

## Volatility of DDT and Related Compounds

William F. Spencer\* and Mark M. Cliath

Vapor pressures of two isomers of DDT and some of their degradation products, measured by a gas saturation method, indicate that the *o,p'* isomers are more volatile than the *p,p'* isomers. The vapor pressure of *o,p'*-DDT is 7.5 times that of *p,p'*-DDT. At 30°C the atmosphere above a surface deposit of technical grade DDT contains approximately 62% *o,p'*-DDT, 16% *o,p'*-DDE, 14% *p,p'*-DDE, and only 8% *p,p'*-DDT. When technical grade DDT is applied to moist soil at concentrations up to 20 µg/g, the atmosphere in and above the soil contains approximately equal quantities of *o,p'*-DDT

and *p,p'*-DDT, but at higher concentrations the ratio of *o,p'*- to *p,p'*-DDT in the vapor phase increases. Dieldrin applied with DDT did not affect DDT vapor density and the interaction between *o,p'*- and *p,p'*-DDT was very slight. Air drying of soil reduced volatility of all compounds. The primary breakdown product of DDT, *p,p'*-DDE, has a higher vapor pressure than the original compound, *p,p'*-DDT, which indicates that much of the DDT now present in the soil may volatilize as DDE.

It is known that DDT is highly persistent in soil, and even though its use has been greatly curtailed in the USA, DDT isomers and their degradation products will be present in the environment for many years. Volatilization and vapor phase transport are important processes in the dissipation of even the so-called "nonvolatile" pesticides such as DDT. Balson (1947) and Dickinson (1956) reported values for vapor pressure of DDT at various temperatures. Several investigators recently measured volatilization losses of DDT from soil under laboratory (Guenzi and Beard, 1970; Farmer *et al.*, 1972) and field (Willis *et al.*, 1971) conditions. All of the above researchers worked with the *p,p'*-DDT isomer. Wichmann *et al.* (1946) found that *o,p'*-DDT disappeared more rapidly than *p,p'*-DDT from open dishes containing finely ground crystalline materials heated at 54°C. Most other research on volatility and behavior of DDT under controlled conditions has been with the *p,p'*-DDT isomer only. However, other isomers and related breakdown products of DDT such as *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, and *o,p'*-DDD become distributed in the environment and are of ecological concern.

This paper reports data on the vapor pressure and relative volatility of some DDT isomers, impurities in the technical material and potential breakdown products, and discusses their behavior when applied to soil.

## MATERIALS AND METHODS

Vapor densities of DDT and related compounds, with and without soils, were determined by a gas saturation method in which N<sub>2</sub> gas was passed through insecticide-treated sand or soil at a sufficiently slow rate to insure equilibrium vapor saturation. The apparatus and the procedures have been described (Spencer and Cliath, 1969). For vapor density without soil, quantities equivalent to 1–2% of either DDT, DDE, or DDD were added to white silica sand. Technical grade DDT obtained from City Chemical Corp., New York, was used for both *o,p'*- and *p,p'*-DDT. The DDE was prepared by alkaline hydrolysis of technical DDT, followed by recrystallization from hot ethanol. The *p,p'*-DDD was "puriss" grade obtained from Aldrich Chemical Co. The DDT or related materials were atomized in benzene onto silica sand in 10-l.

carboys. After evaporating the solvent and placing the sand in 6 × 43-cm glass columns, dry N<sub>2</sub> gas was passed through the columns at flow rates of approximately 3.5 ml/min until sufficient material had been trapped in hexane for analysis by glc. After concentrating in a Kuderna-Danish concentrator, the *o,p'* and *p,p'* isomers of DDT, DDE, and DDD in the hexane were quantified by peak height using a Beckman GC-5 gas-liquid chromatograph with a nonradioactive electron-capture detector. Gas chromatographic conditions were as follows: glass column (1.83 × 3 mm i.d.) packed with 2% DC-200 (12,500 cs) on Anakrom ABS (80/90 mesh), or 3% QF-1 on Anakrom ABS (80/90 mesh); helium carrier gas flow rate was 50 ml per min; temperatures: injection port 200°C, column 185°C, detector 250°C. Chromatographic peaks were confirmed by comparison with relative retention values of standards on the DC-200 column.

To study vapor phase desorption and potential volatility of DDT and related compounds from soil, technical grade DDT was added to Gila silt loam. Gila silt loam is a calcareous, desert soil containing 0.6% organic matter and 18.4% clay, predominately montmorillonite (surface area of approximately 90 m<sup>2</sup>/g). Sufficient technical DDT to provide concentrations from 2.5 to 120 µg/g was applied in hexane to 1100 g autoclaved Gila silt loam. The hexane was evaporated, the water content of the soil adjusted to 7.5%, and the soil placed in glass columns. Measurements were begun about 10 days to 3 weeks after soil treatment. Each run required approximately 10 days at 30°C, with the N<sub>2</sub> carrier gas adjusted to 100% relative humidity. Soil assays for DDT before and after a series of runs indicated no significant change in DDT concentration occurred during the incubation or measurement period.

Soils treated at 40, 60, and 120 µg/g were subsequently air-dried to 2.2% water to provide data on the effect of soil water content on vapor density of DDT. The N<sub>2</sub> carrier gas was adjusted to 50% relative humidity for the measurement at 2.2% soil water content.

The competitive effect of other pesticides on the vapor density of DDT was measured by applying 10 µg/g of either *p,p'*-DDT or *o,p'*-DDT to Gila silt loam with and without the concurrent addition of an accessory pesticide. The so-called accessory pesticide was either the other isomer of DDT or recrystallized dieldrin, 99% HEOD. The insecticides, either alone or in combination, were added in hexane to autoclaved Gila silt loam, the hexane evaporated, water content adjusted to 7.5%, and the soil placed in glass columns. Vapor density measurements were initiated 10 days after DDT was added to

USDA, Agricultural Research Service, Soil and Water Conservation Research Division, University of California, Riverside, California 92502.

Table I. Saturation Vapor Density in Sand Columns and Apparent Vapor Pressure of DDT and Related Compounds at 30°C

Chemical	Vapor density ng/l.	Vapor pressure <sup>a</sup> mm × 10 <sup>-7</sup>
<i>p,p'</i> -DDT	13.6	7.26
<i>o,p'</i> -DDT	104	55.3
<i>p,p'</i> -DDE	109	64.9
<i>p,p'</i> -DDD	17.2	10.2
<i>o,p'</i> -DDE	(104) <sup>b</sup>	(61.6) <sup>b</sup>
<i>o,p'</i> -DDD	(31.9) <sup>c</sup>	(18.9) <sup>c</sup>

<sup>a</sup> Calculated from vapor density, w/v, with the equation:  $P = w/v \cdot RT/M$ . <sup>b</sup> Atmosphere probably not saturated with *o,p'*-DDE. <sup>c</sup> Atmosphere probably not saturated with *o,p'*-DDD. The sand column was prepared with *p,p'*-DDD which contained sufficient *o,p'*-DDD as an impurity to produce this vapor density.

Table II. Vapor Composition in Association with a Mixture of 1-2% Technical DDT and Silica Sand at 30°C

Chemical	Vapor density		Conc in tech DDT, %
	ng/l.	% of total	
<i>p,p'</i> -DDT	13.6	8.0	74.6
<i>o,p'</i> -DDT	104	61.7	21.1
<i>p,p'</i> -DDE	24.1	14.3	0.81
<i>o,p'</i> -DDE	26.9	16.0	0.07
Total	168.6		

the soil. Temperature was controlled to  $\pm 0.2^\circ\text{C}$  for all measurements.

#### ABBREVIATIONS

*p,p'*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane); *p,p'*-DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene); *p,p'*-DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane); *o,p'*-DDT (1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane); *o,p'*-DDE (1,1-dichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethylene); *o,p'*-DDD (1,1-dichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl) ethane); HEOD (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene).

#### RESULTS AND DISCUSSION

The measured vapor densities of *p,p'*-DDT on sand were: 2.9, 13.6, and 60.2 ng/l., equivalent to vapor pressures of 1.52, 7.26, and  $33.2 \times 10^{-7}$  mm at 20, 30, and 40°C, respectively. These values are in close agreement with vapor pressures reported for *p,p'*-DDT by Balson (1947) and Dickinson (1956). The calculated constants *A* and *B* in the equation  $\log_{10} P = A$

— *B/T* are 14.24 and 6176, respectively, compared with 14.19 and 6160 (Balson, 1947) and 13.78 and 6010 (Dickinson, 1956).

The saturation vapor densities and apparent vapor pressures of two isomers of DDT, DDE, and DDD at 30°C are shown in Table I. The *o,p'* isomers appear to be more volatile than the *p,p'* isomers. The vapor pressure of *o,p'*-DDT is 7.5 times greater than that of *p,p'*-DDT. Moreover, some of the impurities and potential breakdown products of *p,p'*-DDT, such as *p,p'*-DDE, have much higher vapor pressures than the original compound, *p,p'*-DDT. The DDE and DDD used in the sand columns were mainly the *p,p'* isomers, and the measured vapor densities for *o,p'*-DDE and *o,p'*-DDD resulted from *o,p'* impurities in the *p,p'* materials. Therefore, the values for *o,p'*-DDE and *o,p'*-DDD in Table I are probably low, since it is doubtful that the N<sub>2</sub> atmosphere was saturated with these compounds.

The composition of vapor at 30°C in equilibrium with technical grade DDT added to silica sand at the rate of 1-2% is shown in Table II. These data represent the probable ratios of the four compounds initially evaporating from nonabsorbing surfaces after the application of technical grade DDT. Only 8% of the vapor concentration is *p,p'*-DDT. The vapor density of *o,p'*-DDT is 7.5 times that of *p,p'*-DDT, and even the two isomers of DDE have about twice the vapor density of *p,p'*-DDT. These are equilibrium values and undoubtedly the vapor concentration ratios change as evaporation depletes the constituents present in lesser amounts.

The vapor density of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDE and the percent of the total vapor made up of each constituent as related to application rate of technical DDT to Gila silt loam at 7.5% water content is shown in Table III. At low soil concentrations, the ratio of the vapor density of *o,p'*-DDT to that of *p,p'*-DDT was approximately 0.85 until sufficient technical DDT was added to produce a saturated vapor of *p,p'*-DDT. A saturated vapor of *p,p'*-DDT was reached at approximately 20 μg of technical DDT/g and vapor density of *p,p'*-DDT did not increase further with increasing concentrations of technical DDT. However, the vapor density of *o,p'*-DDT continued to increase, linearly, with concentration of technical DDT and did not reach a saturated vapor density even at 120 μg/g. Considerable quantities of *p,p'*-DDE and even *o,p'*-DDE were present in the soil atmosphere, especially at higher rates of DDT application. At the higher rates, the *p,p'*-DDE vapor density was approximately 25 to 30% as great as that of *p,p'*-DDT. The ratio of *o,p'*- to *p,p'*-DDT in the vapor phase will depend on the makeup of technical DDT with respect to the two isomers. However, the ratio of DDT to DDE or to other metabolites will reflect the rate, or degree, of breakdown of the DDT.

Table III. Vapor Density of *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, and *o,p'*-DDE as Related to Concentration of Technical DDT in Gila Silt Loam at 7.5% Water Content and 30°C

Tech DDT <sup>a</sup> conc, μg/g	Vapor density, ng/l.				Total	Vapor density, % of total			
	<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	<i>p,p'</i> -DDE	<i>o,p'</i> -DDE		<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	<i>p,p'</i> -DDE	<i>o,p'</i> -DDE
2.5	1.11	1.16	0.43		2.70	41.2	43.1	15.7	
5	2.65	2.22	0.60		5.47	48.4	40.6	11.0	
10	6.07	5.26	1.08		12.41	48.9	42.4	8.7	
20	13.95	11.92	2.94	0.45	28.81	47.8	40.7	10	1.5
40	12.11	21.40	3.03	0.70	36.54	32.5	57.5	8.1	1.9
60	13.37	32.74	3.42	0.97	49.53	26.5	64.8	6.8	1.9
120	13.62	67.0	5.41	1.64	86.03	15.5	76.5	6.1	1.9

<sup>a</sup> Technical DDT containing 74.6% *p,p'*-DDT, 21.1% *o,p'*-DDT, 0.81% *p,p'*-DDE, and 0.07% *o,p'*-DDE.

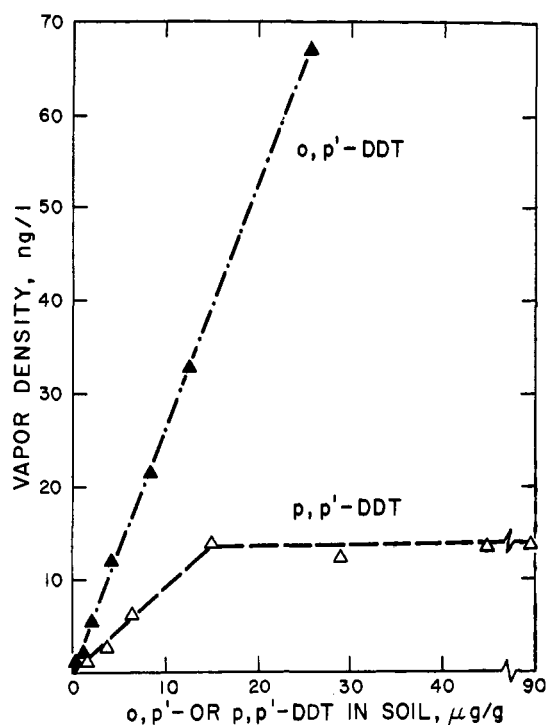


Figure 1. Vapor density of *o,p'*-DDT and *p,p'*-DDT as related to the concentration of each isomer in Gila silt loam at 7.5% water and 30°C. (Concentrations of each isomer calculated from the measured content of technical DDT)

Some of the concentrations of technical DDT in Table III may appear to be unrealistically high, but when DDT is applied as a spray to plants or to the soil surface, the concentration in the surface layer of soil often exceeds even the highest rates used. For example, a 1 kg/ha application of DDT is equivalent to 150  $\mu\text{g/g}$  in the surface  $\frac{1}{2}$  mm of soil. Therefore, soil surface applications would result in initial vapor compositions similar to the highest rate of technical DDT.

Figure 1 shows desorption isotherms relating vapor density of *o,p'*- and *p,p'*-DDT to the actual amounts of each isomer in the soil calculated from their concentrations in the technical DDT. The vapor density of *o,p'* increases much more rapidly with concentration than does that of *p,p'*-DDT. However, when the concentration of each isomer is plotted against relative vapor density,  $d/d_0$  (where  $d$  is the observed vapor density and  $d_0$  is the saturation vapor density without soil), a different picture emerges (Figure 2). This type of plot provides information on relative strengths of adsorption. It required only 15 ppm of *p,p'*-DDT to produce a saturated vapor, whereas approximately 39 ppm of *o,p'*-DDT would be required to produce a saturated vapor of *o,p'*-DDT. This indicates that *o,p'*-DDT is more strongly adsorbed on the soil than *p,p'*-DDT. But, in spite of the increased adsorption, the much higher vapor pressure of *o,p'*-DDT more than compensates for the greater adsorption, and the overall result is essentially an equal or higher vapor density of *o,p'*- than *p,p'*-DDT when technical DDT is added to soil.

A plot of relative vapor density,  $d/d_0$  of *p,p'*- and *o,p'*-DDT vs. the concentration of technical DDT in soil, shows that each isomer essentially acts independently (Figure 3). The vapor density of *o,p'*-DDT is primarily related to the concentration of *o,p'* in the soil and not to the total amount of DDT present. If it were related to the total amount of DDT present, a saturated vapor density for *o,p'*-DDT would have been

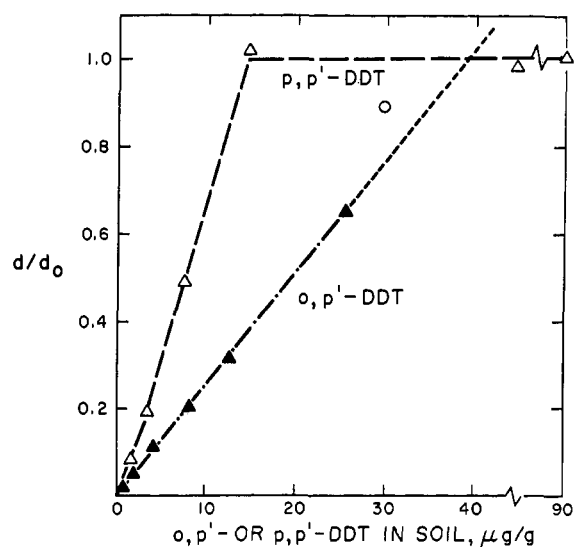


Figure 2. Relative vapor density  $d/d_0$  of *o,p'*-DDT and *p,p'*-DDT as related to the concentration of each isomer in Gila silt loam at 30°C

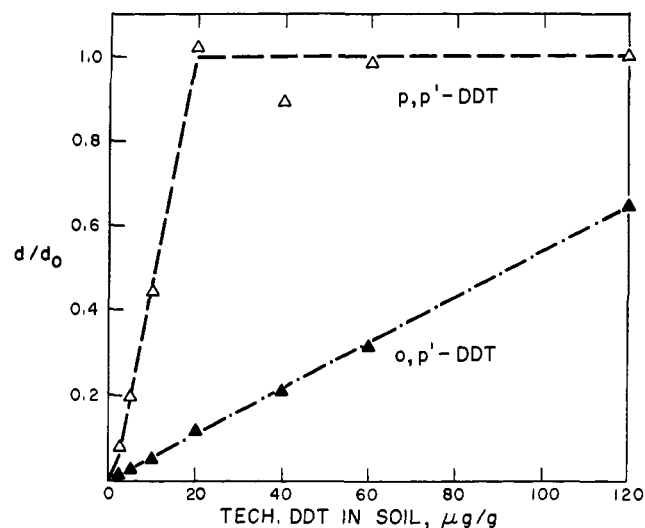


Figure 3. Relative vapor density,  $d/d_0$ , of *p,p'*-DDT and *o,p'*-DDT as related to the concentration of technical DDT in Gila silt loam at 7.5% water and 30°C

Table IV. Vapor Density of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDE as Related to Concentration of Technical DDT in Gila Silt Loam at 2.2% Water Content and 30°C

Tech DDT, $\mu\text{g/g}$	Vapor density, ng/l.				Total
	<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	<i>p,p'</i> -DDE	<i>o,p'</i> -DDE	
40	0.47	0.89	0.28	0.09	1.73
60	0.74	1.06	0.40	0.10	2.30
120	1.66	1.85	0.47	0.17	4.15

reached at the same concentration of technical DDT that produced a saturated vapor for *p,p'*-DDT.

Air drying of Gila silt loam containing 40, 60, and 120  $\mu\text{g/g}$  of technical DDT greatly reduced the vapor density of both isomers of DDT and DDE (Table IV). The vapor density was approximately 21 times greater at 7.5% than at 2.2% soil water content. Marked reductions in vapor density of dieldrin and lindane due to air drying of soil were previously reported by Spencer *et al.* (1969) and Spencer (1970).

**Table V. The Competitive Effect of Accessory Insecticides on the Vapor Density of *p,p'*-DDE and *o,p'*-DDT in Gila Silt Loam at 7.5% H<sub>2</sub>O and 30°C**

Primary DDT isomer in soil, 10 μg/g	Accessory pesticide, μg/g	Vapor density ng/l.		Increase due to accessory pesticide	
		<i>p,p'</i> -	<i>o,p'</i> -	ng/l.	Equivalent to DDT, <sup>a</sup> μg/g
<i>p,p'</i> -DDT	None	7.41			
<i>p,p'</i> -DDT	<i>o,p'</i> -DDT, 10	8.99		1.58	2.1
<i>p,p'</i> -DDT	HEOD, 20	8.08		0.67	0.9
<i>o,p'</i> -DDT	None		29.8		
<i>o,p'</i> -DDT	<i>p,p'</i> -DDT, 10		34.6	4.8	1.6
<i>o,p'</i> -DDT	HEOD, 20		30.7	0.9	0.3

<sup>a</sup> The additional amount of *p,p'*-DDT or *o,p'*-DDT which would result in an increase in vapor density equivalent to that resulting from the accessory pesticide based on a linear relationship between soil DDT concentration and vapor density.

The vapor density associated with 10 μg/g of either *p,p'*-DDT or *o,p'*-DDT applied to Gila silt loam with and without the addition of accessory pesticides and the increase due to the accessory pesticide, if any, are shown in Table V. Adding 20 ppm of dieldrin (HEOD) had no measurable effect on the vapor density of either *p,p'*-DDT or *o,p'*-DDT. There was a small observable interaction between the vapor density of *p,p'*- and *o,p'*-DDT. However, the vapor density of each was increased only slightly by the presence of the other in the soil. This relatively low degree of interaction indicates that the adsorption sites on soil for *p,p'*-DDT may be different from those for *o,p'*-DDT; or possibly the insecticides do not interact more strongly because of the very small percentage of the total available surfaces covered by DDT at these low concentrations. The latter possibility is more probable, as only 0.01% of the total 90 m<sup>2</sup>/g surface would be covered by DDT at these concentrations. The absence of any interactions between HEOD and the isomers of DDT was somewhat unexpected. They are all weakly polar compounds, and if competition for adsorption sites on the soil is important in influencing volatility, one would expect the presence of "like" compounds to increase the vapor density of each. These data indicate that insecticides must be very closely related chemically before the presence of one will increase the volatility of the other.

These findings on relative volatility of the various isomers and breakdown products of DDT have wide application. When technical DDT is added to soil or plant surfaces, the concentration of *o,p'*-DDT in the atmosphere above the surface will be much higher than that of *p,p'*-DDT. As the *p,p'*-DDT degrades in the soil the degradation products, such as DDE and DDD, will increase in the soil, in the soil atmosphere, and in the air above the soil. This means that the actual amount of *p,p'*-DDT volatilized from the soil surface may be relatively small compared to the amounts of *o,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD, even though only small amounts of these materials may be present in the applied technical DDT.

Apparently, the volatilization process results in the dispersion of the more volatile components in the environment away from the point of application and in the accumulation of the less volatile components in the soil. Terriere *et al.* (1966) reported the ratio of *o,p'*-DDT/*p,p'*-DDT in orchard soils was less than in technical DDT, indicating that *o,p'*-DDT is less persistent in soils than *p,p'*-DDT. In experiments on the persistence of termiticides in Hawaiian soils, Bess and

Hylin (1970) found that the residue of technical DDT remaining in the soil after 7 yr was mainly *p,p'*-DDT, even though the technical material contained about 17% *o,p'*-DDT. They suggested that fractionation may have occurred in the soil by differential binding, solubility, or biodegradation. Our data on relative vapor pressures of the various isomers indicate that *o,p'*-DDT probably disappeared more rapidly from the soil than *p,p'*-DDT in both of the above cases because of its higher rate of volatilization.

McCaskill *et al.* (1970) studied the residues of chlorinated hydrocarbons in soybean seed and surface soils from selected counties of South Carolina. Using their data from the "highest residue" fields in each region, the calculated ratios of *o,p'*-/*p,p'*-DDT in soil and in soybean seed were 0.23 and 0.79, respectively. In our experiments, the *o,p'*-/*p,p'*-DDT ratios in solid phase technical DDT and in the vapor phase (for soil concentrations between 0 and 20 μg/g) were 0.27 and 0.85, respectively. This similarity of *o,p'*-/*p,p'*-DDT ratios suggests the possibility that the DDT was adsorbed by the soybean plants from the vapor phase. Even though it is not possible to state definitely that this is the case, the similarity of ratios implies that the relative mobilities of the two isomers are related to their relative vapor pressures in the soil.

Cliath and Spencer (1971) recently obtained evidence to indicate that volatilization of degradation products is a major pathway for dissipation of some organochlorine pesticides from soils. Under aerobic conditions (the usual state of most agricultural soils), much of the DDT applied to soil degrades to DDE. From our data on volatilization of the isomers and breakdown products of DDT, most of the residual DDT now in the soil will probably be volatilized as *o,p'*-DDT or as *p,p'*-DDE. Some *p,p'*-DDT, of course, will be vaporized from the surface, especially for a short time following application. But after DDT applications are discontinued, most of the DDT will probably be volatilized following conversion to DDE or some even more volatile material. Under anaerobic conditions, in the presence of organic matter, DDT is degraded to DDD and other water-soluble metabolites (Guenzi and Beard, 1967). Inducing anaerobic conditions may be one means of altering the relative loss of the various compounds by volatilization.

*o,p'*-DDT has a relatively high degree of estrogenic activity compared with *p,p'*-DDT (Bitman and Cecil, 1971). *p,p'*-DDE has become increasingly implicated as the main organochlorine compound causing egg shell thinness and population decline of raptorial birds in several areas of the world (Ratcliffe, 1970; Cade *et al.*, 1971). Therefore, the ecological significance of the greater mobilities of *o,p'*-DDT and *p,p'*-DDE than of *p,p'*-DDT should be ascertained. If DDE and *o,p'*-DDT volatilize more rapidly, harmful levels of *o,p'*-DDT and *p,p'*-DDE should disappear from ecologically important areas faster than *p,p'*-DDT, or faster than the total DDT analysis would indicate. On the other hand, however, greater volatilization probably means greater movement into areas of low contamination.

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## Evaluation of Herbicides for Possible Mutagenic Properties

Kenneth J. Andersen,\* Edith G. Leighty, and Mark T. Takahashi<sup>1</sup>

One-hundred-and-ten herbicides were evaluated for their ability to induce point mutations in one or more of four different microbial systems. None of the herbicides appeared to cause point mutations in these microbial systems in comparison with known mutagens such as 5-bromouracil or 2-aminopurine. Except for inconclusive evidence relating to four herbicides within one test of one system, mutagenic rates of herbicide-treated organisms did

not differ significantly from spontaneous rates. In this one test, four herbicides were associated with mutation frequencies slightly in excess of the control. The observed increases were small, and the rates of mutation were lower than spontaneous rates of controls in other tests of the same system. Therefore, it appears that the increases observed with these four herbicides were within the normal range of spontaneous rates.

Pesticide chemicals are used widely to control insects, nematodes, plant diseases, and weeds. These chemicals and their residues enter the biological food chain at various points and for varying periods. Some of these compounds may be toxic to desirable organisms such as crops, wildlife, and man. It is uncertain whether some may also be mutagenic. Consequently, they might cause alterations of the genetic material of living cells as a result of chronic exposures below the toxic level. A number of reports are available concerning nuclear (or chromosomal) aberrations in plants, which suggests that herbicides should be evaluated for possible mutagenicity (Northrop, 1963; Suneson and Jones, 1960; Suneson *et al.*, 1965; Wu and Grant, 1966, 1967).

Accordingly, we have undertaken and completed an examination of more than 100 herbicides and other chemicals for their ability to cause point mutations in certain microbiological systems. In this study we have used as evaluation systems eight histidine-requiring mutants of *Salmonella typhimurium*, bacteriophage T<sub>4</sub>, and two rII mutants of bacteriophage T<sub>4</sub>. These test systems have been reported (Crow, 1968) to provide a high probability of detecting genetic damage of the point mutation type, to single genophores, or to the DNA molecule. These tests have been generally recommended as a first step on the evaluation of chemicals for mutagenic properties and have the advantage of being a qualitative and quantitative assessment of a large number of compounds. These tests are designed to detect mutagens causing base substitutions, deletions or additions, or grosser alterations, but do not detect mutations involving DNA transformations or genetic alterations

caused by chromosome breaks or other chromosome changes which are restricted to diploid cells.

### EXPERIMENTAL

**Bacterial Strains and Viruses.** Eight histidine-requiring mutants of *Salmonella typhimurium* were obtained from Bruce N. Ames of the University of California, Berkeley. These mutants involve the C, D, and G gene of the histidine operon in *S. typhimurium* and were designated as either nonsense (amber or ochre), missense, or frameshift mutants (Whitfield *et al.*, 1966). The T<sub>4</sub> bacteriophage and *Escherichia coli* B host were supplied by Robert M. McCombs of Baylor University College of Medicine, Houston, Texas. The rII mutant, AP72, and *E. coli* strain KB were obtained from Sewell Champe of Purdue University, Bloomington, Ind., while the rII mutant N17 was obtained from Ernst Freese of the National Institutes of Neurological Diseases and Blindness, NIH, Bethesda. Mutant AP72 is a transition mutant which involves the transition of the guanine-cytosine pair to adenine-thymine pair, while mutant N17 involves the transition from adenine-thymine pair to guanine-cytosine pair.

**Cultural Conditions.** The mutagenic properties of the test herbicides and chemicals were evaluated with the *Salmonella typhimurium* mutants by measuring the frequency of reversion to histidine independence, using an agar overlay technique according to the procedures described by Ames and Whitfield (1966). The bacteria were exposed to the test compounds on petri plates which were prepared by mixing 0.2 ml of freshly grown cultures (2 × 10<sup>8</sup> bacteria per milliliter) of the mutants with 2 ml of 0.6% agar at 45°C. The soft agar, which contained a trace (0.20 μmol) of histidine as well as the bacterial inoculum, was then poured onto plates of histidine-free minimal agar medium. Approximately 1 to 5 μl of liquid

Columbus Laboratories, Battelle Memorial Institute, Columbus, Ohio 43201.

<sup>1</sup>Present address: Rutgers, The State University, New Brunswick, New Jersey 08903.