DDT: Current Theories on its Mechanism of Poisoning

by F. Matsumura

DDT is perhaps our most potent insecticide, but many questions about the mechanism of its action remain unanswered. How can an "inert" chemical be so toxic to biolgical systems?

As many people already know DDT, which was once a miracle chemical, has recently become a problem child of our society. Perhaps DDT has saved more human lives than any other chemical in our human history, and yet, because of its long-lasting effects upon non-target ecosystems, its usefulness turned into a headache.

The paradox of DDT action is that DDT itself is a chemically unreactive compound as attested by the fact that DDT persists so long in various environments. It is quite puzzling that such an "inert" chemical could be so toxic to many biological systems.

Efforts to find the mechanisms of DDT-poisoning actually started as soon as this insecticide was first commercially used. Unfortunately, despite the efforts by many researchers for more than 20 years, the actual poisoning mechanism of DDT in animals remains as a mystery. The only facts we know for sure are that it initially attacks the nervous systems of animals and that the end result of DDT-poisoning is disruption of the ion-transport mechanism (Narahashi and Haas, 1967; Matsumura and O'Brien, 1966a). This disruption may ultimately cause death through respiratory failure or secondary poisoning by neurotoxins which are produced by the animals themselves under DDT poisoning (Sternburg, 1963). The key question then is how DDT upsets the function of nervous system to cause such effects.

It appears to be the intact DDT molecule itself that reacts with the nervous system, for researchers have recovered only unchanged DDT from the nervous system of poisoned insects (Matsumura and O'Brien, 1966b; Eaton and Sternburg, 1967).

Besides being unreactive, DDT and other chlorinated hydrocarbon insecticides have another interesting characteristic in common. That is, towards electrons they have high affinities, which become evident when researchers began adopting an electron-capturing device for detecting organic compounds in the effluent gas from the gas-chromatographic systems (e.g., electron-capture detectors). They discovered that chlorinated hydrocarbon insecticides had outstanding susceptibilities toward this detection method allowing them to study the insecticidal residues at 1 picogram, or parts per billion ranges in various biological materials. The general molecular structure of DDT (Figure 1) is arranged so that electrons are forced to flow from both the phenyl groups toward the $-CCl_3$ position creating two electronegative centers at both p,p' positions. Close examination of other active chlorinated hydrocarbon insecticides also indicates the presence of such electronegative centers for each molecule positioned opposite to each other across the line of symmetry (Soloway, 1965).

As the reader probably noticed already, perhaps the most likely way by which a stable compound such as DDT can react with biological systems is to directly bind with some biologically vital sites and to form a tight complex. The result of such a complex formation would be a loss of normal cell function.

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Because of the exereme lipophilicity of DDT, early workers suspected the lipid components of the cell to be the target substance for DDT. More recent studies, however, indicate that proteins take part in selective binding of DDT (Gunther *et al.*, 1954; Matsumura and O'Brien, 1966 a, b; Holan, 1969). The net result of DDT-protein binding should be that Na^+ and K^+ ions are prevented from passing through the cell membranes.

Then how does this complex formation cause such an end result? The question can only be partially answered because our knowledge of the mechanisms of ion transport as well as of the ultrastructures of cell membranes themselves are limited.

The purpose of this article is to describe the various experimental approaches made mainly in our laboratory and to report the current status of our understanding on this complex problem.

Our initial venture was an attempt to show that DDT can be bound to nerve components. In view of the lipophilic nature of DDT it was suspected from the first that a large quantity of DDT would be simply absorbed by non-vital lipid components. To distinguish the specific binding from non-specific absorption, we decided to always compare the pattern of DDT-absorption to the pattern of its non-insecticidal analog DDE. which is almost as lipophilic at DDT itself.

To study first the general absorption pattern of DDT by various subcellular components of the nervous system, the rat brain was homogenized. The homogenate was then incubated with 10^{-5} M C^{14}-DDT or C^{14}-DDE for 10 minutes. The centrifugal technique adopted to separate subcellular components was that of Marchbanks *et al.* (1966) who modified the method of Whittaker (1959). The fractions were carefully separated a..d all particulate portions were washed once. The Table 1 is a brief summary of the results from matched DDT-DDE absorption tests under the identical experimental conditions. It became evident from the data that the amount of DDT binding to the supernatant fraction was no more than hat of DDE, while more DDT than DDE was found in the nerve ending fraction. The above experiment revealed two important facts: first, soluble fraction does not contain the target substance, and second, DDT is preferentially absorbed by the material(s) that is present in the fraction containing largely the nerve ending particles (NEP).

Table 1. Distribution of C^{14} DDT and DDE among the subcellular fractions from the rat brain: the data are expressed in % of given (5 nanomole DDT or DDE.

Fractions	Absorption DDT %	Absorption DDE %	Difference (DDT-DDE) %
Supernatant (Sp)	3.96	4.63	—0.67
Cell Membrane (CM)	2.63	2.79	—0.16
Myeline Fragment (My)	20.03	19.88	0.15
Nerve Ending Particles (NEP)	37.78	33.65	4.13
Mitochondria (Mt)	1.26	1.53	—0.27
Nucleus (N)	34.38	37.63	—3.25

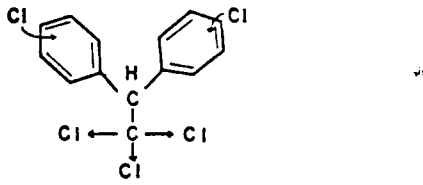


Figure 1 (right). The molecular structure of DDT and its pattern of electron distribution. Note that two electronegative centers are formed at p,p' positions.

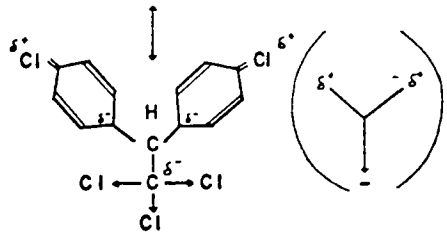
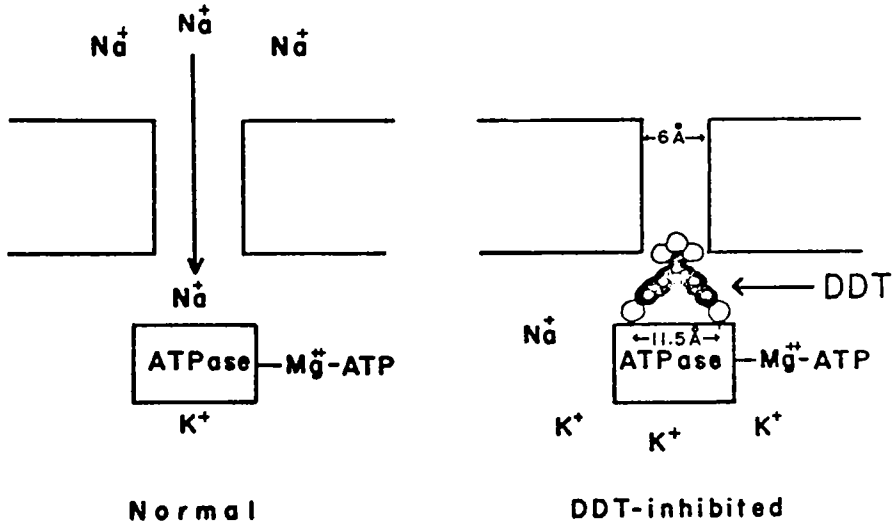


Figure 2 (below). Schematic representation of the synaptic complex and the possible site of DDT attack which is related to DDT-poisoning. Ax: axon, em: ending membrane, ssw: subsynaptic web, sm: synaptic membrane, sv: synaptic vesicle, mt: mitochondrion.



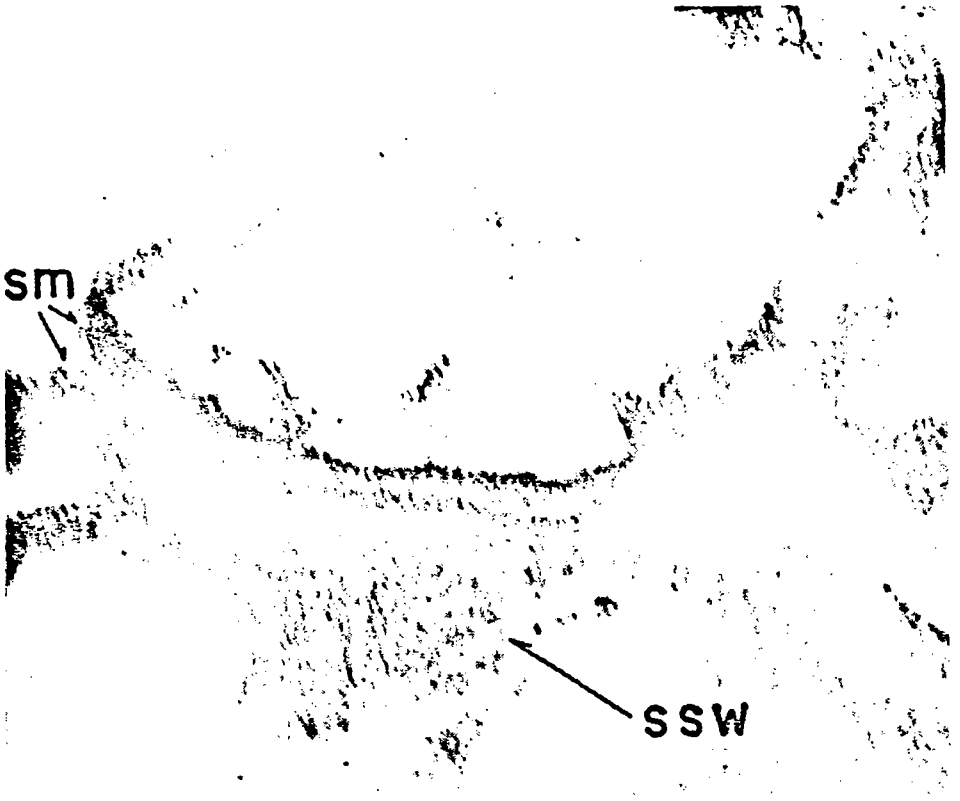
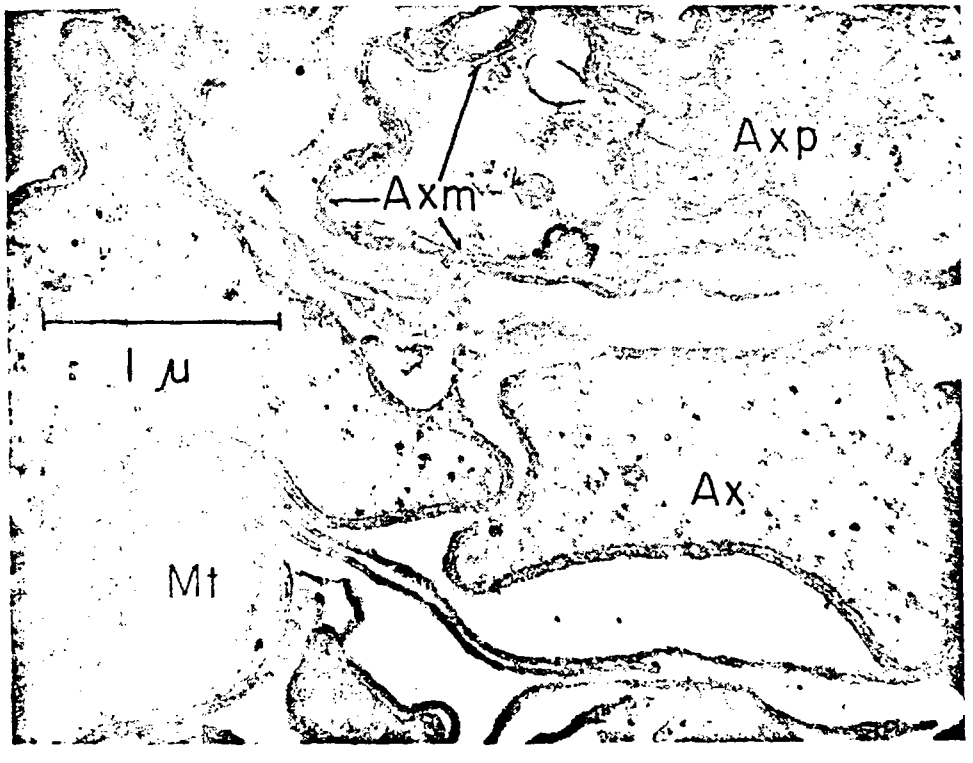


Figure 3. Electron microscopic view of synaptic membranes of the rat brain. Note that the synaptic vesicles as well as the ending membrane have been removed by the treatments of first osmotic shock and second Triton X-100 (De Robertis, 1967). It was found that DDT had the highest affinity toward this portion of the nervous system.

Figure 4. Radioautographic recording of H^3 DDT-binding to the axonic membrane of the abdominal nerve cord of the American cockroach. The dark filamentation represents the DDT molecule. Axp: axoplasm. Axm: axonic membrane.



The next question was the location of DDT binding within the precipitate of NEP fraction. The most helpful technological assistance came from the recent development of an ultra-centrifugal method by E. De Robertis and his associates (ref. De Robertis, 1967) to separate various neural components of the brain. Figure 2 schematically illustrates the actual sites of specific DDT-binding within the junctional complex of the rat brain.

All the experimental results have indicated that the post-synaptic regions of the nervous system play an important role in DDT binding and that the difference in the amounts of DDT and DDE binding does increase with treatments which attempt to purify the post-synaptic region.

Electron microscopic observations of the Triton treated nerve fraction (Figure 3) indeed contained fragmented junctional complexes containing the sub-synaptic webs and synaptic membranes joined by the intersynaptic filaments. A radioautographic technique to actually locate the cites of DDT attack has also been developed to facilitate such studies (Figure 4).

The foregoing experiments were helpful in establishing the order of the relative importance in specific DDT binding among the nerve components. The experimental results thus indicate the position of the primary target within the nervous system. This way of approach does not, however, give an answer to the question of why such formations of DDT-complex leads to disruption of normal cell functions.

To relate the action of DDT to inhibition of a certain system which normally performs an important role in cell function, at least three requirements were considered to be important: (1) the system must be concerned with the mechanisms of ion transport in the nervous system. (2) it must be DDT specific and it should be susceptible to DDT at low DDT concentrations, and (3) it should be able to form a complex with DDT itself.

Systematic search of such a system in our laboratory led to a finding that there is a certain nerve ATPase (adenosine triphosphatase) which satisfies all the above requirements. The nerve ATPases have been known to hydrolyze ATP (a high energy phosphate) to utilize its energy to regulate the flow of ions across the nerve membrane. Although there are a number of ATPases in the nervous system, not all of them showed a special susceptibility toward DDT. One ATPase system we

found in the synapses of the rat brain (exactly the same synaptic fraction as being described above) showed 1000 times more sensitivity toward DDT as compared to DDE. Moreover the concentration range of DDT to cause nerve disruption was in the same order as that required to inhibit the nerve ATPase.

The close correlation of the overall phenomenon of ATPase inhibition by DDT and nerve symptoms of DDT poisoning suggests that the site of DDT attack is very much similar to the nerve ATPase, if not the ATPase being the actual target system.

The whole mechanisms by which these nerve ATPases facilitate ion transport are still unknown. Biochemical evidences, however, indicate that some ATPases require Na^+, K^+ and Mg^{++} in a certain proportion for their maximum activity; implying that the ATPases control the relative concentrations of Na^+ and K^+ by the aid of Mg^{++} and ATP. That is, when the sodium ion rushes in and the potassium ion leaks out of the membrane (excitation) this ATPase system gets activated by the change in the ion composition, and thereby acts as the brake (turn off mechanism) for the continuing inflow of Na^+ ion, or as the triggering device (activation mechanism) for outflow of K^+ ion. In addition, this mechanism or related mechanisms also can act as the "pump" to expell unwanted Na^+ ions from inside of the nerve membrane (Fig. 5).

By the virtue of the rigid spacial requirements for the insecticidal activity of the DDT-like compounds, the approximate shape of the target site on the nerve membrane has long been speculated by many researchers (e.g. Holan, 1969). Fig. 5 illustrates the size requirements for the DDT target and its possible relationship to the ATPase molecule. DDT by its particular shape and the pattern of electron distribution binds with the ATPase and thus blocks the channels for specific ion passage.

Except for the established fact that DDT does inhibit the nerve ATPase *in vitro*, the bulk of the above "ATPase" theory has to be yet confirmed by actual experimental evidence. One factor which does not favor the above theory is that the extent of inhibition of ATPases is not too great in the nervous systems of actually DDT-poisoned animals *(in vivo)*. That is to say, we could not observe high degrees of ATPase inhibition even in insects which were already in the state of complete paralysis as the result of DDT.

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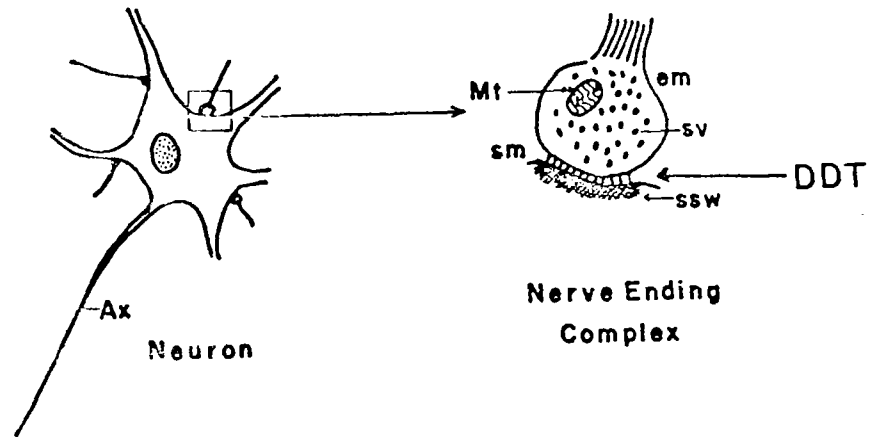
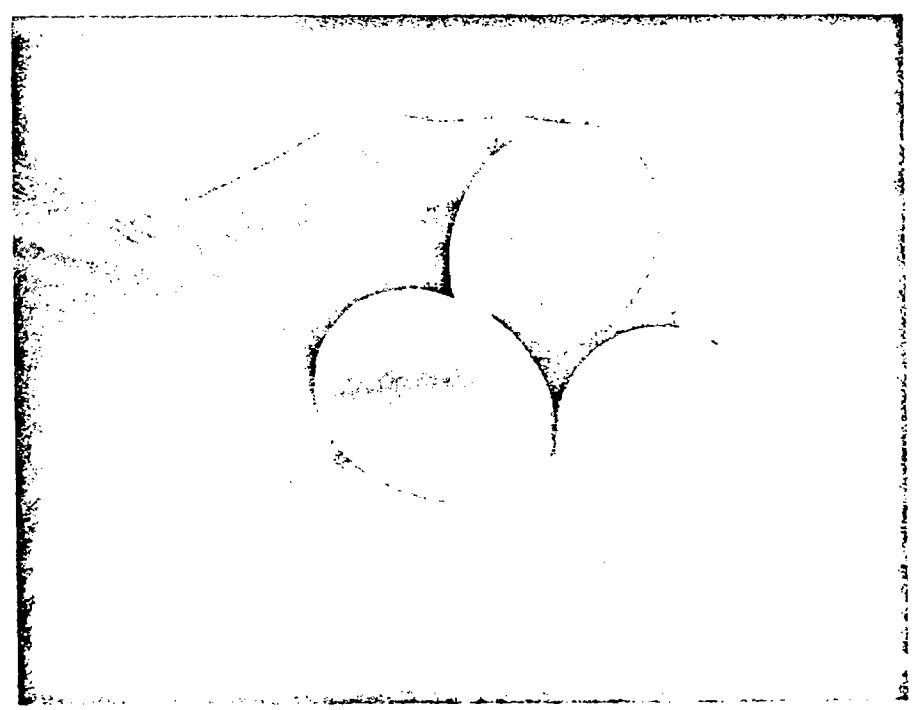


Figure 5. Schematic diagram explaining our current thinking on DDT-blockage of the ion transport mechanism across the nerve membrane. When the external ions (mainly Na^+) start flowing into (i.e. excitation) the cell through the ionic channel (sodium channel) the ATP system becomes activated by the increase of Na^+ and the decrease of K^+ ions. It is not known whether there are two channels (one for Na^+ and another for K^+) or the same chanel is used for both ions. The picture left is drawn to illustrate the hypothetical sodium channel only. The DDT molecule can exactly fit into the potassium channel which should be bigger than that for the sodium ion, and thus blocks the outflow process of K^+ ion (inhibition of K^+—excitation mechanism). DDT also attacks ATP-systems involving Na^+ channel in the same manner as it does to K3 ATP-systems except that the channel for Na^+ is smaller and the -C(Cl3) end of DDT cannot plug the hole. Thus Na^+ turn off mechanism. The DDT molecule is draw here in the manner that both p,p'positions of phenyl ring are attached to the ATP system and the -C(Cl3) end is fitted to mechanism. The DDT molecule is drawn here in the manner that both p,p'positions the K^+ channel.



Picture 6. DDT also weakens the egg shells of many birds, thus endangering the survival of these species.

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poisoning. However a close parallelism of the electrophysiological symptoms of DDT-poisoning and low degrees of ATPase inhibition was always observed in the nervous system of poisoned animals (unpublished data). This problem of "low degree inhibition" of ATPase *in vivo* (in live animals) has been interpreted by our group to suggest two alternative possibilities: (1) the actual portion of the specific ATPases that are involved in the process of DDT-poisoning is very small in comparison to other non-target ATPases, or (2) ATPases are not the actual site of DDT attack but there is a very similar, functionally analogous system (e.g. an ATP receptor protein) that is susceptible to DDT. The latter possibility is quite attractive since all the data obtained *in vitro* (at enzyme-levels) strongly indicate that some ATP requiring system should be involved in DDT-poisoning. Such examples of two analogous target systems can be found in nature; e.g. the case of the acetylcholine receptor proteins vs. acetylcholinesterases in the synaptic complex: e.g. nicotine specifically attacks the former because of its molecular similarity to acetylcholine while it does not inhibit the acetylcholinesterase *in vitro*.

Whatever the outcome of the future examination of such hypotheses, two things are alereadv certain at this stage: first, DDT attacks membrane ATPases, and second, it forms a tight complex with certain membrane components to alter the rates of ion exchange processes across the membrane.

The distribution of membrane ATPases is wide among various parts of any animal system. Furthermore enough evidence has accumulated to show that various biological membranes do possess similar, basic structures and properties. Recent experimental work in our laboratory, for instance, indicates that dieldrin, another potent chlorinated hydrocarbon insecticide, upsets the ion transport mechanisms in the liver cells (Wang and Matsumura, 1969). The experimental results from another research group also indicate that various chlorinated hydrocarbon insecticides can inhibit ATPases (Koch, 1969; Koch *et al.*, 1969). It is entirely possible that DDT and related insecticides inhibit the ATPases and/or ATP-requiring systems in various biological membranes through complex formation and cause the state of ion inbalance which in turn results in unexpected side effects in those systems. Studies on the action mechanism of DDT, therefore, could shed a light upon the various biological effects of other chlorinated hydrocarbon insecticides which are the major contaminants of our environment.

(References will be sent on request.)

NATURE, ART and MATHEMATICS *continued from page 24*

somewhat at random. For a smaller error, however, the arrangement will be seen to be limited to a more regular appearance. Approximation, as recognized by Galileo, involves the choice and number of parameters; it is of paramount importance in using mathematical descriptions of natural phenomena. Ptolemy's successful description of observed planetary paths was made possible by virtually an approximation series involving epicycles. By appropriate choices of radii and of frequencies, indeed, one can produce even a square orbit.

The role of mathematics in art, I believe, is similar to that in science: in both instances it is a convenient servant of man—not an autocratic queen of experience.

Under no circumstances should we overlook the fundamental role of nature itself. The same phenomenon can be regarded from both scientific and artistic aspects, which converge in the very commonness of the single exper. nce. Is it not possible that underlying relations, due to substratum forces, may emerge out of our similar methodology? Wonder as to the ever-increasing mystery of nature may be more inspiring than beauty itself—the appeal of the unknown greater than that of the known!

Suggestive of the possibility of some such deeper understanding are some pictures exhibited about ten years ago in Basel. In each case a famous painting was set along side a scientific photograph. Two comparisons are shown here: Honegger's "Composition" (Picture 2a) and a hexiacontrain crystal (Picture 2b), Taüber's "Circular Movements" (Picture 3a) and rubber latex (Picture 3b). What is the basic explanation for such remarkable similarities? (In most instances the work of art predated the scientific discovery.) Evidently the artist is unconsciously seeing something of scientific significance, the scientist something of artistic worth. What may emerge out of such convergences? Our artificial two cultures are Janus-like aspects of one world.

In conclusion, let us bear in mind the significance of man himself—more humanly distinctive, I believe, in his conscious search for reality than in the fumbling grasping of surrealism. And yet, efficient reason, though necessary, is itself not sufficient; the totality of humanity requires due regard for man's own personality, for his spirituality. Man needs to discover—and rediscover—experiential relations to nature, to man, and to God! Otherwise we may be merely performing meaningless antics. Recall the picture showing the hero Hercules fighting the giant Antaeus. Hercules had been advised that his opponent could be defeated only by being strangled in midair. The frantic struggles ("antics") of the giant would then be of no avail; only when he touched the ground woold his strength be instantly renewed. To me, this is a parable of the scientist. As long as he speculates in the rare atmosphere of isolated theory, he may merely be performing intellectual antics; his scientific strength is renewed only when he touches the bedrock of experience. Do not be hypnotized by the strangeness of the Cheshire cat's grin—it, too, finally vanishes.

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Report
of the
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on
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and
Their Relationship
to
Environmental Health

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sures occur, opportunities to make meaningful observations of health consequences should not be overlooked.

Extrapolations from occupationally exposed populations to the general population always require qualification. The most significant difference between the two groups is that the general population includes large numbers of very young, very old, ill and disabled, while such persons are found rarely or not at all among the occupationally exposed. These individuals are often least resistant to the effects of toxic agents: it is therefore apparent that dose-response relationships based on studies of formulators or other occupational groups may be extrapolated to the general population only with great caution.

Analytical complexities

An appreciation of some of the more important analytic and interpretive problems peculiar to the organochlorines is essential for perspective in this area.

A recently-discovered source of error in analysis of DDT and related materials by gas chromatography is the presence of polychlorinated biphenyls (PCB). Since 1929, polychlorinated biphenyl liquids, resins, or solids have been spread widely in our environment in oils, hydraulic fluids, adhesives, plastics, building materials, fuels, fire retardants, heat transfer agents, electrical equipment, paper, and many other industries. The presence of PCB has caused serious analytical errors in the nonspecific gas chromatographic analysis of the chlorinated hydrocarbons (Jensen, 1966; Richardson, 1969; Reynolds, 1969). It is clearly necessary to confirm qualitatively the determination of DDT residues and not to be confused by gas chromatographic peaks that overlap DDT but represent totally different materials (Schechter, 1968). Such false peaks have been reported in gas chromatograms from some wildlife samples, along with organochlorine pesticides (Roburn, 1965). Cod liver oil from Norway gave rise to gas chromatographic peaks in the region expected for DDT, DDE, and TDE, but paper chromatography indicated the presence of halogenated compounds which were not known pesticides at all (Eidelman, 1963). In samples for the Nature Conservancy in Britain, compounds were detected interfering with detection of p,p'DDT and p,p'TDE (Harrison, 1966). Since 1966, the presence of PCB has also been noted in the British (Holmes, *et al.* 1967; Holden and Marsden, 1967; Robinson, undated reference cited in Richardson, 1969), Dutch (Koeman, *et al.* 1969), and North American (Risebrough, *et al.* 1968) environments.

Polychlorobiphenyls have been identified in fish, wildlife, and the environment as cited above, but no attempt appears to have been made to look for them in human tissues or excreta. Pooled samples of

[illegible] from Colorado Springs and eight individual samples [illegible], Calif., were positive for PCB (Risebrough, 1969; personal communication). Past reports of levels of chlorinated hydrocarbon pesticides might thus have been too high as a result of the presence of PCB. A further source of error might be introduced by storage of specimens in plastic containers (Quinby, 1966). ← G.E.?

Analyses of environmental samples must be supported by qualitative and quantitative confirmation of the results. Too many papers have been published that incorporate no such quality controls; nor have potential sources of error been considered. Especially when the residues are reported as positive near the lower limits of detection of the gas chromatographic method (which is often the case), the results may be absolutely misleading. The mass of material in question is often also insufficient for qualitative and quantitative corroboration of the results by other techniques of analysis.

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Complexities of Terminology

A large body of information has been published regarding the health consequences of human exposure to pesticides. Review of these publications reveals that the scientific terminology is often imprecise and