causes a decrease in the phosphorylation product—ATP. This reduction in available free phosphate would be directly proportional to the reduction in total ATP produced.

The amount of inhibition present in the cloacal bladder and intestinal mucosa of all species indicates that DDT may have a pronounced effect upon absorption of metabolites due to a lack of energy required for active transport across the cellular membrane. In this study there was marked inhibition of intestinal mucosa and cloacal bladder ATPase after *in vitro* treatment with 53 μM DDT. It is possible that treatment with equal concentrations of DDT *in vivo* would cause a serious inability to effectively utilize metabolites.

The action of the lowest concentration (0.53 μM) of DDT in the kidney of all species studied except *Pseudemys scripta* resulted in low remaining (Na^+,K^+)-dependent ATPase activity. Assuming that the (Na^+,K^+)-dependent ATPase activity in the turtle kidney is similar in function to that of other vertebrates, the inability to reabsorb electrolytes from the glomerular filtrate due to enzyme inhibition by DDT may cause serious electrolyte imbalances.

The observed action of DDT in all tissues assayed might be related to the ability of the compound to alter the cellular membrane configuration by binding with the fat portion of the membrane. Since ATPase is a structural part of the membrane, the active site of the enzyme would be altered. Movement of substances by active transport would be blocked. If the uptake of DDT by turtles in a natural environment reaches tissue concentrations equal to those found in this study, the resulting ATPase inhibition may be sufficient to impair organ function.

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Mutagenicity of Ethylene Chlorohydrin. A Degradation Product Present in Foodstuffs Exposed to Ethylene Oxide

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Ethylene chlorohydrin (2-chloroethanol) is mutagenic for *Salmonella typhimurium* in which microorganism it induces mutations of the base-substitution type. This chemical also preferentially inhibits the growth of DNA polymerase deficient bacteria which is taken to indicate an ability to react with the DNA of living cells. In view of the possible presence of ethylene chlorohydrin in foodstuffs exposed to ethylene oxide, the potential hazard of human exposure to this chemical requires examination.

Ethylene oxide (EO) is widely used as a fumigant to sterilize foodstuffs, textiles, medical instruments, and a variety of other objects (Merck Index, 1968; Fishbein, 1969). EO has wide applications in agriculture as a pesticide (Merck Index, 1968; Fishbein, 1969; Fishbein *et al.*, 1970) and in the tobacco industry to shorten the aging process and to reduce the nicotine content of tobacco leaves (see Fishbein, 1969; Fishbein *et al.*, 1970). The toxicity and mutagenic potential of EO are well recognized (Fishbein *et al.*, 1970); however, because of its volatile nature, it has generally been assumed that EO was safe, not leaving a residue (see, however, Fishbein, 1969; Fishbein *et al.*, 1970). It has been found, however, that under conditions for effective fumigation, EO reacted with moisture and chloride ions to form ethylene chlorohydrin (2-chloroethanol), a nonvolatile (bp 129°) toxic substance found in foodstuffs exposed to EO (Wesley *et al.*, 1965; Ragelis *et al.*, 1968; Fishbein, 1969). In the present report it is shown that ethylene chlorohydrin possesses mutagenic properties and that it is presumably able to react with the DNA of living cells. These results confirm and extend the earlier mention of the mutagenicity of ethylene chlorohydrin (Voogd *et al.*, 1972). Because of these findings and the strong positive correlation between mutagenic and carcinogenic potentials (Ames *et al.*, 1973a; Miller and Miller, 1971; Rosenkranz, 1973), it is suggested that the possible health hazards resulting from the presence of ethylene chlorohydrin in foodstuffs be evaluated and that fumigation procedures using EO which keep the level of ethylene chlorohydrin generated to a minimum be devised.

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DESCRIPTION AND RATIONALE OF THE ASSAYS

Mutagenesis Assay. The procedure for detecting revertants to histidine prototrophy was essentially that described by Ames (1971). The *Salmonella typhimurium* strains used (TA 1530, TA 1535, and TA 1538) were kindly provided by Dr. Bruce N. Ames. Strains TA 1530 and TA 1535 are essentially isogenic; they have a base-pair change in the histidine G gene and can be used to detect mutagens causing base-pair changes. In addition, both strains contain the uvr B mutation (Ames, 1971; Ames *et al.*, 1973b). However, strain TA 1535 differs from TA 1530 in that it is a deep-rough strain deficient in cell envelope li-

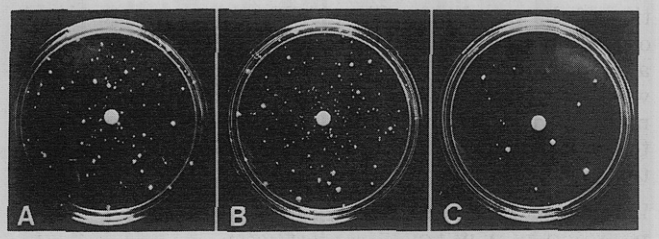

Figure 1. Mutagenicity of ethylene chlorohydrin for *Salmonella typhimurium* strains. Histidine-free nutrient agar plates were inoculated (see text) with strains TA 1530 (A), TA 1535 (B), and TA 1538 (C), respectively. A paper disk containing 10 μl of ethylene chlorohydrin was deposited onto the surface of each plate. The plates were incubated at 37° for 54 hr and then examined for the appearance of histidine-independent colonies (mutants). Note the appearance of mutants in a zone surrounding the ethylene chlorohydrin containing disks in the plates inoculated with *S.typhimurium* TA 1530 and TA 1535 but not TA 1538. This indicates that ethylene chlorohydrin induces base substitution but not frameshift mutations.

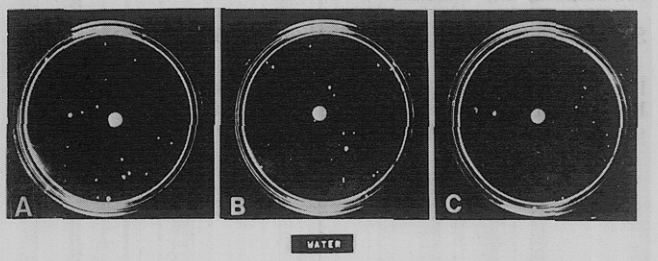

Figure 2. *Control plates for the experiment described in Figure* 1. The procedure used was identical with the one described in Figure 1 except that the filter disks were impregnated with sterile water. A, B, and C refer to strains TA 1530, TA 1535, and TA 1538, respectively.

popolysaccharides which increases its permeability to large molecules (Ames *et al.*, 1973b). Strain TA 1538 has a mutation in the D gene of the histidine operon. It can be used to detect frameshift mutations and it probably results from the deletion of a single-base pair; in addition, it is deep-rough and has the uvr B mutation (Ames *et al.*, 1973b).

For qualitative testing, a sterile paper disk impregnated with the test compound was placed onto the surface of agar plates that already contained the test strain in the top agar layer. This is a slight modification of the procedure of Ames (1971) who deposited the test substance directly onto the surface of the agar; however, it was found that this permitted a more exact amount of test substance to be deposited and it also allowed for more uniform diffusion. The plates were incubated at 37° in the dark for 54 hr whereupon bacterial colonies (revertants) were enumerated. The quantitative assay in which known amounts of the test compound are incorporated into the agar overlay was performed as described by Ames *et al.* (1973a,b).

Assay Using DNA Polymerase-Deficient *Escherichia coli.* This assay is based upon the assumption that cells exposed to agents (mutagens or carcinogens) which alter the cellular DNA attempt to repair this damage. The extent of cellular survival is then dependent among other factors upon growth conditions, concentration, and stability of the test agent and duration of exposure. The enzyme DNA polymerase I has an essential role (Rosenkranz, 1973) in the DNA repair process and therefore cells lacking this enzyme (pol A_1^- cells) are more sensitive than their pol A^+ parent to the growth inhibitory action of agents that react with the cellular DNA (Slater *et al.*, 1971). Based upon these findings a bioassay procedure to detect agents which react or otherwise alter the cellular DNA was devised (Slater *et al.*, 1971). The procedure described previously (Slater *et al.*, 1971) was used except that the incubation time was reduced to 8 hr (37°) and zones of inhibition were determined. Determination of the size of the zone of inhibition was repeated after the plates were kept at 23° for an additional 24 hr.

Table I. Mutagenicity of Ethylene Chlorohydrin for *Salmonella typhimurium* **Strains**

Chemical	Amount per plate, μl	Revertants per plate TA 1530	TA 1535	TA 1538
Ethylene chlorohydrin	5	75	82	14
	10	125	143	13
	15	169	213	12
	17.5	201	223	13
	20	240	281	11
Methylmethanesulfonate	10	264	104	26
N-Acetoxy-N_2-fluorenylacetamide	2.5 (μg)	23	17	586
H_2O	10	17	14	13

RESULTS

Mutagenesis Assay. Both by the qualitative and quantitative assays, it was found that ethylene chlorohydrin was mutagenic for *Salmonella typhimurium* TA 1530 and TA 1535 but not for TA 1538 (Figures 1 and 2 and Table I) and that this activity was dependent upon concentration. Tests with known mutagens revealed that the assay was functioning properly; thus, methylmethanesulfonate was also mutagenic for TA 1530 and TA 1535 but not for TA 1538, while *N*-acetoxy-N_2-fluorenylacetamide was mutagenic for TA 1538 but not for the other two strains. This confirms earlier findings by Ames and his collaborators (1973a,b).

Effect on the Growth of pol A_1^- Bacteria. Ethylene chlorohydrin preferentially blocked the growth of DNA polymerase deficient *E. coli*, a property it shares with the known mutagens and carcinogens propane sultone, *N*-methyl-*N*-nitroso-*N'*-nitroguanidine, and 4-nitroquinoline *N*-oxide (Table II). On the other hand, methicillin, chloramphenicol, and colistin, which are known to act on cellular structures other than DNA, inhibited the two strains to the same extent (Table II).

The relative inhibitory activities of these substances can be assessed by comparing ratios of the areas of the zones of inhibition on pol A_1^- and pol A^+ strains. These ratios are independent of concentrations provided identical amounts are used for each strain. A ratio of 1.00 is indicative of a lack of activity (*e.g.*, chloramphenicol) while values in excess of 1.00 indicate some preferential inhibition of the pol A_1^- strain. By these criteria, ethylene chlorohydrin has a relative activity which is of the same order of magnitude as that of the mutagens 4-nitroquinoline *N*-oxide and methylmethanesulfonate (Table II).

DISCUSSION

It is shown by two independent procedures that ethylene chlorohydrin is mutagenic and able to alter the DNA of living cells. This confirms the earlier mention (Voogd *et al.*, 1972) of the mutagenicity of this substance. The exact mechanism by which this occurs is as yet unclear. The mutagenic specificity indicates, as expected, that ethylene chlorohydrin induces base substitution but not frameshift mutations; however, confirmation of this must await the results of chemical studies which are presently underway. Preliminary results indicate, however, that exposure of DNA to 2-chloroethanol results in drastic alterations of the physical chemical properties of this biopolymer.

Because elevated levels of ethylene chlorohydrin (1000 ppm) can be found in foodstuffs sterilized with EO (Fish-

Table II. Effect of Ethylene Chlorohydrin on the Growth of DNA Polymerase Deficient *E. coli*[a]

Group	Substance	Amount	Diam. of zones of inhibition (mm)		Relative activity (area pol A_1^-/ area pol A^+)
			pol A^+	pol A_1^-	
I	Ethylene chlorohydrin	10 μl	7.7	9.8	1.62
	Propane sultone	50 μg	11.9	18.9	2.52
	N-Methyl-*N*-nitroso-*N'*-nitroguanidine	50 μg	21.8	30.8	2.00
	4-Nitroquinoline *N*-oxide	50 μg	28.1	34.5	1.51
	Methylmethanesulfonate	10 μl	45	54	1.44
II	Methicillin	30 μg	28.5	28.3	
	Chloramphenicol	30 μg	28.8	28.8	1.00
	Colistin	10 μg	16.1	16.0	

[a] The agents in group I served as "positive controls." They are known to be mutagens and/or carcinogens and to inhibit the growth of DNA polymerase deficient bacteria preferentially. The substances in group II serve as "negative controls." They are known to affect structures and functions other than the cellular DNA.

bein, 1969; Fishbein *et al.*, 1970), it would seem that its possible deleterious effect on human health deserves serious investigation.

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Phytotoxicity, Absorption, and Translocation of 4-Aminopyridine in Corn and Sorghum Growing in Treated Nutrient Cultures and Soils

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There were no visible phytotoxic effects when 1-month-old corn (*Zea mays*) or sorghum (*Sorghum vulgare*) seedlings were grown 1 week in nutrient cultures containing 0.1–100 ppm of 4-aminopyridine. Autoradiograms of 1-month-old plants grown 1 week in treated nutrient solutions containing 5 or 10 ppm of 4-[^{14}C]aminopyridine revealed a general distribution of radioactivity throughout the plants, with higher concentrations in the roots, lower stem, and leaf sections nearest the stalks. The quantity of 4-[^{14}C]aminopyridine translocated into shoot tissues of 2- and 3-month-old plants was inversely proportional to age, with most of the radioactivity in these older plants contained in the lower sections. Carbon-14 was not detected in corn seeds, but trace amounts were detected in seeds of sorghum plants. Shoots of 1-month-old corn plants grown in treated soils contained only small quantities of radioactivity, indicating that the compound was highly adsorbed onto soil colloids and thus relatively unavailable for root absorption.

Damage to ripening cereal grains by flocks of feeding blackbirds is a problem of economic concern in the United States (Neff, 1949; Neff and Meanley, 1957). Recent surveys in major corn-producing states revealed that direct losses attributable to birds were about 6 million bushels during both 1970 and 1971 (Stone *et al.*, 1972; Stone, 1972).

One of the most effective means found to date for reducing blackbird damage to corn involves the use of 4-aminopyridine, a chemical frightening agent (De Grazio *et al.*, 1971), whose utility in reducing problems caused by birds was first demonstrated by Goodhue and Baumgartner (1965). Cracked corn treated with this compound and broadcast in fields (De Grazio *et al.*, 1972) causes birds that ingest treated baits to fly erratically and emit distress calls, thereby inducing other members of the flock to abandon the area.

As with other toxic chemicals intended for soil application in the vicinity of food or feed crops, it is necessary to understand the fate of 4-aminopyridine in soils and its phytotoxicity, translocation, and metabolism in plants, in order to evaluate potential hazards from its use. With a

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