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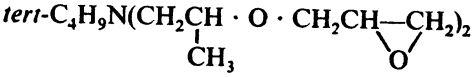
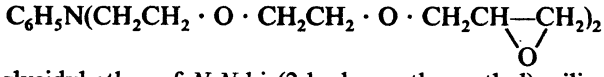
LETTERS TO THE EDITOR

CARCINOGENICITY OF CERTAIN GLYCIDYL DERIVATIVES

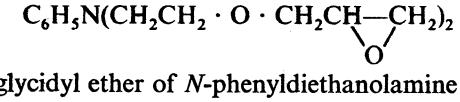
Sir,—Several bi- and monofunctional epoxides, a class of compounds used industrially for a wide variety of purposes, have been shown to be carcinogenic when injected subcutaneously into rats or mice (Walpole, 1958; Hine *et al.* 1958; Weil *et al.* 1963). We recently tested five epoxides, all of the glycidyl type, for carcinogenicity (Fig. 1). These were: Diglycidyl ether of *N,N*-bis(2-hydroxypropyl)*tert*-butylamine (I); diglycidyl ether of *N,N*-bis(2-hydroxyethoxyethyl)aniline (II); diglycidyl ether of *N*-phenyldiethanolamine (III); monoglycidyl ether of *N*-phenyldiethanolamine (IV); *N,N*-diglycidyl-*p*-toluenesulphonamide (monomer and some polymer) (V).



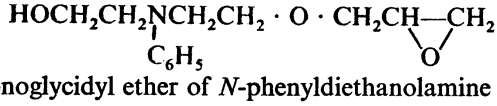
Diglycidyl ether of *N,N*-bis(2-hydroxypropyl)*tert*-butylamine (I)



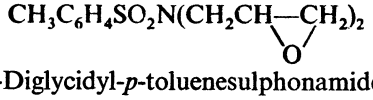
Diglycidyl ether of *N,N*-bis(2-hydroxyethoxyethyl)aniline (II)



Diglycidyl ether of *N*-phenyldiethanolamine (III)



Monoglycidyl ether of *N*-phenyldiethanolamine (IV)



N,N-Diglycidyl-*p*-toluenesulphonamide (V)

FIG. 1. Chemical structures of glycidyl derivatives tested for carcinogenicity.

One hundred male mice of the Chester Beatty Stock strain were randomized into 5 groups of 20 and, from the age of 6 wk onwards, received subcutaneous injections into the right flank at weekly intervals with the compounds dissolved in polyethylene glycol of average molecular weight 400 (PEG 400) or with the latter solvent alone. Details of treatment are given in Table 1. Treatment was continued for 1 yr, except in the case of compound I where injections ceased after 9 wk because of toxicity. One mouse in this group developed

an anaplastic sarcoma in the injection-site area after 10 months. In each of the remaining three test groups, two injection-site sarcomata arose after latent intervals ranging from 10 to 19 months (Table 1). In addition, two benign injection-site tumours, a fibroma and a lipoma, occurred in mice treated with compound II.

Table 1. *Induction of injection-site tumours in groups of 20 Chester Beatty Stock male mice*

Compound	No. of weekly subcutaneous injections*	No. surviving at month			No. of injection-site tumours		Induction time of malignant tumours (months)
		8	12	16	Benign	Malignant	
Polyethylene glycol 400 (control)	52	20	18	11	0	0	
I	9†	12	9	2	0	1‡	10
II	52	18	13	2	2§	2‖	14, 16
IV	52	19	17	6	0	2‖	10, 19
V	52	16	11	7	0	1‡	10

*Each injection consisted of 10 mg compound/0·2 ml of polyethylene glycol 400.
†Treatment stopped because of systemic toxicity and early death.
‡Sarcoma.
§One fibroma and one lipoma.
‖Sarcomata.

When mice died, or were killed, with injection-site tumours, or because they were sick, a full post-mortem examination was carried out. Lung adenomas, hepatomas, generalized and localized lymphomas were seen in all groups, including the control group treated with polyethylene glycol only. There were no obvious differences in the incidence or onset of appearance of these neoplasms between the various groups.

In a second experiment, 24 male rats of the Chester Beatty Stock strain were given subcutaneous injections into the right flank, of 30 mg compound III in 0·5 ml arachis oil weekly for 3 wk. Injections were then suspended for 4 wk because of ulceration at the injection-site before resumption at the level of 15 mg III/0·25 ml arachis oil, which was then given weekly for 44 wk. Of 24 rats, 18 developed sarcomata at the injection-site after intervals ranging from 9 to 17 months. No other neoplasms were encountered. In this experiment there was no control group treated with arachis oil only. However, previous experience, and the experience of others, indicates that no such carcinogenic response would have been expected from the injection of arachis oil alone.

Except in the case of III the carcinogenic responses observed were weak, even though the strain of mouse used is known to be sensitive to the effects of injected carcinogens.

A priori one would expect primary epoxides to be more active as biological alkylating agents than secondary epoxides. If one accepts the contention of Walpole (1958) that "a direct chemical interaction with some cellular component is an essential feature of the carcinogenic process with these agents", then one would expect primary epoxides to be more active as carcinogens. Such evidence as there is (Table 2) from carcinogenicity experiments tends to fulfil this expectation. However the volume of evidence is small, and includes data from experiments on various species of animals given test substances by different routes and in non-comparable doses.

Table 2. *Relationship between carcinogenicity and nature of epoxide groups*

Nature of epoxide	Test species	Route of administration	No. of compounds giving positive results/ no. of compounds tested	Reference
		One alkylating radical		
Primary	Rat	Subcutaneous	7/10	Walpole, 1958
do.	Mouse	do.	1/1	Present experiment
do.	do.	Skin application	0/5	Weil *et al.* 1963
Secondary	do.	do.	0/3	do.
		Two alkylating radicals		
2 Primary groups	do.	Subcutaneous	3/3	Present experiment
do.	Rat	do.	1/1	do.
do.	Mouse	Skin application	2/6	Weil *et al.* 1963
1 Primary and 1 secondary Group	do.	do.	1/2	do.
2 Secondary groups	do.	do.	1/5	do.
2 Primary groups	Rat	Subcutaneous	2/2	Hine *et al.* 1958
Low molecular weight with 2 primary groups	Mouse	Skin application	1/1	do.
High molecular weight with 2 primary groups	do.	do.	0/1	do.
do.	Rabbit	do.	0/2	do.

Considering all the results at present available it would appear that the highest level of carcinogenic activity is to be expected in compounds of relatively low molecular weight with primary rather than secondary terminal epoxy groups. This is perhaps a factor which should be taken into account in the choice of compounds for industrial purposes.

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FOOD LABELLING

Sir,—It is of interest to note that BIBRA's Director, Dr. L. Golberg, has written to the Ministry of Agriculture, Fisheries and Food in connexion with the use of the words 'artificial' and 'natural' in labelling regulations.

The Food Standards Committee, in its report on colouring matters in 1954, made some very tangible points in regard to the relative safety of colourings natural to foods and synthetic substances used for colouring food. Furthermore, careful reading of this report leads to the interesting conclusion that the Committee was concerned to protect the consumer from, among other things, uninteresting food, in other words they recognized the need for consumer appeal and therefore for added colour.

Another interesting point emerging is that although the Committee preferred natural colourings to artificial colourings it is clear that they were considering colourings natural to food. No-one would wish to argue this point and the arguments against the natural versus artificial thesis arise from extrapolation of this concept to all natural colourings. In including cochineal, for example, in its recommendations the Committee were presumably recognizing any risk as being comparable with that of including some of the synthetic dyestuffs for which data were scanty. Perhaps the Report was inadvertently the source of the current misconception. Incidentally I wonder how many of the advocates for natural colourings in food would accept cochineal as a legitimate colouring if they were aware of its natural (!?) origin.

It is indisputable that a natural colouring, and a colouring natural to a food cannot be absolutely excluded from this, could well be toxicologically unacceptable for use in food and it is therefore to be deplored that regulations might be introduced which would perpetuate current misconception.

There is at least one foodstuff on the market which contains a permitted colouring matter of natural origin and which is quite correctly labelled as containing no 'artificial' colouring. So far as I can ascertain the natural ingredient containing the natural colouring is added to this compounded food primarily for colouring purposes. There is nothing wrong in this but why should the legislation encourage manufacturers to use an untested colouring, of variable (because of natural origin) composition in food so as to compete successfully in a misinformed market at the expense of other equally reputable manufacturers who may be using (deadly!) synthetic colourings which comply with carefully drafted specification and which have survived the battles of the toxicological lists (perhaps on the hillsides at Carshalton)?

It seems to me equally inappropriate to require a manufacturer to declare, in a place other than among the ingredients, the presence of a permitted food additive when we are basing our food legislation on permitted lists of substances of demonstrated safety. In other words if it is as safe as to be permitted why the fuss? If not, what is it doing in the food, let alone on the label? For those who recognize that the eating of food, natural or otherwise, is a calculated risk there are the more comprehensive data in the list of ingredients.

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