REFERENCES

1. R. O. Recknagel, E. A. Glende and A. M. Hruskewyzcz, in *Free Radicals in Biology* (Ed. W. A. Pryor), Vol. III, pp. 97–132. Academic Press, New York (1977).
2. R. P. Mason, in *Reviews in Biochemical Toxicology* (Eds. E. Hodgson, J. R. Bend and R. M. Philpot), Vol. I, pp. 151–200. Elsevier North Holland, Amsterdam (1979).
3. E. S. Reynolds and M. T. Moslen, in *Free Radicals in Biology* (Ed. W. A. Pryor), Vol. IV, pp. 44–49. Academic Press, New York (1980).
4. C. R. Wolf, W. G. Harrelson, W. M. Nastainczyk, R. M. Philpot, B. Kalyanaraman and R. P. Mason, *Molec. Pharmac.* **18**, 553 (1980).
5. J. L. Poyer, P. B. McCay, E. K. Lai, E. G. Jansen and E. R. Davis, *Biochem. biophys. Res. Commun.* **94**, 1154 (1980).
6. E. Albano, K. A. T. Lott, T. F. Slater, A. Stier, M. C. R. Symons and A. Tomasi, *Biochem. J.* **204**, 593 (1982).
7. C. R. Wolf, D. Mansuy, W. Nastainczyk, G. Deutschmann and V. Ullrich, *Molec. Pharmac.* **13**, 698 (1977).
8. H. J. Ahr, L. J. King, W. Nastainczyk and V. Ullrich, *Biochem. Pharmac.* **29**, 2855 (1980).
9. H. Shah, S. P. Hartman and S. Weinhouse, *Cancer Res.* **39**, 3942 (1979).
10. V. L. Kubic and M. W. Anders, *Life Sci.* **26**, 2151 (1980).
11. L. R. Pohl, R. V. Branchflower, R. J. Highet, J. L. Martin, D. S. Nunn, T. J. Monks, J. W. George and J. A. Hinson, *Drug metab. Dispos.* **9**, 334 (1981).
12. B. A. Mico, R. V. Branchflower, L. R. Pohl, A. T. Pudzianowski and G. H. Loew, *Life Sci.* **30**, 131 (1982).
13. B. A. Mico and L. R. Pohl, *Biochem. biophys. Res. Commun.* **107**, 27 (1982).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
16. Y. Yasukochi and B. S. S. Masters, *J. biol. Chem.* **251**, 5337 (1976).
17. B. S. S. Masters, C. H. Williams and H. Kamin, *Meth. Enzym.* **10**, 565 (1967).
18. S. B. West, M. Huang, G. T. Miwa and A. Y. H. Lu, *Archs Biochem. Biophys.* **193**, 42 (1979).
19. U. K. Laemmli, *Nature, Lond.* **227**, 680 (1970).
20. F. P. Guengerich, *J. biol. Chem.* **253**, 7931 (1978).
21. D. A. Haugen and M. J. Coon, *J. biol. Chem.* **251**, 7929 (1976).
22. J. S. French and M. J. Coon, *Archs Biochem. Biophys.* **195**, 565 (1979).
23. G. D. Knott, *Comput. Progm. Biomed.* **10**, 271 (1979).
24. I. G. Sipes, G. Krishna and J. R. Gillette, *Life Sci.* **20**, 1541 (1977).

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# Structure–genotoxicity relationship for aliphatic epoxides

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In a recent commentary on the role of reactive metabolites in the carcinogenicity of halogenated ethylenes Bolt *et al.* [1] have discussed the relationship between the stability of the corresponding epoxides and activity (both carcinogenic and mutagenic) in the light of Henschler's structural theory [2]. They have also presented data for the oncogenic effects of halogenated ethylenes in comparison with covalent macromolecular binding, mutagenicity in bacterial tests and carcinogenicity in animal bioassays. The interpretation of this data and of genotoxic response more generally outlined by these authors, particularly with regard to the *optimum* (as opposed to minimum) stability of the epoxide ring for genotoxic response, parallels closely that put forward independently by the present authors shortly afterwards [3]. Here, we wish to develop this theme still further.

The essential feature of our approach to the mutagenicity

Table 1. Structure–activity relationships for haloethylenes

| Compound | Two-centre energy of the corresponding epoxide (eV) | Mutagenicity* | Oncogenicity† |
|---|---|---|---|
| $CCl_2{=}CCl_2$ | −14.89 | 100 | 0 |
| $CCl_2{=}CHCl$ | −14.1 | 232 | 0 |
| $CCl_2{=}CH_2$ | −13.16 | 229 | 0.15 |
| $CHCl{=}CHCl$ | −14.38 | 100 | ND |
| $CHCl{=}CH_2$ | −13.4 | 663 | 18.2 |
| $CH_2{=}CHF$ | −13.24 | ND | 14.3 |
| $CH_2{=}CF_2$ | −12.89 | ND | 1.81 |

* Per cent spontaneous mutation rate for the arginine operon of *E. coli* $K_{12}$ [2].
† Per cent foci theoretically produced by 1 mole metabolites per kg body wt [1].
ND = no experimental data available.

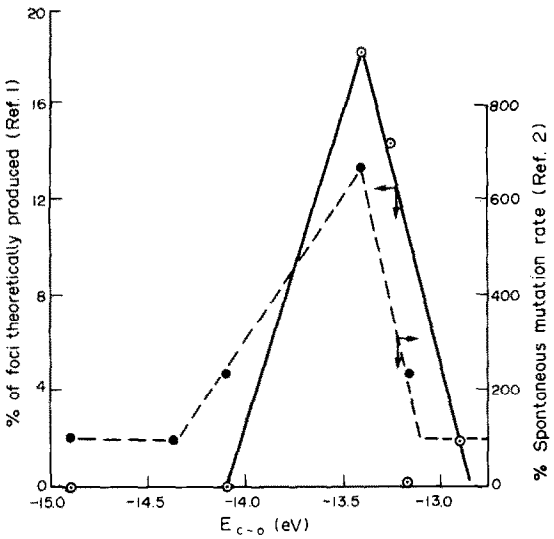
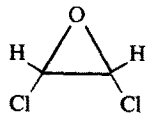
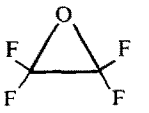
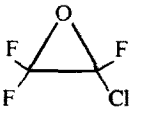
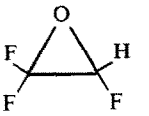
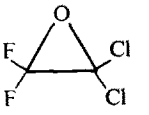
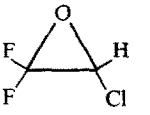
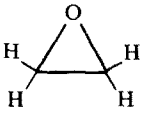
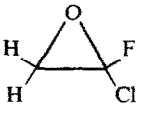



Fig. 1. Per cent spontaneous mutation rate for the arginine operon of *E. coli* $K_{12}$ (●) and per cent foci theoretically produced by 1 mole metabolites per kg body wt (○) for fluoro- and chloro-ethylenes as a function of the calculated two-centre energy of the corresponding epoxides.

of the chlorinated ethylenes reported by Greim *et al.* [2] is that there is a *quantitative* relationship between the *experimental* mutation rate and the *calculated* stability of the epoxide ring, and that the form of this relationship can be understood in terms of a balance between DNA binding and enzymic detoxification. At the time of our previous communication there was no consistent *in vivo* data comparable to that of Greim *et al.* [2] which could be examined in the same way. The recent results by Bolt *et al.* [1], however, rectify this situation. They compare the oncogenic effects, defined therein as the percentage pre-neoplastic foci observed in new-born Wistar rats, for a similar series of haloethylenes. The purpose of the present communication, then, is to present a structure–activity relationship of the type previously given [3], to show that this relationship fully supports the ring stability arguments proposed by Bolt *et al.* [1] and to indicate the striking similarity between the *in vitro* and *in vivo* activity patterns. In addition, we compare the reported mutagenic activity of a number of other aliphatic epoxides with that predicted by our model and consider the activity of nine other derivatives for which no data are available.

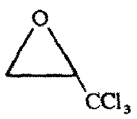
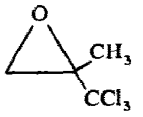
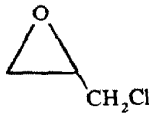
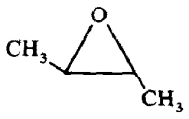
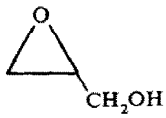
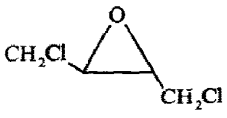
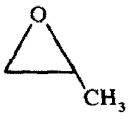
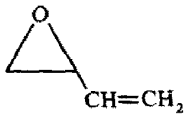
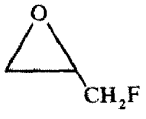
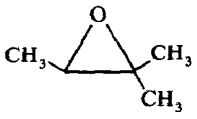
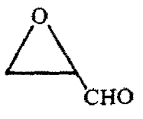
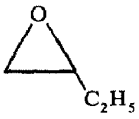
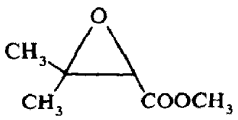
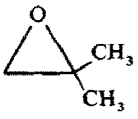
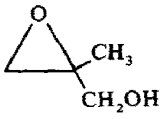
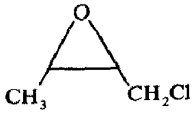
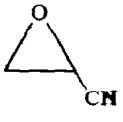
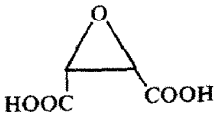
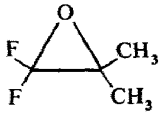
In Fig. 1 we have plotted both the mutagenic data of Greim *et al.* [2] and the oncogenic data of Bolt *et al.* [1] against the calculated two-centre bond energies, $E_{C\text{-}O}$, of the weaker of the two C–O bonds of the corresponding epoxides [3]. The numerical details are collected in Table 1. The similarity between the two patterns of activity is quite striking. Not only do we find vinyl chloride with the maximum activity in both cases, but the stability range within which active molecules fall is very nearly the same. The observed threshold band for oncogenicity ranges from −14.1 to −12.9 eV, compared with −14.5 to −12.8 eV for mutagenicity, and it is suggested that compounds which fall within these limits are *potentially* hazardous. In view of the close similarity between the two activity patterns we suggest that the origins for maximum activity previously outlined [3] hold in both cases. With regard to the reported oncogenic activity the only anomalous point in Fig. 1 is that for vinylidene chloride. However, we note that the dose used by Bolt *et al.* [1] was 200 times *less* than the other haloethylenes in view of its acute toxicity. The correlation between activity and stability proposed here suggests that the percentage of pre-neoplastic foci at an exposure con-

Table 2. Structure–activity relationships for untested compounds

| Compound | Two-centre energy (eV) | Oncogenicity* |
|---|---|---|
|  | −14.38 | 0 |
|  | −16.44 | 0 |
|  | −15.47 | 0 |
|  | −14.83 | 0 |
|  | −14.73 | 0 |
|  | −13.91 | 5.0 |
|  | −13.68 | 11.5 |
|  | −13.02 | 5.6 |

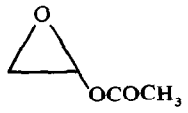
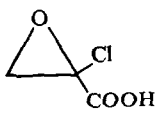
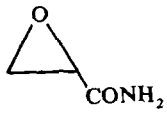
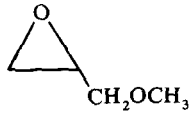
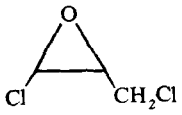
* Per cent foci theoretically produced by 1 mole metabolites per kg body wt predicted from the continuous line in Fig. 1.

Table 3. A comparison of the predicted mutagenicity of aliphatic epoxides with published literature data

| Compound | Mutagenicity | | Compound | Mutagenicity | |
|---|---|---|---|---|---|
| | Experiment* | Model† | | Experiment* | Model† |
|  | + [6, 7] | + |  | + [7] | − |
|  | + [6–9] | + |  | − [7] + [11] | + |
|  | + [6, 7, 10] | + |  | + [9] | + |
|  | + [6–8] | + |  | + [7, 12] | + |
|  | + [6, 7] | + |  | − [11] | − |
|  | + [7, 10] | + |  | + [13] | + |
|  | + [7, 8] | + |  | ND | − |
|  | − [7] | − |  | ND | − |
|  | + [14] | + |  | ND | + |
|  | ND | − |  | ND | − |

*(continued)*

Table 3 (*continued*)

| Compound | Mutagenicity | | Compound | Mutagenicity* | |
|---|---|---|---|---|---|
| | Experiment* | Model† | | Experiment* | Model† |
|  | ND | + |  | ND | + |
|  | ND | + |  | ND | + |
|  | + [14] | + | | | |

* References are given in brackets.

† The predictions are based on the structure–activity relationship shown by the continuous line in Fig. 1.

ND = no experimental data available.

centration of 2000 ppm, the dose used for the other five compounds, would be nearly 10 (as opposed to 0.15 reported at 100 ppm) in the absence of toxic effects. Thus we conclude that for the chlorinated ethylene epoxides, at least, there is a close relationship between *in vitro* and *in vivo* activity.

The structure–activity relationship shown by the continuous line in Fig. 1 can be used to predict the number of foci theoretically produced under identical conditions for eight other haloalkenes, including ethylene. The results are listed in Table 2. As before [3], we emphasise that these relate *specifically* to the derived epoxides, and that the oncogenic activity of the alkenes *per se* might be quite different.

We have suggested previously that activity patterns of the type shown in Fig. 1 strictly speaking apply to a restricted series of compounds, in this case the chloroalkenes. However, it might be of interest to compare the activities of a wider range of substituted alkenes predicted on the basis of these relationships with the reported mutagenicity, with specific reference to the corresponding oxiranes. The results are given in Table 3. For present purposes all epoxides that fall within the threshold band (−14.5 to −12.8 eV) are taken to be active (+) although it is important to emphasise that this threshold is arbitrary and that the (theoretical) activity for some compounds may be overestimated.

Nevertheless, as shown in Table 3, the agreement with experimental results is good. Of 16 epoxides for which there are data, our structure–activity relationship correctly assigns the activities of 14. For one, namely, 2,3-epoxybutane, the experimental results appear to be ambiguous. The only incorrect prediction is that for 2-trichloromethyl propylene oxide.

In conclusion then we have shown that there is a correlation between the mutagenicity and oncogenicity of certain haloalkenes based on the stability of the corresponding oxiranes. The proposed structure–activity relationship is used to predict the oncogenicity of eight other haloalkenes *under identical conditions* and the mutagenicity of a further 16 aliphatic epoxides for which data are available for comparison. Thus, empirical models of this type might be useful for predicting the genotoxicity of epoxides *per se* or those formed *in vivo* from alkene precursors. Indeed, there is evidence that alkenes themselves are formed as intermediates in the metabolism of a variety of compounds. An example of this is urethane, which is thought to give rise to a putative epoxide via vinyl carbamate [4]. On the other hand, in cases such as the α-halohydrins, epoxides may be formed independently of the alkene [5]. Finally, we believe that an approach such as this might be of value in the prediction of the genotoxicity of a wider range of compounds.

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REFERENCES

1. H. M. Bolt, R. J. Laib and J. G. Filser, *Biochem. Pharmac.* **31**, 1 (1982).
2. H. Greim, G. Bonse, Z. Radwan, D. Reichert and D. Henschler, *Biochem. Pharmac.* **24**, 2013 (1975).
3. R. B. Jones and W. C. Mackrodt, *Biochem. Pharmac.* **31**, 3710 (1982).
4. H. C. Pitot, *A. Rev. Med.* **30**, 2 (1979).
5. A. R. Jones and G. Fakhouri, *Xenobiotica* **9**, 595 (1979).
6. K. Hemminki and K. Falck, *Toxic. Lett.* **4**, 103 (1979).
7. D. R. Wade, S. C. Airy and J. E. Sinsheimer, *Mutation Res.* **58**, 217 (1978).
8. K. Hemminki, K. Falck and H. Vainio, *Archs Toxic.* **46**, 277 (1980).
9. H. Bartsch, C. Malaveille, A. Barbin and G. Planche, *Archs Toxic.* **41**, 249 (1979).
10. M. J. Wade, J. W. Moyer and C. H. Hine, *Mutation Res.* **66**, 367 (1979).
11. C. De Meester, M. Mercier and F. Poncelet, *J. appl. Toxic.* **2**, 284 (1982).
12. C. De Meester, F. Poncelet, M. Roberfroid and M. Mercier, *Biochem. biophys. Res. Commun.* **80**, 298 (1978).
13. U. Rannug, R. Gothe and C. A. Wachtmeister, *Chem. Biol. Interact.* **12**, 251 (1976).
14. S. A. Kline, E. C. McCoy, H. S. Rosenkranz and B. L. Van Duuren, *Mutation Res.* **101**, 115 (1982).