, CdCl<sub>2</sub>, ZnCl<sub>2</sub>, and the metalloid NaAsO<sub>2</sub>, have been shown to induce QOR (2, 14). Antimony belongs to the same periodic group as arsenic and is similar to arsenic both chemically and biologically (46). By analogy with NaAsO<sub>2</sub>, Sb<sub>2</sub>O<sub>3</sub> possibly acts through the ARE (2, 14). Of the inorganic compounds tested, CuCl induced most strongly, being the only one to significantly increase enzyme activity. The toxicity of copper, when present in unusually high concentrations, has been attributed to its participation in redox cycling, which can produce hydroxyl radicals (42). Given that hydroxyl radicals can induce HQOR1 gene expression via NF-κB (42, 47), this might account for the relatively strong induction by CuCl. The induction by MoO<sub>3</sub> is unusual in that it is biphasic. This may reflect the complex and varied effects that molybdenum can have on cellular metabolism (48 –51).

Finally, induction by ACN, a Michael reaction acceptor, is unique in that enzyme activities closely parallel RNA levels. This chemical is widely used industrially and is thus a potential human health hazard (52, 53). It has been shown to be a moderately strong inducer in Hepa 1c1c7 cells (7, 11, 22, 24, 54). The relatively strong induction by ACN at the enzyme activity level may reflect a contribution by the HQOR2 gene, which would not be detected by our RNase protection assay (19). Alternatively, the expression of unidentified QORs or other diaphorases could be altered by xenobiotic exposure such that, in response to chemicals other than ACN, these enzymes may drop while HQOR1 is elevated.

In summary, we have quantified the induction of the endogenous HQOR1 gene, in the human cell line HepG2, upon exposure to a variety of industrial compounds. The response of the HQOR gene was generally modest and was different from that expected based on previous results with a proposed (murine) model system (2, 3, 7). These results emphasize the necessity of examining the endogenous HQOR1 gene directly.


