into account the high cellular turnover rate of the intestinal mucosa, should pay attention to choosing the right system(s).

Department of Pharmacology
Faculty of Pharmacy
State University of Utrecht
Catharijnesingel 60
3511 GH Utrecht
The Netherlands

PAUL BORM*
ANK FRANKHUIJZEN-SIEREVOGEL
JAN NOORDHOEK

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* To whom correspondence should be addressed.

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Structure–mutagenicity relationships for chlorinated ethylenes: a model based on the stability of the metabolically derived epoxides

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A number of chlorinated ethylenes are known to be mutagenic in certain bacteria [1], while vinyl chloride, in particular, has been shown to be carcinogenic in laboratory animals [2] and also in humans [3]. It is believed that the genotoxic activity of these compounds depends on the metabolic transformation by monofunctional oxygenases to the corresponding epoxides, the essential feature of which is a three-membered oxirane ring. The chemical reactivity of epoxides towards biological macromolecules such as DNA is well known [4] and depends, in this case, on both the number and distribution of the chlorine atoms in the oxirane ring. Furthermore, there is recent evidence relating DNA binding with mutagenic activity, as shown by Hemminki [5], for example. The detoxification of epoxides, which protects organisms from the deleterious effects of these compounds, is also known to occur. It is controlled mainly by enzymes such as glutathione transferase and epoxide hydrolase. *The net genotoxic* response of a biological system is a balance between activation (represented by DNA binding) and detoxification (represented by conjugation or diol formation); and this varies with biological species because of the different enzyme levels present.

Despite the complexity of genotoxic behaviour itself, *the activity pattern for a limited series of compounds* might be related to certain molecular properties of the compounds in a somewhat less complicated way. Greim *et al.* [1] have noted a rather simple *qualitative* relationship between the mutagenicity of a series of chlorinated ethylenes and the molecular symmetry of the related epoxides. The purpose of this investigation is to develop this notion still further by presenting a *quantitative* structure–activity relationship for the same alkenes based on the calculated electronic structure of the corresponding epoxides, and to use this relationship to predict the mutagenicity of other haloalkenes for which no experimental data exist at present.

Theoretical model for epoxide activity. While the chemical reactions involved in the activation and deactivation of epoxides are quite different, they have one important feature in common; namely, the rupture of the oxirane ring. Given that genotoxic response is a balance between activation and detoxification, it is of interest to explore the possibility that the *pattern of mutagenic activity* for a series of similar compounds is determined largely by the ease or otherwise of ring opening. Now the rupture of the oxirane

ring, which under both chemical and physiological conditions always takes place by fission of a C–O bond, is clearly a function of its stability and, in particular, the stability or strength of the weakest C–O bond. Thus, neglecting features such as membrane solubility and steric hindrance, the simplest theoretical model for the mutagenicity of the alkenes relates mutagenic activity directly to the energy of the weakest C–O bond of the corresponding epoxides. This is the relationship we seek to investigate.

While the details of the relationship are purely *empirical*, for there is no *a priori* procedure for determining them, the underlying basis of our model suggests a particular functional form. In the extreme case of very stable epoxides, the intrinsic reactivity towards both DNA and detoxifying enzymes will be low, and the level of mutagenic activity would be expected to be correspondingly low. Very unstable epoxides, on the other hand, are likely to be removed either by hydrolysis or conjugation, so depleting the concentration capable of binding to DNA, again leading perhaps to a low level of mutagenic activity. In the case of aflatoxin, for example, Callen and Ong [6] have observed an increase in genetic activity in the presence of 3,3,3-trichloropropylene oxide (TCPO). It is suggested that inhibition of epoxide hydrolase by TCPO is responsible, causing an increase in the availability of the putative metabolite, aflatoxin-2,3-epoxide, for binding to DNA. In between these two extremes of oxirane ring stability, DNA binding, and hence mutagenic activity, would be expected to reach a maximum. These very general arguments suggest that the pattern of mutagenic activity as a function of C–O bond energy will be peaked and approximately parabolic in form, with the precise details depending on the biological conditions.

Epoxide ring stability. In principle, bond energies should be obtainable from molecular spectra; for the chlorinated alkene epoxides, however, there is an insufficiency of accurate data. In the present communication, therefore, we use instead theoretical C–O energies derived from molecular orbital calculations. The particular method we have chosen is the MINDO/3 procedure described in full by Dewar *et al.* [7–11]. It has been shown to be both reliable and flexible and the particular quantities of interest to us here are the two-centre energies of the weakest C–O bonds which are determined by calculation.

In the absence of accurate experimental structures for the epoxides we are concerned with in this study, we have used two sets of molecular geometries in the electronic structure calculations. The first assumes 'standard' bond lengths and angles for all the epoxides. The second set uses fully optimised geometries based on minimising the total molecular energy with respect to all independent structural variables. The latter structures are very much more expensive in computer time, but from a comparison of the results for the two sets we can assess both the general reliability of our approach and the need or otherwise for accurate molecular structures in empirical relationships of this type.

Results and discussion. In Fig. 1 we have plotted the mutagenicity data for chloroethylenes [1] against the calculated two-centre bond energies (E_{C-O}) of the weakest C–O bond of the corresponding epoxides. For completeness and for comparison with other compounds later on, we list these data in Table 1. As shown, the relationship between mutagenic activity and bond energy follows the general pattern suggested by our model; namely, that activity is low at the extremes of ring stability (C–O bond energy) with a maximum at some intermediate value, which in this case corresponds to vinyl chloride. There are minor variations in the activity patterns for the two sets of structures such as a narrowing of the active band for the optimised structures, but it would be imprudent to ascribe any significance to the specific differences between the two. Indeed, for predictive purposes, it would be most sensible to consider a 'composite' curve of the type indicated by the dotted line in Fig. 1, which represents a crude 'upper limit' for activity, rather than either of the separate correlations. In Table 2 we list the 'predicted' activities of nine haloalkenes, based on this 'composite' curve for which there is no experimental data. Once again the selection of substituents is purposely restricted so that possible complicating stereochemical and lipophilic effects and further metabolism of these substituents is minimised. We have noted that only three of these compounds are predicted to be mutagenic; they are $CF_2{=}CHCl$, $CH_2{=}CHF$ and $CH_2{=}CH_2$. Each one shows a moderately high level of

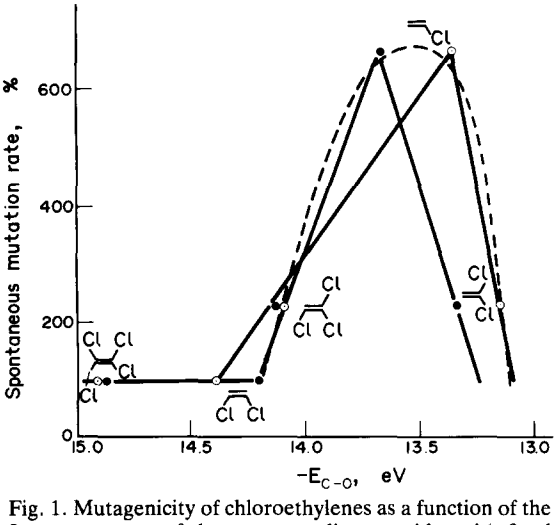

Fig. 1. Mutagenicity of chloroethylenes as a function of the 2-centre energy of the corresponding epoxides with fixed (○) and optimised (●) geometries. The dotted line represents the composite curve.

Table 1. Structure–activity relationships for chlorinated ethylenes

Compound	2-Centre energy (eV)	Activity*
$CCl_2{=}CCl_2$	−14.89	100
$CCl_2{=}CHCl$	−14.1	232
$CCl_2{=}CH_2$	−13.16	229
$CHCl{=}CHCl$	−14.38	100
$CHCl{=}CH_2$	−13.4	663

* Per cent spontaneous mutation rate for the arginine operon of *E. coli* K_{12} taken from [1].

Table 2. Structure–activity relationships for untested compounds

Compound	2-Centre energy (eV)	Activity*
$CF_2{=}CF_2$	−16.44	100
$CF_2{=}CFCl$	−15.47	100
$CF_2{=}CFH$	−14.83	100
$CF_2{=}CCl_2$	−14.73	100
$CF_2{=}CHCl$	−13.91	500
$CH_2{=}CH_2$	−13.68	650
$CH_2{=}CFH$	−13.24	500
$CH_2{=}CFCl$	−13.02	100
$CH_2{=}CF_2$	−12.89	100

* Per cent spontaneous mutation rate predicted from the composite curve in Fig. 1.

activity, but ethylene is the most active, being roughly comparable to vinyl chloride. It is important to emphasise that these conclusions relate *specifically* to the derived epoxides. The mutagenicity of the alkenes *per se* may be quite different and will require further investigation to determine whether metabolic activation to the corresponding oxiranes is a crucial factor. It should be remembered that these predictions relate solely to the arginine operon in *E. coli* K_{12} and cannot be applied to other organisms which might exhibit greater or lesser sensitivities to epoxides. Such differences in sensitivity could lead to activity curves with different widths, heights and asymmetries so that 'cross-correlation' from one system to another would be invalid. We have been unable to find any examples of simple genotoxic aliphatic epoxides (i.e. no more than four carbon atoms in the chain) in the literature whose calculated two centre energy is either greater than -14.5 eV or less than -12.8 eV, and therefore suggest that this may constitute an important threshold band for epoxide genotoxicity.

In summary, we have established an empirical structure–activity relationship for the mutagenicity of certain haloalkenes, based on the calculated stability of the corresponding epoxides. The overall form of the relationship can be understood in terms of a simple theoretical model wherein mutagenic activity results from a balance between DNA binding and detoxification by enzymes such as glutathione transferase and epoxide hydrolase. The activity patterns for two different sets of geometries are sufficiently close to support our theoretical model and to suggest that correlations of this type are relatively insensitive to small changes in molecular geometry. Finally, the mutagenic activity for nine other haloalkenes is predicted, based on the present relationships.

I.C.I. plc
P.O. Box 11
The Heath
Runcorn
Cheshire WA7 4QE
U.K.

R. B. Jones
W. C. Mackrodt

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