

Does the extreme skin sensitization potency of benzoquinone result from special chemistry?

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Background: Benzoquinone is known to be an extreme skin sensitizer. Until now there has been no consideration of whether it owes its extreme skin sensitization potency to unique aspects of its chemistry or simply to its high reactivity as a Michael acceptor.

Objectives: To answer these questions, we have applied two mechanistic approaches to estimate a theoretical potency value for benzoquinone.

Patients/Methods: A mechanistic read-across (MRA) approach, using known potency data for moderate sensitizers in the Michael acceptor domain, and a published quantitative mechanistic model (QMM) for skin sensitization potency of Michael acceptors, all in the moderate and weak range, were used.

Results: The read-across approach gives a theoretical local lymph node assay (LLNA) EC₃ value of 0.04%, and the QMM approach gives a theoretical value of 0.013%, compared with an experimental value of 0.010%.

Conclusions: The good agreement between the theoretical and experimental EC₃ values supports the strength of the MRA and QMM approaches, and provides strong evidence that the extreme sensitization potency of benzoquinone can be attributed solely to its high reactivity as a Michael acceptor.

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The need to develop non-animal methods for prediction of toxicity, including skin sensitization, in the light of new legislation, is now well recognized (1). Much progress has been made in mechanism-based methods for prediction of skin sensitization based on reaction chemistry (2–10).

For risk assessment purposes the need is not only to predict on a yes/no basis, and not only to predict a potency category, but to be able to reliably predict potency on a quantitative basis relative to other known chemicals. As an illustration, consider a compound X that is envisaged for use as a preservative in a consumer product such as a shampoo. Its properties as a preservative would almost automatically suggest the likelihood of its being a strong or even extreme skin sensitizer. However,

other extreme sensitizers, for example 5-chloro-2-methyl-4-isothiazolin-3-one (methylchloroisothiazolinone, INCI) [EC₃ = 0.009% (11)], are already used safely in many consumer products at low concentrations that cause adverse effects with acceptably low frequency under normal consumer exposure conditions.

To illustrate the point above, suppose we can confidently predict that compound X, for use in a shampoo, is no more than 10 times as potent as a known extreme sensitizer Y used safely in shower gels at y%. If we have consumer use data indicating that shower gel exposure and shampoo exposure situations are similar, then we could conclude that use of compound X in shampoo at 0.1 × y% may be safe.

Ultimately, the toxic effect of any chemical results from its chemical properties, and the

mechanism-based approach addresses the questions as to which chemical properties influence the biological system so as to produce the toxicity, and how this influence can be modelled. Mechanism-based models are, in general, preferable to other approaches, although depending on how much is understood about the toxic endpoint they are not always possible, because of their underlying logic and transparency. This not only improves credibility, but also enables knowledge of when the model is applicable and when it is not (i.e. the applicability domain is most clearly definable in mechanistic terms).

The requirement for a chemical to covalently modify skin protein(s) has long been established as a key event in the induction of skin sensitization (12). The skin sensitization properties of most, if not all, known contact allergens can be rationalized in terms of electrophilicity or pro-electrophilicity of the allergens, enabling them to bind to proteins. More details on the determinants of skin sensitization potential can be found in reference (4). The mechanism-based approaches applied here are based on this key concept.

Benzoquinone, widely used in the dye, textile, chemical, tanning and cosmetic industries (13) is known to be an extreme skin sensitizer (14). Until now there has been no consideration of whether it owes its extreme skin sensitization potency to unique aspects of its chemistry or simply to its high reactivity as a Michael acceptor. Here we apply mechanism-based approaches, using known potency data for moderate sensitizers in the Michael acceptor domain to estimate a theoretical potency value for benzoquinone, aiming thereby to demonstrate the potential value of these approaches and to better understand the basis of benzoquinone's extreme sensitization potency.

Methods

Published local lymph node assay (LLNA) data on Michael acceptors, and a published quantitative mechanistic model (QMM) correlating electrophilic reactivity with LLNA potency were used to arrive at predicted LLNA values for benzoquinone, using the mechanistic read-across (MRA) approach and the QMM approach, as described below.

MRA may be defined as estimating the toxicity for a given compound, by comparing it with related compounds whose toxicity or effects are known. This simple definition implies two issues: how to know which compounds are related and which are not, and what parameters to use for the comparison. These are difficult issues when the chemical mechanism of toxicity is not well understood and when a (quantitative) structure

activity relationship [(Q)SAR] has been established based on statistically selected parameters. In such cases relatedness can only be defined in terms of statistically based similarity indices, which in our experience can often fail to pick up important mechanistic subtleties, even when these are obvious to organic chemists. In contrast, where the chemical basis of the toxic effect is well understood, a mechanism-based approach can avoid these difficulties. For skin sensitization, if a compound can be assigned to a reaction mechanistic domain, its sensitization potential can be assessed by comparison with known sensitizers in the same domain.

QMM is a recently introduced term (15) for a long-established approach. A QMM is an equation relating toxicity (or more generally an effect) and physicochemical parameters, which can be derived mathematically from the known mechanism of action, applying established principles of chemistry and physics. An example is the QMM for skin sensitization potency of Schiff base domain sensitizers (16).

Results and Discussion

Estimation of EC3 for benzoquinone, by the MRA approach

The argument that benzoquinone sensitizes predominantly by Michael addition can be tested, using data on known Michael acceptor sensitizers to assess whether its potency is consistent with its Michael acceptor reactivity, as follows:

Recent experimental work to quantify electrophilic reactivity towards peptides (17) does not provide a clear answer, since benzoquinone reacts too quickly for a rate constant to be measured under the experimental conditions. However, the question can be addressed by an MRA approach using linear free energy principles.

Consider a Michael acceptor of general formula $XCH=CHCOY$. The carbon atom shown in bold type is the point of attack by the nucleophile. If steric effects are negligible for the compounds considered, reactivity will depend on the inductive effects of the groups X and Y. These inductive effects can be modelled by σ^* values. On this basis, a theoretical EC3 value for benzoquinone can be estimated from EC3 values of cinnamic aldehyde (cinnamal, INCI; cinnamaldehyde) and related compounds.

For benzoquinone, the X and Y groups are not separate but are connected through the ring structure. In this situation the simplest approach, following the principles of Perrin et al. (18), is to use σ^* values for separate groups with similar inductive effects (Fig. 1). For benzoquinone, the X group in the above general formula is a carbonyl

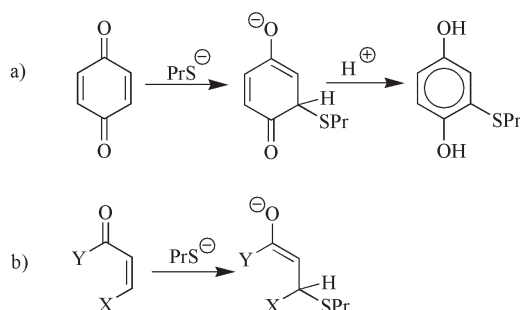


Fig. 1. Michael addition to benzoquinone. (a) Michael addition to benzoquinone, illustrated for thiolate anion as nucleophile. (b) General representation of the rate determining step. X modelled by $-\text{COCH}=\text{CH}_2$, similar in electronegativity to $-\text{CHO}$, $\sigma^* = 2.15$. Y modelled by $-\text{CH}=\text{CHCHO}$, $\sigma^* = 1.59$.

group attached to a vinylene group. In terms of electronegativity, a double bond ($\sigma^* = 0.56$ for vinyl) is similar to hydrogen ($\sigma^* = 0.49$), so to a good approximation the X group in benzoquinone can be modelled by $-\text{CHO}$ ($\sigma^* = 2.15$). The Y group in benzoquinone is a vinylene group attached to a carbonyl group, so to a good approximation the Y group in benzoquinone can be modelled by $-\text{CH}=\text{CHCHO}$ ($\sigma^* = 1.59$, calculated from the listed σ_m value by the formula $\sigma^* = (0.106 + \sigma_m)/0.217$, as given by Perrin et al. (18)).

The sensitivity of pEC3 to variation in the Y group can be estimated from data on cinnamic aldehyde (Y = H, $\sigma_Y^* = 0.49$) and benzylidene acetone (Y = Me, $\sigma_Y^* = 0.00$).

For cinnamic aldehyde, the compilation of Gerberick et al. (14) gives a value of 3.1% for the EC3. Benzylidene acetone has an EC3 value of 3.7% (14).

The corresponding pEC3 values, compared with σ_Y^* values are:

	σ_Y^*	pEC3
cinnamic aldehyde	0.49	1.63
benzylidene acetone	0.00	1.60
difference	0.49	0.03

Thus, replacing the Me group of benzylidene acetone by $-\text{CH}=\text{CHCHO}$ ($\sigma^* = 1.59$) should increase the pEC3 value by $1.59 \times 0.03/0.49 = 0.10$.

The sensitivity of pEC3 to variation in the X group can be estimated from data on benzylidene acetone (X = Ph, $\sigma_X^* = 0.75$) and 1-(p-methoxyphenyl)pent-1-ene-3-one (X = p-MeOPh, $\sigma_X^* = 0.60$).

The corresponding pEC3 values, compared with σ_X^* values are:

	σ_X^*	pEC3
benzylidene acetone	0.75	1.60
1-(p-methoxyphenyl)pent-1-ene-3-one	0.60	1.31
difference	0.15	0.29

Thus, replacing the Ph group of benzylidene acetone by $-\text{CHO}$ ($\sigma^* = 2.15$) should increase the pEC3 value by $(2.15 - 0.75) \times 0.29/0.15 = 2.70$.

Replacing the X and Y groups of benzylidene acetone by $-\text{CHO}$ and $-\text{CH}=\text{CHCHO}$, modelling benzoquinone, gives a calculated pEC3 value of $1.60 + 2.70 + 0.10 = 4.40$ for benzoquinone. Thus, the calculated EC3 is 0.004%. Bearing in mind the extrapolative nature of this calculation and its reliance on the reported EC3 values for cinnamic aldehyde, benzylidene acetone and 1-(p-methoxyphenyl)pent-1-ene-3-one, the agreement with the reported EC3 value of 0.01% (the difference being a factor of 2.5) is good. EC3 values are usually considered to be accurate within a factor of ca. 2 (19). It is worth noting that the difference is in the direction of the experimental potency being lower than the calculated potency, this being the opposite to what would be expected if benzoquinone is more potent than can be attributed to its Michael acceptor properties.

This exercise demonstrates that the extreme sensitization potency of benzoquinone is consistent with it sensitizing via the Michael acceptor mechanism, its potency relative to other Michael acceptors being in line with its calculated reactivity relative to other Michael acceptors.

Estimation of EC3 for benzoquinone, by the QMM approach

Kinetics of benzoquinone reaction with glutathione in an aqueous solution at pH 6 have been reported by Butler and Hoey (20). The rate constant is $1.1 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$. Converting to pH 7.4 (as used in the Schultz assay (21)) gives a calculated 'Schultz assay rate constant',

$$k_{\text{GSH, pH 7.4}} = 1.1 \times 10^5 \times 10^{(7.4-6)} \\ = 2.8 \times 10^6 \text{ M}^{-1}\text{s}^{-1}.$$

Applying equation 9 from reference (17)

$$\log k_{\text{cys}} = 1.15 \log k_{\text{GSH, pH 7.4}} - 0.13$$

to convert to reactivity with the Cys peptide used by Roberts and Natsch (17), gives:

$$\log k_{\text{cys}}(\text{benzoquinone}) = \\ 7.3 (\text{units of } k \text{ being } \text{M}^{-1}\text{s}^{-1}).$$

Applying the Roberts and Natsch QMM equation (equation 6 from reference 17):

$$\text{pEC3} = 0.24 \log k_{\text{cys}} + 2.11$$

gives a predicted pEC₃ value of 3.9 for benzoquinone. This corresponds to EC₃ = 0.013%, in very good agreement with the reported value for benzoquinone of 0.010% (22).

We conclude that benzoquinone's extreme sensitization potency can be ascribed completely to its high reactivity as a Michael acceptor.

Conclusions

The first aim of the work presented here was to test the ability of MRA and QMM to estimate the toxic potency of a chemical of interest. For this purpose we firstly used skin sensitization data on three known Michael acceptors to estimate a potency value for another Michael acceptor, benzoquinone, by comparison of reactivity-relevant structural features, and we secondly used a kinetics-based QMM for skin sensitization potency of Michael acceptors to estimate the potency of benzoquinone by reading off from its experimentally determined rate constant.

Because benzoquinone is much more reactive than the mechanistically related compounds for which sensitization and kinetic data are available, application of MRA and QMM has to be by extrapolation, as illustrated for the QMM approach in Fig. 2. Nevertheless, we show here that the MRA approach, based on using three moderate sensitizers for comparison of chemical properties, gives an estimated value out by only a factor of 2.5 from the experimental value. The QMM approach, using more known data points from which to extrapolate, should be more accurate, and this is found to be the case – the estimated EC₃ value of 0.013% is not significantly different from the reported experimental value of 0.010%.

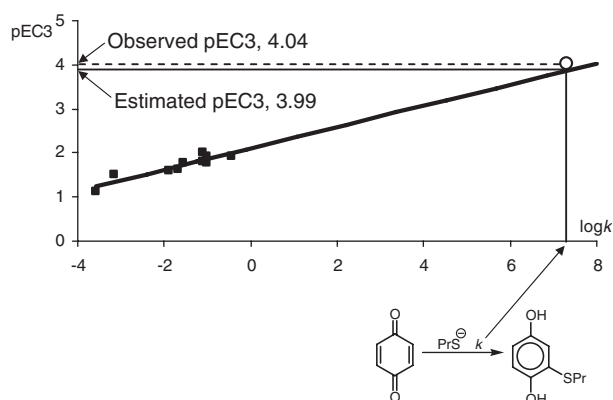


Fig. 2. QMM for Michael acceptors. Comparison of extrapolated estimate with experimental LLNA potency for benzoquinone. Points shown as filled squares represent the data used in reference 17 to develop the QMM, $pEC_3 = 0.24 \log k + 2.11$. The diagonal line is the regression line representing this equation. Data for benzoquinone were not used in deriving this line. The point shown as an open circle represents benzoquinone.

These findings provide strong support for the applicability of MRA and QMM to the risk assessment paradigm. Before applying these techniques to a new compound it is of course essential to make sure that the compound belongs to the same reaction mechanistic domain as the reference compounds. Where this is not obvious, it can be confirmed by appropriate laboratory chemistry.

The second aim was to investigate whether the extreme sensitization potency of benzoquinone is due to unique aspects of its chemistry or whether it can be attributed solely to its high reactivity as a Michael acceptor. The present findings strongly support the latter interpretation.

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