[CANCER RESEARCH 33, 458–459, March 1973]

Preferential Effect of Dichlorvos (Vapona) on Bacteria Deficient in DNA Polymerase[1]

Herbert S. Rosenkranz[2]

Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

SUMMARY

Bacteria deficient in DNA polymerase are much more susceptible than are normal cells to the lethal action of dichlorvos [Vapona, *O,O*-dimethyl *O*-(2,2-dichlorovinyl) phosphate], a widely used insecticide. The results are taken to indicate that dichlorvos is capable of altering the cellular DNA.

The anticholinesterase compound DDVP[3] is widely used as an insecticide in agriculture (9, 10), as an antimosquito agent for eradicating malaria (3, 4, 11, 17), as a disinfectant of commercial aircraft (16), and for the control of flea infections (10). Hence, human exposure to and inhalation of this pesticide occurs frequently. As normally used, this substance has not exhibited acute toxicity except for the occasional lowering of plasma cholinesterase levels (2–4, 6, 10, 12, 19, 21, 22). However, it was shown recently that DDVP and related phosphoric acid esters reacted with purified DNA and that this reaction was due to the methylation of guanine residues of DNA (8, 15). If the reaction were also to occur *in vivo*, then DDVP could present a real hazard to health in view of the established relationship between the ability of agents to alkylate DNA and their potential carcinogenicity and mutagenicity.

In this report it is shown (Tables 1 and 2) that bacteria (*Escherichia coli*) deficient in DNA polymerase are more sensitive to DDVP than are the parents from which they were derived. This is a property also exhibited by other DNA-alkylating agents (Table 2, Group 2) that are known to be carcinogens as well. On the other hand, substances that do not act on the cellular DNA did not inhibit the DNA polymerase-deficient strain preferentially (Table 2, Group 1).

DNA polymerase has been implicated in the DNA repair process (1, 7); bacterial strains lacking this enzyme are more sensitive than their parents to agents that react with the cellular DNA (1, 5, 18, 20), presumably, because they are unable to repair efficiently the damage to their DNA. The present data suggest that DDVP reacts with the DNA of living cells—a reaction which already has been demonstrated *in vitro* (8, 15). A reexamination of the potential hazard (carcinogenicity and mutagenicity) to human health of this widely used substance seems, therefore, imperative.

Table 1

Effect of DDVP on the growth of bacteria deficient in DNA polymerase

Bacteria (*E. coli* W3110 *thy*$^-$ (*pol A*$^+$) and its DNA polymerase-deficient derivative *E. coli* p3478 (*pol A*$_1^-$) (1) in Medium HA (13, 14) containing 5 μg of thymine per ml were brought to the early exponential growth phase, at which time portions of each culture received DDVP (6.4×10^{-3} M). At intervals, serial dilutions (0.1 ml) of cell cultures were plated on agar plates (Medium HA plus 5 μg thymine per ml) (13).

Time (hr)	Additions	No. of viable bacteria/ml with *Pol A*$^+$	No. of viable bacteria/ml with *Pol A*$_1^-$
0	None	1.1×10^8	1.4×10^8
0.5	None	1.8×10^8	2.3×10^8
1	None	4.3×10^8	4.5×10^8
2	None	9.3×10^8	8.7×10^8
0	DDVP	1.1×10^8	1.4×10^8
0.5	DDVP	7.1×10^7	2.6×10^7
1	DDVP	4.3×10^7	5.0×10^6
2	DDVP	1.0×10^7	1.5×10^5

ACKNOWLEDGMENTS

DDVP was kindly provided by the Shell Chemical Co., San Ramon, Calif.

REFERENCES

1. DeLucia, P., and Cairns, J. Isolation of an *E. coli* Strain with a Mutation Affecting DNA Polymerase. Nature, *224:* 1164–1166, 1969.
2. Durham, W. F., Gaines, T. B., McCauley, R. H., Jr., Sedlak, V. A., Mattson, A. M., and Hayes, W. J., Jr. Studies on the Toxicity of *O,O*-Dimethyl-2,2-dichlorovinyl Phosphate (DDVP). A.M.A. Arch. Ind. Health, *15:* 340–349, 1957.
3. Funckes, A. J., Miller, S., and Hayes, W. J., Jr. Initial Field Studies in Upper Volta with Dichlorvos Residual Fumigant as a Malaria Eradication Technique. Bull. World Health Organ., *29:* 243–246, 1963.
4. Gratz, N. G., Bracha, P., and Carmichael, A. A Village-Scale Trial with Dichlorvos as a Residual Fumigant in Southern Nigeria. Bull. World Health Organ., *29:* 251–270, 1963.
5. Gross, J., and Gross, M. Genetic Analysis of an *E.coli* Strain with a Mutation Affecting DNA Polymerase. Nature, *224:* 1166–1168,

[1] This investigation was supported by a grant from the Dr. George A. Carden, Jr. Special Fund for Cancer Research.

[2] Research Career Development Awardee of the National Institute of General Medical Sciences (5-K3-GM-29,024).

[3] The abbreviation used is: DDVP, *O,O*-dimethyl- *O*-(2,2-dichlorovinyl)phosphate.

Received September 1, 1972; accepted November 16, 1972.

Table 2

Effects of agents on the growth of a DNA polymerase-deficient strain of E. coli

The procedure used has been described (18). Bacteria [*E. coli* W3110 *thy*$^-$ (*pol A*$^+$), the parent strain, and *E. coli* p3478 (*pol A*$_1^-$), the DNA polymerase-deficient strain (1)], were spread onto the surface of agar plates [Medium HA plus 5 μg thymine per ml (13, 14)]. When the plates had dried, discs impregnated with the substances to be tested were deposited on the surface of the agar; after incubation at 37° for 16 hr, the diameters of the zones of inhibition were measured.

Group	Agent	Amount	Diameter of zone of inhibition (mm)	
			pol A$^+$	*pol A*$^-$
1	Chloramphenicol	30 μg	24	24
	Streptomycin	10 μg	17	17
	Kanamycin	30 μg	19	19
	Erythromycin	15 μg	9	9
2	Methylmethanesulfonate	0.13 μmole	42	59
	Ethylmethanesulfonate	0.11 μmole	4	30
	N-Methyl-*N*-nitrosourea	0.05 μmole	0	16
	N-Methyl-*N*-nitrosourethan	0.02 μmole	0	22
	N-Ethyl-*N*-nitrosourethan	1 μmole	2	33
3	DDVP	0.06 μmole	21	26

1969.

6. Hayes, W. J., Jr. Safety of DDVP for the Disinfection of Aircraft. Bull. World Health Organ., *29:* 629–633, 1961.
7. Kelly, R. B., Atkinson, M. R., Huberman, J. A., and Kornberg, A. Excision of Thymine Dimers and Other Mismatched Sequences by DNA Polymerase of *Escherichia coli*. Nature, *224:* 495–501, 1969.
8. Löfroth, G. Alkylation of DNA by Dichlorvos. Naturwissenschaften, *57:* 393–394, 1970.
9. Mattson, A. M., Spillane, J. T., and Pearce, G. W. Dimethyl 2,2-Dichlorovinyl Phosphate (DDVP), an Organic Phosphorus Compound Highly Toxic to Insects. J. Agr. Food Chem., *3:* 319–321, 1955.
10. National Communicable Disease Center: Public Health Pesticides. Pest Control, *38:* 1–24, 1970.
11. Quarterman, K. D., Lofte, M., and Schoof, H. F. Initial Field Studies in Upper Volta with Dichlorvos Residual Fumigant as a Malaria Eradication Technique. I. General Considerations. Bull. World Health Organ., *29:* 231–235, 1963.
12. Rasmussen, W. A., Jensen, J. A., Stein, W. J., and Hayes, W. J., Jr. Toxicological Studies of DDVP for Disinfection of Aircraft. Aerospace Med., *34:* 593–600, 1963.
13. Rosenkranz, H. S., Carr, H. S., and Morgan, C. Unusual Growth Properties of a Bacterial Strain Lacking DNA Polymerase. Biochem. Biophys. Res. Commun., *44:* 546–549, 1971.
14. Rosenkranz, H. S., Carr, H. S., and Rose, H. M. Phenethyl Alcohol. I. Effect on Macromolecular Synthesis of *Escherichia coli*. J. Bacteriol., *89:* 1354–1369, 1965.
15. Rosenkranz, H. S., and Rosenkranz, S. Reaction of DNA with Phosphoric Acid Esters: Gasoline Additive and Insecticides. Experientia, *28:* 386–387, 1972.
16. Schoof, H. F., Jensen, J. A., Porter, J. E., and Maddock, D. R. Disinfection of Aircraft with Mechanical Dispenser of DDVP Vapour. Bull. World Health Organ., *24:* 623–628, 1961.
17. Schoof, H. F., Mathis, W., Taylor, R. T., Brydon, H. W., and Goodwin, W. J. Studies with Dichlorvos Residual Fumigant as a Malaria Eradication Technique in Haiti. I. Operational Studies. Am. J. Trop. Med. Hyg., *15:* 661–669, 1966.
18. Slater, E. E., Anderson, M. D., and Rosenkranz, H. S. Rapid Detection of Mutagens and Carcinogens. Cancer Res., *31:* 970–971, 1971.
19. Stein, W. J., Miller, S., and Fetzer, L. E., Jr. Studies with Dichlorvos Residual Fumigant as a Malaria Eradication Technique in Haiti. III. Toxicological Studies. Am. J. Trop. Med. Hyg., *15:* 672–675, 1966.
20. Town, C. D., Smith, K. C., and Kaplan, H. S. DNA Polymerase Required for Rapid Repair of X-ray Induced DNA Strand Breaks *in Vivo*. Science, *172:* 851–854, 1971.
21. Witter, R. F. Effect of DDVP Aerosol on Blood Cholinesterase of Fogging Machine Operators. A.M.A. Arch. Ind. Health, *21:* 7–9, 1960.
22. Witter, R. F., Gaines, T. B., Short, J. G., Sedlack, V. A., and Maddock, D. R. Studies on the Safety of DDVP for the Disinfection of Commercial Aircraft. Bull. World Health Organ., *24:* 635–642, 1961.

Preferential Effect of Dichlorvos (Vapona) on Bacteria Deficient in DNA Polymerase

Herbert S. Rosenkranz

Cancer Res 1973;33:458-459.