Epoxides—*is there a human health problem?*

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ABSTRACT The purpose of this review is to consider whether epoxides represent a hazard to human health. Possible means of occupational and non-occupational exposure are discussed with reference to the production and uses of industrially important compounds and other epoxides, such as naturally occurring plant and fungal products. In addition to epoxides themselves, unsaturated compounds that may be metabolised *in vivo* to epoxides are included, since this appears to be a further important means of exposure. The toxicology, in particular carcinogenicity and mutagenicity, is discussed, along with a brief outline of the biochemistry such as metabolism, binding to cell constituents, and DNA repair mechanisms. The question of interactions between different epoxides *in vivo* is also raised.

During the past 30 years there has been an ever increasing number of reports on the toxicity, mutagenicity, and carcinogenicity of epoxides. Much of the current interest in these compounds has been generated by the finding that an epoxide seems to be the ultimate carcinogenic metabolite for several polyaromatic hydrocarbons. But even from the earliest studies it was realised that the same properties that made epoxides so useful in industrial processes could also pose potential hazards for biological systems. And yet it is still unclear exactly how big a problem they are in terms of human health.

Epoxides are ubiquitous—they occur naturally, in industry, the environment, and *in vivo* as metabolites in certain biochemical pathways. Several of these compounds are quite toxic, a few even carcinogenic, at least in animals, but because epoxides vary greatly in reactivity and molecular geometry they are not all equally hazardous.

In this review the term epoxide refers to any molecule containing the three-membered ring

—C—C—, where one oxygen atom bridges two carbon atoms. The nomenclature is confusing,¹ but the compounds are referred to here by their most commonly used names.^{2,3}

Epoxides can be solids, liquids, or gases. Ethylene

oxide, the simplest aliphatic epoxide, is a gas whose vapours are explosive in mixtures with air, but can be inactivated by mixing with CO₂ or fluorocarbons (Freon). Under certain conditions ethylene oxide will polymerise to a liquid or solid. Propylene oxide is a liquid with a low boiling point, so that it can occur as a gas with properties similar to ethylene oxide. Some epoxides are liquids with much higher boiling points, such as epichlorohydrin, diepoxybutane, and styrene oxide, while glycidyl oleate is an oil. 9-10-Epoxystearic acid and fusarenon-X, two naturally occurring epoxides, are solids. The most important characteristics of epoxides arise from their electrophilic and lipophilic nature and the reactivity of the epoxide ring. Ring-opening reactions entail attack by a nucleophile, such as NH₂, OH⁻, Cl⁻, or a thiol—in fact reactions with OH⁻ and S⁻ groups, both enzymically and non-enzymically, are two of the major *in-vivo* mechanisms for eliminating these compounds.

The emphasis of this review is more towards the aliphatic compounds, as these are industrially the most important epoxides and since there are several comprehensive reviews on the polyaromatic hydrocarbons and their epoxides (arene oxides).⁴⁻⁹

Occurrence and exposure—epoxides

Epoxides are produced industrially in vast quantities. The reactivity of the epoxy group makes them particularly useful as alkylating or cross-linking agents. The most widely used are the small aliphatic com-

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Table 1 Industrial uses and production figures—epoxides^{10 11}

Epoxide	Industrial uses	Approximate quantity produced (country, year)
Diepoxybutane	Curing of polymers; cross-linking textile fibres; prevention of microbial spoilage; chemical intermediate	
Diglycidyl resorcinol ether	Liquid epoxy resin; reactive diluent in production of other epoxy resins; curing of polysulphide rubber	5000 kg (Japan, 1975)
Epichlorohydrin (ECH)	Production of synthetic glycerin; preparation of unmodified epoxy resins, ECH elastomers, glycidyl ethers, modified epoxy resins, wet-strength resins for the paper industry, water treatment resins; cross-linking starch in food; paint solvent	156.5 × 10 ³ kg (USA, 1973) 41 × 10 ³ kg (Japan, 1974) No longer produced in UK
Epoxidised soya-bean oil	Stabiliser and plasticiser in PVC industry	46.5 × 10 ³ kg (USA, 1974)
Epoxy resins	Surface coatings; castings and potting for tooling and encapsulating electronic circuits; adhesives, especially in metal-to-metal bonding; textile treating agents to improve wearability and crease-resistance; laminating especially for structural use in aircraft	99 × 10 ³ kg (USA, 1973) ¹² 113 × 10 ³ kg (USA, 1974)
Ethylene oxide	Production of ethylene glycol used mainly in antifreeze products; polyethylene terephthalate polyester fibre and film production; manufacture of non-ionic surface active agents, diethylene glycol, triethylene glycol, ethanalamines, choline and choline chloride, and other organic chemicals; fungicide for treating books, and dental, pharmaceutical, medical, and scientific equipment, drugs, leather, motor oil, paper, soil, bedding for experimental animals, clothing, furs, furniture, and vehicles; sterilisation of foodstuffs; to accelerate maturing of tobacco leaves	865 × 10 ³ kg (Europe, 1972) 415 × 10 ³ kg (Japan, 1974) 119.5 × 10 ³ kg (UK, previously) 1892 × 10 ³ kg (USA, 1973) 1896 × 10 ³ kg (USA, 1976) 2005 × 10 ³ kg (USA, 1977)
Glycidaldehyde	Cross-linking agent in finishing of wool; oil tanning and fat liquoring of leather and surgical sutures; protein insolubilisation, tested as a vapour phase disinfectant (inactivates foot and mouth virus)	No evidence of commercial production or use in USA
Propylene oxide	Production of polyether polyols for polyurethane foams; polypropylene glycol for unsaturated polyester resins; conversion to dipropylene glycol, glycolethers, and synthetic glycerin; fumigant for materials from plastic medical instruments to food	903 × 10 ³ kg (Europe, 1973) 131 × 10 ³ kg (Japan, 1974) < 50.8 × 10 ³ kg (UK, 1977) 796 × 10 ³ kg (USA, 1973) 812 × 10 ³ kg (USA, 1976) 862 × 10 ³ kg (USA, 1977)
Styrene oxide	Diluent in epoxy resins; preparation of agricultural and biological chemicals, cosmetics, surface coatings; treatment of textiles and fibres; production of phenyl ethyl alcohol	100 × 10 ³ kg (Japan, pre-1976) Smaller quantities are now being produced
Vinyl cyclohexene dioxide	Reactive diluent for other diepoxides and for epoxy resins derived from bisphenol A and ECH; chemical intermediate	

Table lists the main industrial epoxides and some of their uses, past and present. Production figures (not available for all the compounds) give an idea of the annual output for particular countries over past ten years.

pounds—ethylene oxide, propylene oxide, epoxybutane, and epichlorohydrin—and the compounds used in the epoxy resin industry, such as the glycidyl ethers. Table 1 indicates the various uses to which epoxides have been put and their production figures. Tanaka *et al*¹¹ have compiled a list of epoxides used in the epoxy resin industry along with their manufacturers.

Although a few are useful as end products—for example, as sterilising agents—they are most valuable as intermediates and as stabilisers. As intermediates they are used to manufacture surface active agents, special solvents, synthetic resins, cements, adhesives, and fine chemicals. The largest use of ethylene oxide is in the production of ethylene glycol for antifreeze—an estimated 67% of the total in Japan in 1974.¹⁰ In the synthesis of epoxy resins epichlorohydrin is a principal epoxidising agent.

Many commercial products, such as polyvinyl chloride (PVC) and trichloroethylene tend to degrade slowly with the evolution of HCl. However, the addition of very small amounts (often less than 1%) of various epoxy compounds, usually in combination with other classes of compounds, prevents

this.^{13 14} The stabilisation mechanism is not well understood, but the role of acid acceptor is generally ascribed to the epoxide. There are many patents for using epoxides as stabilisers, but probably only a few of these have found commercial application. One brand of technical grade trichloroethylene is stabilised with 0.22% epichlorohydrin and 0.20% epoxybutane.¹⁵ Epoxidised soyabean oil is used at levels of 1.5–3.0% as a heat and light stabiliser for many PVC formulations, and at higher concentrations as a plasticiser in PVC used in cars, furniture, floor coverings, packaging films, and coatings for wire. The use of epoxy compounds as polymer stabilisers and plasticisers has been reviewed by Port¹⁶ and the stabilisation of 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene was discussed by Szczeszek.¹⁷

OCCUPATIONAL EXPOSURE

Because of the vast quantities handled, by far the highest risk of exposure to epoxides will be in the work place where these compounds are produced or used.

Propylene oxide and in particular ethylene oxide

are widely used as sterilising agents (table 1). Despite other advantages, the time for sterilisation and aeration with these gases is much longer than for some other methods and has been described as more difficult to control,¹⁸ thus increasing the likelihood of exposure.¹⁹

Workers come into contact with epoxide stabilisers in chemicals that they handle in large quantities—for example, in trichloroethylene used for degreasing machinery and drycleaning.

Although there is little information on the number of people occupationally exposed or the concentrations handled, production figures show that many employees may be affected in many industrial situations, and that the concentrations of chemicals may be significant for chronic, if not acute, exposure.

Threshold limit values have been set for several epoxides²⁰ but even if these values, which vary from country to country, were adhered to, they have usually been set on the basis of irritant properties and other obvious clinical symptoms, with little consideration for potentially more serious long-term toxic effects.

Exposure may be reduced by proper ventilation, protective clothing, respirators, alarm systems, and personnel monitoring. Segal *et al*²¹ have described a personal monitoring badge capable of detecting direct alkylating agents such as diepoxybutane and glycidaldehyde in the lower ppb range. The toxicity, hazards, and safe handling of materials used in the epoxy resin industry have been discussed by Borgstedt and Hine.²²

Table 2 Examples of non-occupational exposure to epoxides

Epoxide	Occurrence	Examples of exposure
Cholesterol epoxide	Naturally occurring in vivo	Isolated from human skin after irradiation with a mercury arc lamp or ultraviolet ^{23, 24}
Dieldrin	Pesticide	Intake by occupationally exposed workers 0.72-1.10 mg/man/day compared with 0.025 mg/man/day for the general population; trace amounts in neonates and low concentrations in human milk; barely detectable in air, but found in rain water, soil (retention is highest with high organic content), and some drinking and surface waters; trace amounts in foods ²⁵
Endrin	Pesticide	Much less persistent than dieldrin, so not stored in large amounts in plant and animal tissue, rarely found in food ²⁵
Ethylene oxide	Sterilised products	In rubber catheters after 48-h aeration still 0.1 mg/g and in plastic catheters 0.4 mg/g ²⁶ ; some sterilised pharmaceutical plastics cause cell culture toxicity ²⁷ ; polyethylene, gum rubber, and plasticised PVC take up high concentration: of EO ²⁸ ; polyethylene and Teflon appear to need very little aeration, natural rubber about 90 min and PVC about 6.5 h to show no toxicity ²⁹ ; accidental exposures reported with disposable gloves, anaesthesia masks, gauze dressings, hospital linen, and footwear ³⁰
	Sterilised food	Residues in foods such as spices, cocoa, flour, dried egg powder, desiccated coconut, dried fruits, and dehydrated vegetables ³¹ ; levels in bread sterilised in sealed plastic bags were negligible after 3 days; meat meal contained traces after 24 h; fish and bone meal were free after 12 h ¹⁰
	Cigarettes	Commercial charcoal filters remove ethylene oxide from smoke in various brands of cigarettes ³²
Cis-9, 10-epoxystearic acid	Naturally occurring	Glyceride is a constituent of the oil of sal nuts from the tree <i>Shorea robusta</i> ; also found in some malvaceous seed oils; acid has been found in several sunflower seed oil samples (up to 0.5%) after prolonged storage ¹⁰
Fusarenon X	Naturally occurring mycotoxin	Formed by fungi such as <i>Fusarium nivale</i> , <i>F. episphaeria</i> , <i>F. oxysporum</i> , and <i>Giberella zaeae</i> , which are found worldwide as plant pathogens; strains have occasionally been isolated from scabbed wheat grains in Japan where 20% of domestic wheat and barley samples are contaminated by such fungi ¹⁰ ; <i>Fusarium</i> sp found on wheat, barley, and oat kernels grown in Canada ³³ ; many species very common in rice fields of California ³⁴
Heptachlor epoxide	Pesticide	0.01-0.2 ppm detected in agricultural soils and values at the lower end of this scale in city soils; negligible quantities of both compounds in surface waters; <1 ppm of epoxide detected in food; in UK in mid-60s, epoxide in total diet was calculated as generally less than 0.0005 mg/kg body weight; heptachlor was not detected in food; traces of epoxide found in adipose tissue of general population in most countries; also found in blood and fat of stillborn infants and in human milk ²⁵
Heptachlor		Residues in sterilised materials such as cellulose acetate film, wood shavings, paper cups, rubber tubing, nylon film, polycarbonate film, and polyvinylidene chloride film after 3-h aeration were about 2000 mg/kg ³⁵ ; also residues of more than 1500 mg/kg in some polyethylene products
Propylene oxide (PO)	Sterilised products	Residues in foods as under ethylene oxide and also where used as an insecticidal and fungal fumigant in bulk foods like cocoa, gums, processed spices, starch, and processed nut meats that are further processed to a final food ³¹
	Sterilised foods	No residual PO detected in sucrose, glucose, table salt, and sodium glutamate; dried fish, wheat and soyabean flours contained lowest levels; 1000-2600 mg/kg found in several spices; 4000-9000 mg/kg found in oils, lard, butter, mayonnaise, and shortening; after 24-h aeration no propylene oxide in linseed, soya, and olive oils but still 4000-5000 mg/kg in lard and oleic acid (residue tolerance level in the USA is 700 mg/kg and 300 mg/kg for bulk foods for processing) ¹⁰
Triethylene glycoldiglycidyl ether	Clinically prescribed	Antineoplastic agent given by intravenous injection or into pleural or peritoneal cavity (not active by mouth) ¹⁰

NON-OCCUPATIONAL AND CLINICAL EXPOSURE

Non-occupational and clinical exposure will generally be to much lower levels than in industrial settings but may be more difficult to identify (table 2). The most obvious non-occupational exposure occurs as a direct result of the industrial use of epoxides—for example, after accidental release of large quantities into the atmosphere or from continuous low levels escaping from the factory. The possible occurrence of epoxides in air pollution has been discussed by Kotin and Falk³⁶ and Van Duuren.³⁷ Members of a worker's family may be exposed if he returns home without washing or changing his clothes.

The use of sterilised products such as wrappings, tubings, medical equipment, and food, which contain residual amounts of ethylene oxide or propylene oxide, also results in exposure. Whitbourne and West¹⁸ showed that although many materials can absorb up to 30 000 ppm of the gas during sterilisation, with adequate aeration before use the gas was eluted from almost all of the hundreds of materials tested. Nonetheless, there are still many reports of accidental exposure to residual gas in sterilised items.³⁰ An additional problem with foods is the formation of glycol or halohydrin residues. Since gaseous sterilisation requires the presence of some moisture to be effective, glycol formation would seem inevitable. Both ethylene oxide and propylene oxide also combine with chlorine from the natural inorganic chloride content of food, forming the corresponding chlorohydrins.²⁷ These chlorohydrins are non-volatile and chemically unreactive as they persist through normal food-processing conditions. They are, however, toxic and mutagenic.^{38, 39}

A reasonably efficient way of contaminating air, soil, water, and food, and therefore eventually man is by the widespread use of non-biodegradable pesticides. Some of these are epoxides or compounds that are readily converted to epoxides in the soil, such as aldrin and its epoxide dieldrin, and heptachlor and heptachlor epoxide. The use of some of these compounds, however, is now restricted or banned in many countries.

The formation of epoxides as a result of heating certain vegetable oils in air during cooking has also been reported.^{40, 41}

Epoxides occur naturally in some plant oils used in the food industry, and as mycotoxins. Triglycerides containing epoxy fatty acids are well distributed in seed oils of plant families such as the Compositae, Dipsaceae, Euphorbiaceae, and Malvaceae.¹⁰ Mycotoxin epoxides, such as the 12,13-epoxytrichothecenes, produced by various species of *Fusarium*, *Myrothecium*, *Trichoderma*, and *Cephalosporium*, are found in foodstuffs for animal and

human consumption.^{42, 43} Nearly all the trichothecenes show high biological activity. In a review of steroid epoxides in biological systems, Kadis⁹ listed the naturally occurring epoxides that may be involved in steroid metabolism.

Several epoxides have been tested as anticancer agents because of their cytotoxic effects,^{2, 44} and one that has been used clinically is triethylene glycol diglycidyl ether.¹⁰

Occurrence and exposure—potential epoxides

In addition to the industrial and other environmental epoxides already discussed there is a growing awareness of their role as metabolic intermediates. When an unsaturated compound enters the body, one means of making it more soluble and therefore more readily excretable is to hydroxylate it by first adding an oxygen across the double bond to form an epoxide ring. This metabolic route has now been shown for many polyaromatic hydrocarbons, such as benzo(a)pyrene, benz(a,h)anthracene, and phenanthrene, and there is growing evidence that it occurs in many of the simpler aliphatic molecules such as vinyl chloride and trichloroethylene.⁴⁵ In most cases the epoxide metabolite appears to be much more toxic than the parent compound. Therefore, for completeness, it is also necessary to consider exposure to potential epoxides.

PRODUCTION AND USES

Table 3 lists a few examples of industrial unsaturated compounds that may be metabolised to epoxides in vivo, along with their uses and production figures. As with epoxides themselves, vast quantities of these potential epoxides are used in a wide range of industrial processes, again affecting many workers.

OCCUPATIONAL EXPOSURE

Styrene, which can be metabolised to styrene oxide, is one of the most widely used raw materials for plastics. Barsotti *et al*⁴⁶ reported levels of almost 200 ppm during polymerisation processes and 20-50 ppm for other processes. It has been estimated that about one-tenth of the styrene used during the curing of polyester resins evaporates into the atmosphere. Peak levels during laminating may exceed 500 ppm.⁴⁷ Heating commercial polystyrene, during cutting for example, may give off styrene in amounts from several hundred to a few thousand ppm.^{49, 50}

The National Institute for Occupational Safety and Health, USA, estimated that 282 653 workers might be exposed in various industries in the United States to significant amounts of trichloroethylene. In Sweden some 10 000 workers have been "more or

Table 3 *Industrial uses and production figures—potential epoxides*^{10 11 25 46 47}

Potential epoxides	Industrial uses	Approximate quantity produced (country, year)
Acrylonitrile	Synthetic rubber, plastics, organic synthesis	689 × 10 ⁶ kg (USA, 1976) 744 × 10 ⁶ kg (USA, 1977)
Aldrin	Insecticide for crops, termite control	4.5 × 10 ⁶ kg (USA, 1971)
Benzene	Chemical intermediate in manufacture of cyclohexane, ethylbenzene, phenol, maleic anhydride, detergent alkylate, aniline, dichlorobenzenes, DDT; solvent in paint manufacture and rubber cements	4812 × 10 ⁶ kg (USA, 1976) 5102 × 10 ⁶ kg (USA, 1977)
Butadiene (1,3-)	Rubber industry	1474 × 10 ⁶ kg (USA, 1976) 1447 × 10 ⁶ kg (USA, 1977)
Ethylene	Fuel in welding; anaesthetic; added to manufactured gas as an illuminant; conditioning and colouring of fruits and blanching of vegetables	9991 × 10 ⁶ kg (USA, 1976) 11 × 10 ⁶ kg (USA, 1977)
Heptachlor	Insecticide for crops (use now restricted)	2.7 × 10 ⁶ kg (USA, 1971)
Propylene	Chemical intermediate	4426 × 10 ⁶ kg (USA, 1976) 5696 × 10 ⁶ kg (USA, 1977)
Styrene	Manufacture of a wide variety of elastomers and plastics; solvent and crosslinking agent for polyester resins	2844 × 10 ⁶ kg (USA, 1973) ⁴⁶ 2032 × 10 ⁶ kg (USA, 1975) ⁴⁷ 2857 × 10 ⁶ kg (USA, 1976) 3093 × 10 ⁶ kg (USA, 1977)
Trichloroethylene	Vapour degreasing of fabricated metal parts; chain terminator for PVC production; drycleaning; solvent in textile industry; extractant in food processing (decaffeinated coffee—now discontinued in USA); chemical intermediate; anaesthetic	9 × 10 ⁶ kg (Japan, 1974) 193 × 10 ⁶ kg (USA, 1974)
Vinyl chloride	Production of vinyl chloride homopolymer and copolymer resin; production of methyl chloroform; additive to specialty coatings; component of certain propellant mixtures in pesticides and hair sprays (now discontinued); in the past as a refrigerant, extraction solvent for heat-sensitive materials, intermediate in synthesis of sulphur drugs	> 2.6 × 10 ⁶ kg (USA, 1974) ¹⁷ 2.6 × 10 ⁶ kg (USA, 1976) 2.6 × 10 ⁶ kg (USA, 1977) 7.0 × 10 ⁶ kg (expected worldwide, 1978)

Table lists a few examples of unsaturated compounds, which are widely used in industry, and for which there is evidence to suggest that they are metabolised to epoxides *in vivo*.

less constantly" exposed to trichloroethylene during the past 20 years of its production and use.⁵¹ About 5000 medical, dental, and hospital personnel in the United States may be routinely exposed when trichloroethylene is used as an anaesthetic.⁵² The range of concentrations in an operating theatre under routine working conditions was found to be 5.5-550 mg/m³ (1-103 ppm) near the anaesthetist, and 1.6-8 mg/m³ (0.3-1.5 ppm) near the surgeon.^{53 54} The anaesthetic grade of trichloroethylene, unlike the technical grade solvent, does not appear to be stabilised by epoxides.

Vinyl chloride is another potential epoxide, and world-wide employment in the monomer and PVC industries is over 70 000 workers. Those employed in industries making use of PVC are believed to number millions.⁵⁵ Possible means of exposure during production of PVC have been described.⁵⁶ Of all the compounds mentioned in this review, however, the hazards of vinyl chloride are probably the best recognised.

NON-OCCUPATIONAL AND CLINICAL EXPOSURE (see table 4)

Again the most obvious environmental exposure occurs as a direct result of industrial production and use. Large populations, living in close proximity to industries such as those manufacturing vinyl chloride and PVC, may be at risk. One study in Los Angeles County in 1966 estimated that hydrocarbon emissions into the atmosphere from organic solvent usage

comprised more than 20% of all hydrocarbon emissions. Of the estimated 590 tons emitted each day, 88 tons were attributed to halogenated hydrocarbons, including trichloroethylene and vinyl chloride.⁵⁷

More general atmospheric pollution results from traffic exhaust, various other types of combustion, cigarette smoke, and the use of aerosols. Benzo(a)pyrene is an obvious example of a potentially toxic air pollutant that is metabolised to an epoxide. Human exposure to polyaromatic hydrocarbons has been reviewed by Bridbord *et al.*⁵⁸

The general population may be exposed to several potential epoxides in various consumer products—for example, to styrene or vinyl chloride leaching into food from plastic containers. Lloyd *et al.*⁵² have listed American consumer goods containing trichloroethylene. Cigarettes are also a source of some compounds that can be metabolised to epoxides.

When taking clinically prescribed drugs, an individual may be receiving a deliberate daily dose of mg quantities of a toxic chemical, and the risks of the drug must be weighed against the benefits. Many clinically used drugs are now known to be metabolised to epoxides, and drugs with structural features prone to metabolic oxidation are abundant (table 4). The formation of these epoxides *per se* may not be a problem in terms of toxicity, mutagenicity, or carcinogenicity, but, as will be discussed in more detail in a later section, may be significant

Table 4 Examples of non-occupational exposure to potential epoxides

Potential epoxide	Occurrence	Examples of exposure
Aflatoxin	Naturally occurring mycotoxin	Common contaminants of feedstuffs, especially peanuts, peanut meal, and grain crops during harvesting and storage; responsible for outbreaks of mycotoxicosis in many species of animals and in humans ⁵⁹
Aldrin	Pesticide	Epoxidised to dieldrin in soil and conversion also occurs readily in plants and animals; very small amounts found in soil and water, but rarely found in foods because of epoxidation ²³ ; application of up to 25 lb/acre of corn soil resulted in retention of 10%, mainly as dieldrin, after 4 yr ⁶⁰
Benzo(a)pyrene	Atmospheric	Constituent of automobile exhaust, city smog and charcoal-cooked foods ⁶¹ ; 2000 tons/day put into the air in the USA
Clinical drugs	Cigarettes Prescribed	0.2-12.2 µg/100 cigarettes ⁶²⁻⁶⁴ Drugs for which epoxide metabolites have been reported: allobarbitol, secobarbital, alphenal, protriptyline, cyclobenzaprine, carbamazepine, cyproheptadine, phylloquinone or vitamin K ₁ , some contraceptive steroids ⁶⁵
Styrene	Consumer products	Drugs for which terminal metabolites indicate metabolism via an epoxide: diethylstilboestrol, diphenylhydantoin, phenoximide, phenobarbital, mephobarbital, metaqualone, lorazepam, imipramine, acetanilide ⁶⁵
Trichloroethylene	Consumer products	Various plastic items, eg polystyrene foam cups or food containers can contain 80-3600 ppm, with 0.02-35 ppm leaching into water, 50% alcohol and yogurt ⁶⁶
Vinyl chloride	Anaesthetic Atmospheric	List of American goods containing TCE ⁶⁷ Maximum of 60 000 patients a year ⁶⁷ Total escaping to atmosphere in USA is 100 × 10 ⁶ kg/yr; more than 1 ppm less than 10% of the time in residential areas close to plants; maximum concentration 33 ppm at 0.5 km from centre of plant; as a propellant in aerosols (now discontinued), a 30-sec burst could give an air concentration of 400 ppm for several hours
	Water	Detected in some municipal water supplies ⁶⁸⁻⁶⁹
	Consumer products	Usually 1-5 ppm, but can be as high as 20 ppm, found in a wide variety of foodstuffs packed in PVC containers ⁷⁰⁻⁷¹
	Cigarettes	Very low concentrations (up to 30 ppb) measured in cigarette smoke ⁷²

when more than one epoxide is present at the same time. As already mentioned trichloroethylene is used as an anaesthetic so that a few patients are exposed for a relatively short time to a high dose of this potential epoxide.

Aflatoxins are produced by fungi of the genus *Aspergillus* and are common contaminants of feedstuffs. Aflatoxin B₁ (one of the most potent hepatocarcinogens known) is an example of a naturally occurring fungal constituent that is not itself an epoxide, but for which there is some evidence to suggest that it may be metabolised via this intermediate.⁷³⁻⁷⁶ Although aflatoxin B₁-2,3-epoxide is a likely candidate for the carcinogenic or acutely toxic lesion or both, there are still several reservations about this hypothesis.⁷⁷

Clearly, non-occupational exposure to both epoxides and their precursors, mainly by inhalation and ingestion, varies enormously according to several factors affecting the individual and his environment, and total exposure from several sources could be quite extensive.

Toxicology

The toxicology and pharmacology for most epoxides is far from fully investigated, and there is little biochemical information on the mechanisms of toxicity, in particular of the type that allows predictions to be made for new compounds.

Absorption through the skin, inhalation, or ingestion can lead to a wide range of toxic effects in

both man and experimental animals, including irritation of surface tissues; sensitisation; changes in liver function, in the circulating blood, and in bone marrow; mutagenicity; and carcinogenicity.

CARCINOGENICITY

Because of their strong electrophilic reactivity and the fact that they are alkylating agents, epoxides have been considered for carcinogenicity. As early as 1950, Boyland⁷⁸ suggested that the series of metabolic phenols and dihydrodiols formed from polyaromatic hydrocarbons might be secondary products of metabolically formed epoxides and that these epoxides might be intermediate in tumour production. Several epoxides have been tested, and some of them have given positive results in animal experiments. Results for many compounds are inconclusive, however, either because of inadequacies in experimental design or conflicting data. There are also quite a few epoxides for which results are negative. Therefore, apart from grouping together those that give consistently positive results (table 5a), no attempt has been made to divide the other tested epoxides into carcinogens or non-carcinogens (table 5b). Arene oxides are not included as the publications in this area are too vast. Elegant studies now implicate 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene as a major ultimate mutagenic and carcinogenic metabolite of benzo(a)pyrene.¹⁰⁰⁻¹⁰⁵ Cavalieri *et al*¹⁰⁶ have listed some polycyclic compounds whose carcinogenicity may be mediated by an epoxide.

The conclusions drawn must be qualified by consideration of such variables as species, strain, sex, dose, route of administration, and vehicle, and allowances made for the possibility of a long latent period. Also there is still no reliable method for extrapolating results from animal experiments to man. With all the compounds that Van Duuren's group found to be carcinogenic, high doses normally had to be given to produce significant tumour response, implying that they are weak carcinogens (see table 5 for refs). He considered that perhaps because of their high reactivity they are readily degraded *in vivo* and do not reach target sites in organs remote from the site of treatment, even when given in high doses.

There is no conclusive evidence for the development of cancer in man as a result of exposure to epoxides *per se*, although recently a warning was issued by the International Federation of Chemical Energy and General Workers' Union in Geneva, linking ethylene oxide with leukaemia. This warning followed a report by Hogstedt *et al*¹⁰⁷ describing three cases of leukaemia and a generally higher incidence of cancer in a relatively small group of workers exposed to several chemicals including ethylene oxide.

Any hazard due to epoxy resins would most likely stem from contact with the skin. Hine *et al*⁹³ commented that cancers of the skin are rarely produced by single contact and there is invariably a long latent

Table 5 *Epoxides tested for carcinogenicity*

Compound	Species	Route of administration	Carcinogenicity	Reference
<i>(a) Epoxides that are positive carcinogens in animal experiments</i>				
Diepoxybutane	Mouse	Skin	+	McCammon <i>et al</i> ⁷⁹
	Rat	SC	+	"
	Mouse	Skin	+	Weil <i>et al</i> ^{80*}
	Mouse	?	+	Kotin and Falk ³⁶
	Mouse	IP	+	Shimkin <i>et al</i> ^{81*}
	Mouse	Skin	+	Van Duuren <i>et al</i> ^{82*} 83
	Mouse	SC	+	Van Duuren <i>et al</i> ⁸⁴
	Rat	SC	+	"
	Rat	GF	—	"
	Mouse	Skin	+	Van Duuren <i>et al</i> ^{82*} 83
Both DL and meso 1,2,3,4-diepoxybutane are carcinogenic in mice by skin application and the DL racemate gives +ve results in rats and mice by SC injection. The L isomer is carcinogenic in mice by IP injection				
1,2,5,6-diepoxyhexane	Mouse	?	+	IARC ^{10*}
	Mouse	Skin	—	Kotin and Falk ³⁶
	Mouse	SC	+	Van Duuren <i>et al</i> ⁸³
	Mouse	SC	+	Van Duuren <i>et al</i> ⁸⁴
	Rat	SC	?+	"
Diethyl-2,3-epoxypropylphosphonate	Mouse	Skin	+	Van Duuren <i>et al</i> ^{85*}
	Mouse	SC	+	Van Duuren <i>et al</i> ⁸⁶
	Mouse	Skin	—	Van Duuren <i>et al</i> ^{87*}
	Mouse	SC	(+ 2 stage)	"
Epichlorohydrin	Mouse	IP	+	"
	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
	Mouse	?	+	Kotin and Falk ³⁶
	Mouse	SC	+	Van Duuren <i>et al</i> ⁸⁶ 88
	Mouse	Skin	—	Van Duuren <i>et al</i> ^{87*}
Condensation product of epichlorohydrin with 4,4'-isopropylidene diphenol (8.1% epoxy O)	Mouse	SC	+	"
	Mouse	?	+	Kotin and Falk ³⁶
Carcinogenic in mice by SC injection and also active as an initiator in this species				
Glycidaldehyde	Mouse	Skin	+	IARC ^{10*}
	Mouse	SC	+	Van Duuren <i>et al</i> ⁸³
	Rat	SC	+	Van Duuren <i>et al</i> ⁸⁴
	Rat	GF	—	"
	Rat	SC	+	"
	Mouse	Skin	+	Van Duuren <i>et al</i> ⁸⁹
	Mouse	Skin	+	Van Duuren <i>et al</i> ^{85*}
Carcinogenic in mice by skin application and in rats by SC injection				
Vinyl cyclohexene diepoxide	Mouse	Skin	+	IARC ^{10*}
	Rat	IP	+	Rose <i>et al</i> ⁹⁰
	Mouse	Skin	+	"
	Mouse	?	+	Weil <i>et al</i> ^{80*}
	Mouse	Skin	+	Kotin and Falk ³⁶
	Rat	GF	—	Van Duuren <i>et al</i> ^{82*}
Carcinogenic in mice by skin application				
IARC ^{10*}				

Table 5—cont

Compound	Species	Route of administration	Carcinogenicity	Reference
<i>(b) Epoxides for which the carcinogenic evidence is inconclusive or negative</i>				
bis-(2,3-epoxycyclopentyl) ether	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
bis-(3,4-epoxy-6-methylcyclohexyl-methyl) adipate	Mouse	Skin	—	"
bis-(2-(2',3'-epoxy-2'-methylpropoxy)ethyl) ether	Mouse	Skin	—	"
bis(2,3-epoxy-2-methylpropyl) ether	Mouse	Skin	+	"
bis(2,3-epoxy-2-methylpropyl) ethyl of carbowax 200	Mouse	Skin	—	"
1,4-bis(2,3-epoxypropoxy) benzene	Mouse	?	+	Kotin and Falk ³⁶
2,2-bis(p-(2,3-glycidyloxy)phenyl) propane	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
Cyclohexene epoxide	Mouse	?	—	Kotin and Falk ³⁶
	Mouse	Skin	—	Van Duuren <i>et al</i> ⁸³
	Rat	SC	—	Van Duuren <i>et al</i> ⁸⁹
Dicyclopentadiene dioxide	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
Endo-dicyclopentadiene dioxide	Mouse	Skin	—	"
Dieldrin	Mouse	Oral	+	Thorpe and Walker ⁹¹
				Walker <i>et al</i> ⁹²
1,2,5,6-diepoxyoctane	Mouse	Skin	—	Van Duuren <i>et al</i> ⁸³
1,2,9,10-diepoxydecane	Mouse	?	—	Kotin and Falk ³⁶
Diepoxydihydromyrcene	Mouse	?	+	"
1,2,6,7-diepoxyheptane	Mouse	Skin	? +	Van Duuren <i>et al</i> ⁸³
1,2,3,4-diepoxy-2-methylbutane	Mouse	?	—	Kotin and Falk ³⁶
1,2,8,9-diepoxyonane	Mouse	?	+	"
1,2,7,8-diepoxyoctane	Mouse	?	—	"
	Mouse	Skin	+	Van Duuren <i>et al</i> ^{85*}
1,2,4,5-diepoxyptane	Mouse	?	—	Kotin and Falk ³⁶
	Mouse	Skin	+	Van Duuren <i>et al</i> ⁸³
Diepoxy piperazine	Mouse	IP	+	Shimkin <i>et al</i> ^{81*}
9,10,12,13-diepoxy stearic acid	Mouse	Skin	—	Van Duuren <i>et al</i> ^{82*}
Diglycidyl ether (di-2,3-epoxypropyl ether)	Mouse	Skin	+	McCammon <i>et al</i> ⁷⁹
	Rat	SC	—	"
	Mouse	?	+	Kotin and Falk ³⁶
Diglycidyl ether of disubstituted glycerine (EPON 562)	Mouse	Skin	+	Hine <i>et al</i> ^{93*}
	Rabbit	Skin	—	"
	Rat	SC	+	"
Modified bis-phenol diglycidyl ethers	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
			+	"
Diglycidyl resorcinol ether	Mouse	Skin	+	McCammon <i>et al</i> ⁷⁹
(1,3-bis(2,3-epoxypropoxy) benzene)	Rat	SC	+	"
	Mouse	?	—	Kotin and Falk ³⁶
	Mouse	Skin	—	Van Duuren <i>et al</i> ⁸³
McCammon's study is not fully reported and cannot therefore be evaluated				IARC ^{10*}
Diisobutylene oxide	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
Di(isodecyl) 4,5-epoxycyclohexane-1,2-dicarboxylate	Mouse	Skin	—	"
2,5-dimethyl-1,2,5,6-diepoxyhex-3-yne	Mouse	SC	+	Van Duuren <i>et al</i> ^{87*}
	Mouse	Skin	—	"
Dodecane epoxide	Mouse	?	+	Kotin and Falk ³⁶
Endrin	Fish	Bath water/oral	—	Eller ⁹⁴
Epoxidised 2-ethylhexyl ester of tall oil fatty acid	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
Epoxidised soyabean oil	Mouse	Skin	—	"
	Mouse	Oral	+	Kotin and Falk ³⁶
1,2-epoxybutane	Mouse	Skin	—	Van Duuren <i>et al</i> ^{85*}
1,2-epoxybutene-3	Mouse	Skin	+	Van Duuren <i>et al</i> ^{82*}
1,2-epoxy-3-caproyloxypropane	Rat	SC	+	Walpole ^{95*}
Epoxycyclooctane	Mouse	Skin	—	Van Duuren <i>et al</i> ⁸³
1,2-epoxy-3-(2,4-dinitrophenyloxy) propane	Rat	SC	—	Walpole ^{95*}
3,4-epoxy-6-methylcyclohexylmethyl-3,4-epoxy-6-methylcyclohexanecarboxylate	Rat	SC	+	Weil <i>et al</i> ^{80*}
	Rat	SC	+	Van Duuren <i>et al</i> ^{85*}
	This compound is carcinogenic in mice by skin application			IARC ^{10*}
3,4-epoxy-6-methylcyclohexylmethyl ester of tall oil fatty acid	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
2,3-epoxy-2-methylpropyl acrylate	Mouse	Skin	—	"
Epoxypropidine	Mouse	IP	+	Shimkin <i>et al</i> ^{81*}
Epoxy resin B ₁	Mouse	Skin	—	Hine <i>et al</i> ^{93*}
	Rabbit	Skin	—	"
	Rat	SC	+	"
9,10-epoxystearic acid	Mouse	Skin	—	Van Duuren <i>et al</i> ^{82*}
	Mouse	SC	—	Van Duuren <i>et al</i> ⁸⁴
	Rat	SC	—	"

Table 5—*cont*

Compound	Species	Route of administration	Carcinogenicity	Reference
9,10-Epoxyoctadecanoic acid cis trans	Rat	SC	—	Van Duuren <i>et al</i> ⁸⁹
	Rat	SC	—	Swern <i>et al</i> ^{96*}
	Rat	SC	—	"
The limited amount of data do not indicate that the compound is carcinogenic				IARC ^{10*}
Ethylene glycol bis-(2,3-epoxy-2-methylpropyl)ether	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
Ethylene oxide	Rat	SC	—	Walpole ^{95*}
	Mouse	Bedding	+	Reyniers <i>et al</i> ⁹⁷
	Mouse	Skin	—	Van Duuren <i>et al</i> ⁸³
Data do not allow an evaluation of carcinogenicity to be made				IARC ^{10*}
Ethyleneoxycyclohexane	Mouse	Skin	—	Van Duuren <i>et al</i> ⁸³
1-ethyl-3,4-epoxycyclohexane	Mouse	Skin	—	"
Fusarenon-X	Rat	Oral	—	Saito and Ohtsubo ⁹⁸
	Rat	SC	—	"
	Mouse	SC	—	"
Inadequately tested, therefore no assessment possible				IARC ^{10*}
Glycidol	Mouse	Skin	—	Van Duuren <i>et al</i> ^{85*}
Glycidyl acrylate	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
Glycidyl laurate	Mouse	SC	+	Swern <i>et al</i> ^{96*}
Glycidyl oleate	Rat	SC	—	Walpole ^{95*}
	Mouse	SC	+	Swern <i>et al</i> ^{96*}
	Insufficient data for evaluation			IARC ^{10*}
Glycidyl stearate	Rat	SC	+	Walpole ^{95*}
	Mouse	SC	—	Swern <i>et al</i> ^{96*}
	Mouse	SC	—	Van Duuren <i>et al</i> ⁹⁹
There was no significant increase in the incidence of local tumours				IARC ^{10*}
Hexadecene epoxide	Mouse	?	+	Kotin and Falk ³⁶
	Mouse	Skin	+	Van Duuren <i>et al</i> ^{85*}
Hexaepoxysqualene	Mouse	Skin	—	Van Duuren <i>et al</i> ^{82*}
p-hydroxydiphenylamine glycidylether	Rat	SC	+	Walpole ^{95*}
Limonene dioxide	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
	Mouse	?	+	Kotin and Falk ³⁶
Limonene monoxide	Mouse	Skin	—	Van Duuren <i>et al</i> ⁸⁵
Methyl diepoxydiallyl acetate	Mouse	?	+	Kotin and Falk ³⁶
Octylene epoxide	Mouse	?	+	"
4-oxatetracyclo-(6.2.1.0 ² . 7 ⁰³ . 5)-undecan-9-ol	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
Propylene oxide	Rat	SC	+	Walpole ^{95*}
	This compound was carcinogenic in a limited study in rats by SC injection. It produced local sarcomas.			IARC ^{10*}
Styrene oxide	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
	Mouse	?	+	Kotin and Falk ³⁶
	Mouse	Skin	+	Van Duuren <i>et al</i> ^{82*}
No significant increases in the incidence of skin tumours were observed				IARC ^{10*}
1,2,4,5,9,10-triepoxydecane	Mouse	Skin	+	Van Duuren <i>et al</i> ^{87*}
	Mouse	SC	+	"
Triethyleneglycoldiglycidyl ether	Mouse	IP	+	Shimkin <i>et al</i> ^{81*}
	Carcinogenic in a limited study in mice by IP injection			IARC ^{10*}
Triisobutylene oxide	Mouse	Skin	—	Weil <i>et al</i> ^{80*}
1,1,3-tris(p-glycidyloxy)phenylpropane	Mouse	Skin	—	"
Vinylcyclohexene monoxide	Mouse	Skin	—	"
(1-vinyl-3,4-epoxycyclohexane)	Mouse	Skin	—	Van Duuren <i>et al</i> ⁸³

SC—subcutaneous; IP—intraperitoneal; GF—intragastric feeding.

* References that include structures.

period. As judged from the few authenticated cases of skin cancer of environmental origin, prolonged contact and relatively poor industrial hygiene practices would be required for the development of such tumours. Bourne *et al*¹⁰⁸ emphasised that no neoplastic skin changes had been observed in man with any of the compounds used in the epoxy resin industry to that date.

The evidence for potential epoxides as human carcinogens is more convincing. Vinyl chloride and benzo(a)pyrene, both metabolised *in vivo* to

epoxides, are now linked with human liver and scrotal cancer respectively^{109–111} and aflatoxin with its possible epoxide intermediate also appears to be a human hepatocarcinogen.^{112 113}

Animal data for benzene, another potential epoxide, are not conclusive for carcinogenic activity. In man exposure to commercial benzene may result in damage to the haematopoietic system, and the development of leukaemia is suggested by many case reports.^{110 114}

Mice given trichloroethylene by gastric intubation

showed an unusual incidence of hepatocellular carcinomas, but this did not occur in rats.¹⁰ To date there are no published reports of any association between trichloroethylene and cancer in man. Since the announcement by the National Cancer Institute¹¹⁵ of trichloroethylene carcinogenicity in mice, several substitutes have been introduced in degreasing, manufacture of decaffeinated coffee, and other uses. These include 1,1,1-trichloroethane ($\text{Cl}_3\text{C}-\text{CH}_3$), dichloromethane (CH_2Cl_2), tetrachloroethylene ($\text{Cl}_2\text{C}=\text{CCl}_2$), and hexachloroethane ($\text{Cl}_3\text{C}-\text{CCl}_3$). All four could be expected to cause liver damage and dichloromethane might also be a liver carcinogen.¹¹⁶ Tetrachloroethylene has already been shown to produce liver cancer in laboratory mice as well as other toxic effects such as fatty liver, liver enlargement, and abnormal liver function. It also causes kidney damage and neurophysiological effects.¹¹⁷

MUTAGENICITY

With the development of short-term testing techniques such as the Ames test, much of the more recent work on epoxides, particularly the arene oxides, has been devoted to the mechanisms of mutagenicity and carcinogenicity. The most popular methods make use of *Salmonella* variants of the Ames test, *Neurospora crassa*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, or various tissue cultures in which transformation or DNA damage can be observed.

Most epoxides, both aliphatic and aromatic, that have been adequately tested, have proved to be mutagenic in one or more systems.^{10 118} Mutagenic assays give some "false-positive" and some "false-negative" results—that is, they have failed to show mutagenic activity for about 10% of established carcinogens, especially some of the weaker ones, and they have shown mutagenic activity for some chemicals that have not so far induced tumours in animal tests.⁷⁶ Because of the known differences between the metabolism in in-vitro tests and the human body, short-term mutagenicity testing on its own cannot be used to predict accurately carcinogenicity in man. It may serve, however, as a useful warning.

Various types of mutation have been detected, but most of the changes to DNA concern base-pair substitutions, which give rise to genetically transmissible alterations.¹⁰ As well as indications of mutagenicity in human cell cultures exposed to epoxides such as diepoxybutane¹¹⁹ and epichlorohydrin,^{120 121} results from blood samples of workers exposed to epoxides or their precursors indicate that this might also be a problem in vivo. An increase (related to years of exposure) in chromosomal aberrations, in particular chromatid and chromo-

some breaks, was found in blood samples from workers exposed to epichlorohydrin.¹²² Although the increase was just statistically significant, however, all the values were within the normal range. Similar results were obtained more recently by Picciano¹²³ from a larger group of workers. Excess chromosome aberrations were also observed in lymphocytes taken from people occupationally exposed to ethylene oxide.¹²⁴

Workers in the vinyl chloride/PVC industry were shown to have an increased level of chromosomal abnormalities compared with controls, which was related to job category, length of employment, experience of exposure to short-term excursion levels of vinyl chloride, and smoking habits.¹²⁵ Chromosome abnormalities were also found in several workers using trichloroethylene as a degreaser.¹²⁶ Chromosomes from cultured blood lymphocytes of 10 workers occupationally exposed to styrene, showed an increase (11-26% incidence) in aberrations compared to those from normal controls (3% or less incidence).¹²⁷

STRUCTURE-ACTIVITY RELATIONSHIPS

Several attempts have been made to find a structure-activity relationship for epoxides, particularly with respect to carcinogenicity and mutagenicity. This would be valuable in predicting the toxicity of a compound.

Van Duuren¹²⁸ concluded that diepoxides are more often carcinogenic than monofunctional epoxides, and some monoepoxides that he found to be carcinogenic have an additional reactive site—for example, an aldehyde group in glycidaldehyde, a double bond in 1,2-epoxybutene-3, or an unsaturated ring in styrene oxide—so that they could be considered as difunctional. Glycidol, an analogue of glycidaldehyde that lacks the aldehyde group, and the saturated analogues of the other two were inactive. In general the tumour yield was lower with monofunctional compounds than with their difunctional analogues. Consideration was also given to the distance between the epoxy functions and the flexibility of the molecules as these would seem to be relevant in any consideration of cross-linking in DNA. The findings suggested that flexibility of the reactive centres is important for carcinogenicity. Because of the substantial number of monofunctional alkylating agents, however, including some epoxides, Van Duuren concluded that bifunctionality is not a prerequisite for carcinogenic activity.

In a comparison of the mutagenicity of diepoxyoctane, a straight chain flexible diepoxide, with that of its cyclic analogue, diepoxyoctane, Huang *et al*¹²⁹ found that in Chinese hamster lung cells

only the former was genetically active and formed adducts with radiolabelled nucleotides. The differences in chemical and genetic activities between the two compounds were attributed to their molecular conformations and the resulting different flexibilities. This is in agreement with the carcinogenicity results of Van Duuren's group,^{85 128} who found that the straight chain diepoxide was positive, while the cyclic compound was negative.

Henschler and Bonse¹³⁰ looked at the dependence of mutagenic effect on electrophilic activity of epoxides of chlorinated ethylenes. Electrophilicity was high in those having an asymmetric chlorine substitution and comparatively low in others bearing symmetric chlorine residues. These workers concluded that the mutagenic potential of these chlorinated compounds is related to asymmetric substitution and high chemical reactivity. Symmetric substitution results in lower chemical reactivity and non-mutagenicity. The reason for the instability of an asymmetrically substituted epoxide is the preponderance of the electron withdrawal effect of the chlorine atom(s). So far the rule is substantiated by positive carcinogenic effects in animal experiments with epoxides of vinyl chloride, vinylidene chloride, and trichloroethylene.

No completely satisfactory correlation of structure with carcinogenic potency has been developed for the polyaromatic hydrocarbons and their metabolites. The current so-called "bay region" hypothesis, however, suggests that metabolic activation in the angular ring of these hydrocarbons to produce diol epoxides, in which the epoxide oxygen is situated in the bay region, is an important pathway in the conversion of these molecules to biologically active intermediates.¹³¹

TOXICITY

The acute toxicity in terms of LD₅₀ or LC₅₀ in experimental animals has been calculated for several epoxides and is generally higher for the lower molecular weight compounds.^{10 132} The mechanisms of acute toxicity and the cause of death are not clear and have been described vaguely as shock, respiratory arrest, and fatigue of the central nervous system.²² Chronic toxicity can mean loss of body weight, gastric irritation, and slight liver damage.

The most commonly recognised effect of epoxides in man, at least from occupational exposure, is primary irritation of surface tissues, including the skin, respiratory and gastrointestinal epithelia, and the conjunctivae. Many workers complain of dermatitis. Such irritation may result from exposure to solid, liquid, or gaseous epoxides.^{80 133} Direct pulmonary irritation with its sequelae of pulmonary oedema and pneumonia is at least partly responsible

for the toxicity of the more volatile materials. Also important in man and a not uncommon problem in industry is sensitisation. This may cause skin reactions after repeated local exposures, or asthma-like reactions of the respiratory tract after repeated inhalation of vapours.

Biochemistry

To assess the toxicity of a particular compound, one would like to know how it enters the body and is distributed, which are the target organs, whether the compound accumulates or is readily excreted, or whether it is metabolised and if so how?

Equally as important in a consideration of the risks to health from foreign compounds are questions such as: how much of the compound can be tolerated, and how often, before damage occurs? How do interactions between more than one compound alter the picture? How can the damage best be monitored and, most important, how can it be minimised or avoided? To begin to answer these questions some understanding of the biochemistry of the compound in question is necessary.

Absorption and tissue distribution

Because of their lipophilic nature, epoxides should have little difficulty in passing through cell membranes, allowing them to be widely distributed in the body. This appears to be the case in the few studies available—for example, with styrene and styrene oxide,¹³⁴ ethylene oxide,^{135 136} and epichlorohydrin.¹³⁷ Inhaled trichloroethylene vapour is rapidly absorbed through the lungs into the blood stream and distributed throughout the body. It is also readily stored in body fat⁶⁷ as are some epoxide pesticides.²⁵

Metabolism

Most xenobiotics are metabolised to make them more readily excretable. The process may entail oxidation, reduction, or hydrolysis, usually in combination with conjugation. There are several enzymes *in vivo* involved in the formation or biotransformation of epoxides (fig 1) as well as the possibility of non-enzymic reactions. Many arene oxides, for example, are readily converted to corresponding phenols or conjugated to glutathione without the involvement of transferases. The three main types of enzyme are the mixed function oxidases, giving rise to epoxides *in vivo*, the epoxide hydratases, and glutathione-S-transferases, which metabolise epoxides to more readily excretable products. Although the whole process is often referred to as "detoxification" it is worth noting that, particularly as a result of the mixed function oxidase step, the initial metabolite may be converted to a more reactive,

Fig 1 Epoxide metabolism. Possible pathways for formation and biotransformation of epoxides. Part of figure enclosed in box is expanded in fig 2. (For reviews see Oesch,¹³⁸ Booth *et al*,¹³⁹ Bend and Hook,¹⁴⁰ Jerina and Bend,¹⁴¹ and Habig *et al*,¹⁸⁰

and therefore potentially more toxic, compound.

Epoxide hydratase is also generally considered as a means of detoxification since the diol products are usually less toxic and more hydrophilic. The dihydrodiols of some polyaromatic hydrocarbons, however, may retain sufficient lipophilic character to serve again as substrates for microsomal mixed function oxidase, giving rise to the diol epoxides now believed to be the ultimate carcinogenic metabolites¹⁴²⁻¹⁴⁴ (see also references in section on carcinogenicity). Epoxide hydratase may therefore have a dual role—inactivating monofunctional epoxides and providing precursor molecules for dihydrodiol epoxide biosynthesis (fig 1). The liver is the main site for metabolism of xenobiotics, but these enzymes have also been found in other tissues, such as lung, kidney, small intestine, skin, and reproductive organs.

Most of the work on these enzyme systems has been carried out with respect to polyaromatic hydrocarbons,⁸ and of the simpler molecules most information exists for styrene oxide because this is a particularly good substrate. Thus it is not clear what part if any the enzymes play in protecting cells from the effects of smaller aliphatic epoxides such as ethylene oxide, propylene oxide, and epichlorohydrin. Possibly these are disposed of non-enzymically, since from the evidence for epoxide hydratase specificity, small highly reactive molecules do not appear to act as substrates.¹³⁸⁻¹⁴⁵

Metabolism is important in any consideration of the likely effects from exposure to an epoxide or its precursors. For example, the velocity of epoxidation may not be significant if subsequent reactions, such as enzymic and non-enzymic hydrolysis or conjugation with glutathione, proceed much faster than possible reactions with critical targets in the cell. To predict toxicity it would be useful to know the stability/

reactivity of the epoxide, its rate of accumulation in the cell, the proportion that reacts by each of the enzymic and non-enzymic pathways, how much epoxide reacts with cell components, and the dose-response relation for adverse effects. Unfortunately most investigations concentrate on only one particular aspect and to complicate matters further, the relative activities of the enzymes differ between species and between tissues in any one species, and with respect to such factors as strain, sex, and developmental stages, all of which makes it extremely difficult to extrapolate from animal experiments to man.

Binding to nucleic acids and proteins

If epoxides in the cell are not disposed of, then, because of their electrophilic nature, they can combine with nucleophilic centres on nucleic acids and proteins (fig 2), and it is this binding that has been implicated in such toxic effects as mutagenesis and carcinogenesis.¹⁴⁶ Whatever the detailed mechanism of malignant transformation, the production of DNA damage appears to be a key step.¹⁴⁷

Several factors may affect binding ability, such as reactivity and whether the epoxide enters the body or is produced in vivo from a precursor. Some epoxides may be only transient intermediates with no time to bind to cell components because they are metabolised immediately, whereas others are so stable that they are excreted as epoxides, as occurs with some clinical drugs.¹⁴⁸ Those with intermediate reactivity will be of interest in cellular interactions. If an epoxide is absorbed into the body it may react enzymically or non-enzymically before it has time to reach critical sites in the cell. On the other hand, such targets may be much more vulnerable to an epoxide produced in vivo by the mixed function oxidase

Fig 2 Interaction of epoxides with nucleic acids and proteins.

system, although in this case an epoxide hydratase may be on hand to mop up the epoxide as it is produced. The evidence so far tends to suggest that the compounds that are metabolised to epoxides *in vivo* are the more toxic, at least with respect to human carcinogenicity.

Several workers have tried to relate the information obtained from binding studies to the mutagenicity or carcinogenicity of a particular compound. Using a series of polyaromatic hydrocarbons, including benzo(a)pyrene, Brookes and Lawley¹⁴⁹⁻¹⁵¹ found a positive correlation between the carcinogenic power of these substances (presumably their reactive metabolites) and the extent of their binding to DNA in mouse skin. This was subsequently confirmed by Duncan *et al.*¹⁵² Duncan and Brookes,¹⁵³ and Buty *et al.*¹⁵⁴ There was no such correlation between carcinogenicity and binding to protein or RNA.

Van Duuren¹²⁸ pointed out that carcinogenic epoxides such as diepoxybutane react at the N7 position of guanine in DNA but so do apparently non-carcinogenic agents, such as ethylene oxide and propylene oxide. Therefore it is difficult to equate reaction at this site with carcinogenesis. Reaction also occurs with other bases, particularly adenine, although the extent of the reaction is generally less than with guanine.¹⁴⁹ Also not all carcinogens react at N7, and in fact it is now believed that reaction at the N7 position of guanine has a negligible effect on the functioning of DNA, and that nucleophilic substitution at O atoms may lead to the formation of promutagenic bases. Alterations at other sites, however, such as N1 of adenine, may also cause distortions in the double helix which interfere with

base pairing.¹⁴⁷

Several investigators are making use of the interaction of alkylating agents with molecules *in vivo* in an attempt to monitor the exposure of workers to various compounds. Ehrenberg *et al* have pioneered a method that measures the alkylation of the amino acids cysteine and histidine in haemoglobin by compounds such as ethylene oxide.^{155 156 181}

Repair mechanisms

If epoxides bind to DNA and cause damage the cell still has one last line of defence. Since the integrity of DNA is vital to an organism, it is not surprising that some advanced enzyme repair mechanisms have evolved so that damage to DNA can be reversed, removed, or tolerated. DNA repair has been reviewed by several workers including Trosko and Chang,¹⁵⁷ Strauss,¹⁵⁸ and Lehmann and Bridges.¹⁴⁷ Most of the detailed studies have been carried out on bacterial systems, but several mammalian systems have provided valuable information. Again much of the relevant work in this area has been done with arene oxides.

There are several recessively inherited human disorders in which the individuals are more prone to develop cancer.¹⁵⁹ Several of these—namely, xeroderma pigmentosum, ataxia telangiectasia, and Fanconi's anaemia—have been shown to be associated with defects in the DNA repair mechanisms. The ability of the cell to repair lesions in DNA may greatly contribute to the resistance of the cell to transformation so that analysis of the molecular defects in these diseases should help to elucidate the molecular nature of changes responsible for cytotoxicity, mutagenicity, and carcinogenicity.

Genetic and immunological aspects

There are several other aspects of the biochemistry of epoxides about which little is known but which might provide valuable information on how some of the toxic effects of these compounds might be avoided.

One area that is poorly understood is genetic susceptibility to the toxic effects, in particular cancer, caused by some epoxides. Differences in inducibility of aryl hydrocarbon hydroxylase, a mixed function oxidase, in various strains of mice correlate well with susceptibility of the animals to carcinogenesis after exposure to certain polycyclic aromatic hydrocarbons.¹⁶⁰ Studies by Kellermann *et al*^{161 162} on patients with lung cancer indicated that bronchogenic carcinoma is associated with high aryl hydrocarbon hydroxylase inducibility. In other studies, however, this correlation could not be found, although in some cases aryl hydrocarbon hydroxylase inducibility did seem to be genetically determined.¹⁶³ The ability to predict how individuals would react to a particular drug or their susceptibility to cancer after exposure to environmental chemicals would be of obvious benefit.

Another area that might prove extremely useful, but in which little is known with respect to epoxides, is immunology. In a recent study using a non-carcinogenic analogue of dimethylbenzanthracene conjugated to bovine serum albumin, Moolten *et al*¹⁶⁴ were able to induce in guinea pigs the formation of antibodies that bound a variety of polycyclic aromatic hydrocarbon carcinogens and protected tissue culture cells from their toxic effects.

From the preceding biochemical discussion it may be seen that the body under normal conditions is equipped to deal with epoxides. The problem of toxicity will arise when (1) there is an inadequate defence mechanism for a particular epoxide, (2) the organism is exposed to too high a dose, or (3) interactions occur that alter the normal metabolic pathways. The first could arise if the epoxide does not act as a substrate for any of the enzymes, if it reacts with other cell components faster than with the enzymes, or if it gets into one tissue that is less able to cope than another. The third possibility is discussed in more detail below.

"DRUG" INTERACTIONS

For xenobiotics inactivated primarily by hepatic oxidative metabolism there are two important types of interaction. Firstly, one compound may inhibit the metabolism of another and secondly, prolonged administration of a drug such as phenobarbital, which induces the liver microsomal system, can stimulate the metabolism of another compound administered concomitantly. The first possibility may result in toxic concentrations of an active

compound building up in various tissues. In the second case the plasma concentration of a drug may be rapidly decreased below its therapeutic level or a xenobiotic may be converted to reactive intermediates faster than these can be disposed of by the cell.

Although the question of interactions has not been widely recognised with respect to epoxides, there are one or two examples of the type of problem that can arise. The effect of synthetic steroid contraceptives on the metabolism of the epilepsy drug carbamazepine was studied in rats. The latter is metabolised via a stable epoxide, which was shown to be a poor substrate for epoxide hydratase.^{165 166} The steroids increased the formation of carbamazepine-10,11-epoxide by inducing the mixed function oxidase system. This induction appeared to be species-dependent, occurring in mice and rats but not in guinea pigs. Also within a species various steroids behave differently.¹⁶⁷ 10,11-Epoxides of carbamazepine, cyproheptadine, and cyclobenzaprine were found to inhibit the hydration of styrene oxide and the latter two the hydration of benzo(a)pyrene-4,5-oxide in liver microsomes of male rats.⁶⁵ Pachecka *et al*¹⁶⁸ using a different strain of rat, also found that cyproheptadine inhibited epoxide hydratase activity with respect to styrene oxide, but reported that cyclobenzaprine epoxide showed a slight stimulatory effect. In an in-vivo study in rats Salmona *et al*¹⁶⁹ found that carbamazepine increased the hydration, but not the formation, of styrene oxide.

Andersen *et al*¹⁷⁰ investigated the increased toxicity of 1,1-dichloroethylene in the presence of various epoxides and concluded that the latter were interfering with the metabolism of a toxic product of 1,1-dichloroethylene microsomal oxidation.

Thomson *et al*¹⁷¹ considered the problem of high risk of malignancy in workers exposed simultaneously to asbestos and to benzo(a)pyrene inhaled in cigarette smoke. Evidence suggested that trace metals (contaminants associated with asbestos) may play a part in benzo(a)pyrene binding to macromolecules and therefore in its carcinogenicity. Most of the metals tested reduced the extent of binding in microsomal incubations containing exogenous DNA. Iron, however, had the effect of increasing the binding to components other than DNA in vitro.

Intolerance to alcohol in people exposed to trichloroethylene was investigated by Bardoděj *et al*.^{172 173} In adult male volunteers exposure to a combination of trichloroethylene and alcohol impaired mental capacity more than exposure to either alone,¹⁷⁴ and there also appeared to be an increase in toxicity to the liver.^{175 176} Radike *et al*¹⁷⁷ have shown synergistic effects between ingested alcohol and inhaled vinyl chloride in the induction

of angiosarcomas in rats. This is presumably due to ethanol stimulating alcohol dehydrogenase and mixed function oxidase enzymes of the liver, both of which metabolise vinyl chloride.

Discussion

Epoxides are to be found in air, soil, water, food, micro-organisms, and certain biochemical pathways, and in vast quantities in industry. In addition the number of unsaturated organic compounds, from which epoxide formation *in vivo* has been shown, is continually increasing. In fact it has been suggested that for polyaromatic hydrocarbons this is an obligatory step in metabolism. Thus the question is not whether exposure occurs, but which compounds are a threat to health and what levels can be tolerated. The fact that most forms of life, including ourselves, are equipped with enzyme systems to dispose of epoxides suggests that cells are designed to cope with a certain level of these compounds, even if only from internal metabolic pathways. Therefore it is important to identify conditions under which an individual's exposure to epoxides or unsaturated precursors is appreciably increased—for example, in certain industrial settings, after accidents where large quantities are released into the atmosphere, by smoking or taking drugs, or in contaminated food. Although the irritating effects of most of these compounds ought to prevent excessive exposure in the work place, it is not clear from the toxicity data what are the long-term effects of regular exposure to concentrations less than those which cause irritation.

In 1978 halogenated alkyl epoxides were selected as one category of chemicals to which the US Environmental Protection Agency should give priority for epidemiological studies and testing of health and environmental effects under the 1976 Toxic Substances Control Act. Selection is based on such factors as production volume, potential human exposure or widespread environmental distribution, and lack of adequate toxicology studies.¹⁷⁸

Sensitive detection methods are required to identify individuals who have been exposed, and some knowledge of dose-response relations is necessary to predict the likely toxic effects of such exposure. The general clinical effects have been quite well described for several aliphatic epoxides, although there is little on the mechanisms causing such effects as skin irritation, depression of the central nervous system, or damage to the haematopoietic system. There are many epoxides that have not been tested for carcinogenicity, but even for those that have been tested, the data are often inadequate to state conclusively whether they are positive or negative in this respect. There is not yet

enough epidemiological data to indicate convincingly whether epoxides in the environment can act as human carcinogens, although there is positive evidence for several of the potential epoxides. Most epoxides that have been tested for mutagenicity are positive in one or more systems, even those that are consistently negative in animal carcinogenicity tests. Several of these compounds have produced detectable chromosome aberrations in workers' blood. Thus it seems that most epoxides can interact with the genetic material in the cell, although the significance of this *in vivo* is poorly understood.

With respect to structure-activity relations only the most general and preliminary comments can be made. It may be possible to rank the compounds on the basis—the more electrophilic, the more reactive and thus the more potentially toxic. Of the aliphatic epoxides tested, features such as difunctionality and flexibility occurred more frequently among those for which there was some evidence of carcinogenicity.

The biochemical information for epoxides is also incomplete and mostly concentrated in one or two areas, such as enzymology, and on one or two particular compounds—for instance, styrene oxide or benzo(a)pyrene oxides. Tissue distribution has only been studied for a few epoxides so that the question of target organs is not well understood, although some of these compounds do not appear to be highly selective. Much work has been done on the enzyme systems involved in epoxide formation and biotransformation, including their activities in various tissues. Since most studies of this type have been done with polyaromatic hydrocarbons, however, it is not clear what part, if any, the enzymes play in the disposal of the smaller aliphatic epoxides. Another interesting question with regard to the enzymes is that of complementary action. Since there are two major pathways for removing epoxides, involving either epoxide hydratase or glutathione-S transferase, are these enzymes independent or do they complement each other with respect to levels in a particular tissue, levels at different stages of development, or the substrates that they metabolise? There is some evidence for all three of these possibilities.

Several types of binding to cell components have been shown for epoxides *in vitro*, but it is not clear which interactions *in vivo* cause detrimental biological effects. Nor is enough known about repair systems to predict how much damage can be tolerated or even which types of damage can be repaired routinely.

Interactions between different epoxides or between an epoxide and another chemical should not be overlooked. A great deal more information is required, however, to assess the resultant toxicity under these conditions. Is there an increased risk to an individual

who is occupationally exposed to styrene or styrene oxide and who is simultaneously undergoing treatment with one of the drugs listed in table 4? Is a smoker more susceptible than a non-smoker to the toxic effects of an epoxide in the atmosphere? Results from workers in the vinyl chloride industry suggest that this may be the case.¹²⁵ Even if interactions do not enhance the acute toxicity, they may potentiate mutagenic or carcinogenic effects of one of the compounds, a problem that might not become apparent for many years. It is also possible to conceive of interactions having a beneficial effect. For example, if a compound that inhibited the activity of the mixed function oxidase system were present at the same time as a polyaromatic hydrocarbon, the latter may not be metabolised to its toxic intermediate.

Conclusions

Both occupational and non-occupational exposure to epoxides occurs, but most of the data are for compounds metabolised to epoxides rather than for epoxides themselves. Much of the information on toxicity of epoxides is qualitative, with little on mechanism of toxicity or dose-response relations.

Most epoxides are toxic to man to some extent (even if the main industrial problem is only irritation of the surface tissues) and ironically some of the more toxic compounds may be those which are metabolised to epoxides *in vivo*. At present there is no convincing evidence that exposure to epoxides *per se* causes cancer in man, but there is evidence for several unsaturated compounds such as vinyl chloride, benzo(a)pyrene, and aflatoxin.

Not enough is known about the possible long-term effects to state with certainty a safe level of exposure to epoxides or their precursors, and more epidemiological research and sensitive methods for measuring human exposure are required.

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