The Mode of Action of Organic Insecticides (1948)

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THE MODE OF ACTION OF ORGANIC INSECTICIDES

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This review of the mode of action of organic insecticides, exclusive of fumigants, was prepared at the request of the Entomology Subcommittee of the Chemical-Biological Coordination Center of the National Research Council. It seemed appropriate to confine the subject matter to organic insecticides inasmuch as these materials are currently of much greater interest in the laboratory and in the field than are the older inorganic insecticides. Furthermore, the literature pertaining to the inorganic materials has been very adequately reviewed by Hoskins (1940). Emphasis in this review has been placed on papers which best represent major contributions and modern viewpoints, and some of the older works, which are of historical interest only, have been omitted. Approximately 500 papers were consulted before selecting the more than 300 references which appear in the bibliography. The material has been organized into nine chapters, each dealing with a recognized insecticide or class of insecticides. A brief review of the chemistry of each material has been presented in order to make the following discussion of toxicological, physiological, and biochemical information more intelligible. Several very interesting insecticides, notably the "veratrine" alkaloids of sabadilla and hellebore, chlordan, chlorinated camphene, xanthone, and tartar emetic have been omitted because of the lack of any comprehensive data on their properties and modes of action.

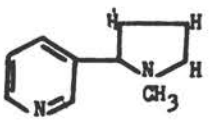
The scientific names of the insects mentioned have been corrected wherever possible to conform with those in the listing of "Common Names of Insects Approved by the American Association of Economic Entomologists" (Muesebeck, C., J. Econ. Entomol. 39, 427, 1946). The journal references in the bibliography are in conformity with the "List of Periodicals Abstracted" of the American Chemical Society, (Chem. Absts. 40, no. 24, pt. 2, 1946). Finally the writer is indebted to the excellent bibliographies of Holman (1940), Gnadinger (1936, 1945), Shepard (1940), Frear (1942), Hoskins and Craig (1946) and Roark (1936 to 1948), U.S.D.A., E-Series Publications, for suggestions as to original source material.

NICOTINE

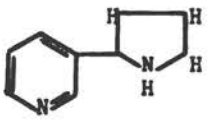
Introduction--Nicotine, as a crude tobacco extract, was used as an insecticide as early as 1763, but the pure alkaloid was not recognized until 1828, and was only synthesized in 1904 by Pictet and Rotschy. Nicotine in the free form, as nicotine sulfate, and in a variety of fixed salts and coordinate compounds has been used as a contact insecticide, a stomach poison, and a fumigant, being especially effective against aphids and other soft-bodied insects. The equivalent of 1,197,000 pounds of free nicotine was utilized for agricultural purposes in the United States in 1944.

Chemistry--Nicotine is levo-1-methyl-2-(3'-pyridyl)-pyrrolidine. The freshly distilled material is a colorless, nearly odorless liquid, b.p. 247°, sp. gr. 1.00925/20°, and vapor pressure 0.0425 mm./25°, 2.8 mm./80° C (Norton et al., 1940; Young and Nelson, 1929). Upon exposure to air, nicotine darkens, becomes more viscous, and develops a disagreeable odor. Because of its basic nature ($Kb_1 = 1 \times 10^{-6}$, $Kb_2 = 1 \times 10^{-11}$, Norton, 1940) nicotine readily forms salts with any acid and dibasic salts with many metals and acids. Nicotine sulfate, $(C_{10}H_{14}N_2)_2 \cdot H_2SO_4$, is widely used as an insecticide as it is more stable and less volatile than the free alkaloid. Nicotine is obtained commercially from the tobaccos Nicotiana tabacum and N. rustica where it occurs in the leaves of the former from 2 to 5 per cent, and in the latter from 5 to 14 per cent. The following additional alkaloids have been recorded from tobacco: nicotimine, anabasine, N-methylanabasine, iso-nicoteine, anatabine, 1-N-methylanatabine, nicotyrine, nicotelline, 2,3'-dipyridyl, nornicotine, and nicoteine; but nicotine generally comprises at least 97 per cent of the alkaloid content of commercial tobaccos (Henry, 1939). Aside from nicotine, only anabasine and nornicotine are of any importance from an insecticidal viewpoint. Anabasine, levo-2-(3'-pyridyl)-piperidine, is a water-white, viscous liquid, b.p. 280.9°/760 mm., sp. gr. 1.0481/20° (Nelson, 1934) which is soluble in water in all proportions and turns brown upon standing in air. The vapor pressure is 2.5 mm./79°. Anabasine is a basic material and readily forms salts with a variety of acids and metals. Anabasine occurs in Anabasis aphylla, a small woody perennial of central Asia, Turkey, and North Africa in concentrations of 1 to 2.6 per cent in the small green twigs, and in concentrations of up to 8 per cent (commonly 1 per cent) in the tree tobacco, Nicotiana glauca, which is common in the southwestern United States. The chemistry and uses have been reviewed by Roark (1941).

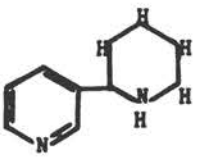
Nornicotine occurs in nature in all three forms, levo-, dextro-, and dl-. It is chemically 2-(3'-pyridyl)-pyrrolidine, b.p. 270-271°, sp. gr. 1.07/20°, and in the pure state is a colorless, viscous liquid. It appears somewhat more stable than nicotine and does not darken readily when exposed to light and air, nor does it have as pungent an odor (Markwood, 1942). Levo-nornicotine comprises 95 per cent of the alkaloid content (1 per cent) of Nicotiana sylvestris, and dextro- and dl-nornicotine are found in the Australian plant Duboisia hopwoodii, although the occurrence is variable. Bottomley et al. (1945) report that of 58 specimens of this plant examined, one had a high content of nornicotine and no nicotine, while the others all contained both alkaloids with nicotine predominating and reaching 3 to 5 per cent in some dried material. Markwood (1942) discovered that a strain of ordinary tobacco contained an alkaloid content of 0.73 per cent, of which 95 per cent was nornicotine and the remainder was nicotine. Bowen and Barthel (1943) found samples of commercial nicotine sulfate which contained as high as 12 per cent nornicotine. Nornicotine, like nicotine, is basic and readily forms salts.



Nicotine



Nornicotine



Anabasine

Toxicology of nicotine and related materials--A comparison of the relative effectiveness of a number of analogues of nicotine is given in table 2. It is evident that slight changes in the nicotine and anabasine molecules may result in marked decreases in insecticidal activity. Optical activity appears to be of importance as levo-beta-nicotine is about five times as toxic as the dextro-form, yet with nornicotine the levo-form is only slightly more effective than the dextro-form. Maximum toxicity is apparently associated with the relative points of attachment of the two nitrogen-containing rings; linkage in the 3,2- or beta, alpha-positions as in the naturally occurring alkaloids, being by far the most effective. A basic structure of two six-membered nitrogen-containing rings, as in anabasine, seems to be more active than the combination of a six-membered with a five-membered ring as in nicotine. The presence of the methyl group on the pyrrolidine nitrogen does not seem important to toxicity, as nornicotine and anabasine are slightly more effective than nicotine.

Much effort has been expended by entomologists in developing fixed nicotine preparations which would be effective stomach poisons for the codling moth and other chewing insects. Hansberry (1942) tested 31 salts of nicotine with acidic and metallic compounds as stomach poisons to the codling moth. He found that the water-soluble salts, such as the laurate, oleate, stearate, and naphthenate, were not effective, but that the water-insoluble salts, such as the Reineckate, silicotungstate, cuprocyanide, and copper ferrocyanide, were highly effective. When these materials were tested as contact insecticides, however, the laurate, oleate, linoleate, and naphthenate were highly effective, while the silicotungstate and Reineckate and other fixed nicotines were ineffective to Aphis rumicis (Hansberry and Norton, 1941). The authors consider that the toxicity of such compounds as contact insecticides is influenced by (1) toxicity of nicotine itself, (2) toxicity of acid part of molecule, (3) increase in toxicity due to good wetting properties, and (4) an unknown activating or synergistic action.

Hansberry et al. (1940) compared the toxicities of nicotine and various fixed nicotines injected internally and as stomach poisons to several species of insects. They concluded that there was no significant difference between soluble and insoluble forms of nicotine or between ingested and injected nicotine.

Since a very large proportion of the nicotine utilized for insecticidal purposes is in the form of nicotine sulfate, studies of the relative toxicity of nicotine as the free alkaloid and the sulfate are of interest. DeOng (1923) found that the toxicity of nicotine and nicotine sulfate to aphids, both as sprays and as fumigants, increased with the pH of the solutions. He ascribed this to the increased volatility of free nicotine over nicotine sulfate and considered both materials to act as fumigants.

Anabasine and nornicotine have been recorded as being more effective insecticides than nicotine. Garman (1933) found anabasine sulfate five times as toxic as nicotine sulfate to Aphis rumicis (A. fabae), while Richardson et al. (1936) found anabasine nearly ten times as effective as nicotine to the same insect. On the other hand, however, Campbell et al. (1933), in tests in aqueous solution with larvae of the mosquito, Culex pipiens, determined that nicotine was 2.6 times as toxic as anabasine and 4.8 times as toxic as methyl-anabasine at the LD_{50}.[1] Racemic nornicotine was about twice as toxic as racemic nicotine to A. rumicis (Richardson et al., 1936). Siegler and Bowen (1946) found that nicotine was much more effective than anabasine or nornicotine when tested against the codling moth, Carpocapsa pomonella. Bottger and Bowen (1946) compared the toxicity of anabasine, nornicotine, and nicotine to several species of mites and insects. Anabasine was the most toxic to the cabbage aphid, pea aphid, nasturtium aphid, and citrus red mite, with nornicotine and nicotine being about equal, while nicotine was the most toxic to the large milkweed bug. The three materials gave approximately equal results against the celery leaf tier and red spider.

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[1]Median Lethal Dosage

Table 1--Relation between dissociation constants of various alpha-substituted-N-methyl pyrrolidines and their toxicity to Aphis rumicis and Tribolium confusum (Craig, 1933)

Compound	Relative MLC[1] 24 hours	Dissociation constant
	A. rumicis	
1-nicotine	1	9×10^{-7}
dl-nicotine	2.9	9×10^{-7}
2-(p-chlorophenyl)-N-methylpyrrolidine	11	5×10^{-6}
2-(p-methoxyphenyl)-N-methylpyrrolidine	21	8×10^{-6}
2-phenyl-N-methylpyrrolidine	34	6.3×10^{-6}
2-n-butyl-N-methylpyrrolidine	45	6×10^{-5}
2-n-propyl-N-methylpyrrolidine	95	6×10^{-5}
	T. confusum	
2-phenylpyrrolidine	8 (nicotine = 1)	4×10^{-5}
N-phenylpyrrolidine	13	2×10^{-10}
pyrrolidine	36	1.3×10^{-3}
N-n-butylpyrrolidine	56	2.3×10^{-4}
N-methylpyrrolidine	310	1.5×10^{-4}
pyridine	1130	10^{-10}

[1]Median Lethal Concentration

Richardson and Shepard (1930a) have shown free nicotine to be more toxic than ionized nicotine in solution. They investigated the effects of hydrogen-ion concentration on the toxicity of nicotine in aqueous medium to larval Culex pipiens and found that the speed of toxic action was directly related to the concentration of undissociated nicotine molecules, although the nicotinium ion was somewhat toxic since toxicity was observed at pH = 2, where ionization is nearly complete. At a pH = 5, the free nicotine base was 5 to 7 times as toxic as nicotine sulfate. The toxicity of free nicotine also increased with increasing pH, reaching a maximum at the highest (most alkaline), i.e., where nicotine was almost completely undissociated. This change in toxicity with change in pH was ascribed as largely due to the dissociation of the pyrrolidine nitrogen. Craig (1933) and Craig and Richardson (1933) found a direct correlation between the dissociation constants and the toxicities to A. rumicis of a series of alpha-substituted-N-methylpyrrolidines, as is shown in table 1. Nicotine, the least dissociated, was the most toxic. This was not corroborated, however, in two other series of compounds related to nicotine tested against Tribolium and Thermobia domestica (Craig, 1931; Kirchner, 1939), where no good correlation between dissociation and toxicity was found. This work was the outgrowth of the theory of Hixon and Johns (1927) who developed the idea that the polar properties of a radical formed a measure of its electron-sharing ability or chemical reactivity.

Table 2--Relative toxicities of some derivatives of nicotine

Compound	24 hr. MLC to Aphis rumicis nicotine = 1	Reference
levo-beta-nicotine [levo-1-methyl-2-(3'pyridyl)-pyrrolidine]	1	Hansberry and Norton (1940)
dextro-beta-nicotine	5	
levo-nornicotine [levo-2-(3'-pyridyl)-pyrrolidine]	0.5	
dextro-nornicotine	0.7	
dl-beta-nornicotine	1	Richardson et al. (1936)
dl-alpha-nornicotine	31	
dl-beta-nicotine	2	
dl-alpha-nicotine	31	
anabasine [levo-2-(3'-pyridyl)-piperidine]	0.1	
nicotyrine [1-methyl-2-(3'-pyridyl)-pyrrole]	13	Richardson and Shepard (1930)
meta-nicotine	10	
dihydro-meta-nicotine	100	
methyl-meta-nicotine	ca. 33	
3-pyridylethyl-N-ethyl amine	100	
3-pyridyl-n-butyl-N-methyl amine	ca. 1600	
phenyl-n-butyl-N-methyl amine	ca. 1600	
2,2'-dipyridyl	100	Smith et al. (1930)
2,3'-dipyridyl	100	
3,3'-dipyridyl	>1000	
3,4'-dipyridyl	300	
4,4'-dipyridyl	750	
2,2'-pyridyl-piperidine	100	
2,3'-pyridyl-piperidine	50	
3,2'-pyridyl-piperidine	<5	
3,3'-pyridyl-piperidine	50	
4,4'-pyridyl-piperidine	1000	
methyl-3,2'-pyridyl-piperidine	20	
2,2'-dipiperidyl	100	

Table 2--Relative toxicities of some derivatives of nicotine (Continued)

Compound	24 hr. MLC to Aphis rumicis nicotine = 1	Reference
2,3'-dipiperidyl	100	
3,3'-dipiperidyl	100	
3,4'-dipiperidyl	<10	
4,4'-dipiperidyl	2100	
levo-2-p-tolylpyrrolidine	50	Starr and Richardson (1936)
dextro-2-p-tolylpyrrolidine	40	
pyrrole	>250	Tattersfield and Gimingham (1927)
pyrrolidine	20	
pyridine	125	
piperidine	ca. 25	
benzyl pyridine	2.5	
pyridyl-N-benzylchloride	ca. 12	

Levine and Richardson (1934) found that potassium salts had a synergistic effect on the paralytic action of nicotine on Periplaneta americana, while sodium salts had little effect. For example, with nicotine alone, the least concentration producing paralytic effects on injection was 0.001 per cent, while with nicotine in 0.1 M potassium chloride solution, effects were observed at 0.0001 per cent nicotine. Treating eggs of Musca domestica, Apple (1941) obtained the following median lethal concentrations: nicotine alone, 0.517 per cent; nicotine in N sodium chloride solution, 0.405 per cent; and nicotine in N calcium chloride solution, 1.273 per cent.

Quantitative toxicology of nicotine--The available data on the quantitative toxicology of nicotine to a number of species of insects is assembled in table 3. The silkworm, Bombyx mori, is apparently extremely susceptible to nicotine but most of the other species are not. From these data it is apparent that nicotine is only moderately toxic to most insects, as compared with the newer organic insecticides. The toxicity does not seem to vary greatly with the mode of administration, in common with other good contact insecticides.

Entrance of nicotine into the insect body--McIndoo (1916) attempted to trace nicotine in the insect body (honeybee) by treating histological preparations of poisoned insects with phosphomolybdic acid and observing the precipitates formed. He concluded that nicotine applied as spray solutions enters the insect body by way of the tracheae, probably as a vapor, and does not pass through the integument. Thus the poison has ready access to the central nervous system which is very richly supplied with tracheoles. As a stomach poison, however, nicotine appeared to be readily distributed to all the insect tissues. Later work by Richardson et al. (1934) and by Glover and Richardson (1936) has definitely proven that nicotine can penetrate directly through the insect integument. These workers, using Periplaneta americana, larval corn earworm, Heliothis obsoleta (H. armigera), and Melanoplus femur-rubrum, exposed to nicotine vapor in such a manner that no spiracles could be involved, were able to quantitatively

Table 3--Determinations of LD_{50} values for nicotine to various insects

Species	LD_{50} in micrograms per gram body weight	Method and site of administration	Reference
Periplaneta americana	ca. 500	Aqueous, external on thoracic terga	Calculated from Yeager et al. (1942)
Periplaneta americana	1200	Vapor as fumigant	Glover and Richardson (1936)
Blatella germanica	2150	Contact	Simanton according to Hansberry et al. (1940)
Lygaeus kalmii	3200	Contact	Simanton according to Hansberry et al. (1940)
Aphis rumicis	48	Contact	Simanton according to Hansberry et al. (1940)
	LD_{100}		
Lucilia sericata male	370	Subcutaneous injection	Calculated from McIndoo (1937)
Lucilia sericata female	650-890	Subcutaneous injection	Calculated from McIndoo (1937)
Calliphora erythrocephala male	1080	Subcutaneous injection	Calculated from McIndoo (1937)
Calliphora erythrocephala female	1090	Subcutaneous injection	Calculated from McIndoo (1937)
Carpocapsa pomonella larva	850	Subcutaneous injection	Calculated from McIndoo (1937)
Prodenia eridania larva	1980	Subcutaneous injection	Calculated from McIndoo (1937)
Phormia regina full-grown larva	331	Subcutaneous injection	Calculated from McIndoo (1937)
Bombyx mori larva	1440	Subcutaneous injection	Calculated from McIndoo (1937)
	LD_{50}		
Bombyx mori larva	10	Oral	Hansberry et al. (1940)
Bombyx mori larva	2.0	Injection into blood	Hansberry et al. (1940)
Protoparce quinquemaculata larva	>4650	Oral	Hansberry et al. (1940)
	ca. 2000	Injected into blood	Hansberry et al. (1940)

Table 3--Determinations of LD_{50} values for nicotine to various insects (Continued)

Species	LD_{50} in micrograms per gram body weight	Method and site of administration	Reference
Ascia rapae larva (Pieris rapae)	>3640	Oral	Hansberry et al. (1940)
	ca. 1500	Injected into blood	Hansberry et al. (1940)
Hyphantria cunea larva	>1020	Oral	Hansberry et al. (1940)
	>2500	Intra-abdominal	Hansberry et al. (1940)
Leptinotarsa decemlineata adult	>570	Oral	Hansberry et al. (1940)
Leptinotarsa decemlineata larva	>1010	Oral	Hansberry et al. (1940)

recover nicotine from wings, legs, fat body, digestive tract, ventral nerve cord, and hemolymph. This penetration readily took place through the wing, leg, or body wall. In P. americana it was found that the LD_{50} of gaseous nicotine was 1.2 mg. per gram body weight, and that the largest amount was recovered from the cuticle with about equal quantities in the muscles, fat body, digestive tract, and nerve cord. Thus the cuticle appears to play an important role in concentrating and transporting nicotine into the body of the insect. Richardson (1945) compared the rate of penetration of nicotine from aqueous solutions of varying pH (and of consequent dissociation) through the integument of P. americana. Nicotine (0.05 M) at pH 9.3 (4 per cent dissociated) produced nearly 100 per cent kill in sixteen minutes immersion, while at pH 2.8 (99.9 per cent dissociated) 83 per cent of the insects were normal after immersion. Richardson speculates that the degree of susceptibility of insects to nicotine may be largely a result of differences in rate of penetration into the body, slow penetration permitting detoxification. The roach seemed to absorb nicotine through the integument much more rapidly from air than from aqueous solution. Thus with a concentration of 0.27 mg. nicotine per liter of air, the insect absorbed 3.3 micrograms per gram body weight per minute, while with a concentration of 324 mg. nicotine per liter of water (or 1200 times the air concentration) the insect absorbed only 1.8 micrograms per gram body weight per minute.

O'Kane et al. (1933) applied minute drops of 100 per cent nicotine to a variety of body regions of larvae of Tenebrio molitor and to Periplaneta americana. The toxic symptoms developed much more quickly when the applications were made to non-sclerotized areas than when made on sclerotized structures, indicating a more rapid penetration through the thinner cuticle. Portier (1930) placed nicotine solutions on the antennae of Vanessa atalanta and Satyrus actea. The alkaloid apparently penetrated the antennal nerves and trachea and acted upon the ganglia of the central nervous system to produce violent convulsions.

Wigglesworth (1944, 1945) has shown that disruption or disorientation of the outer cement and wax layers of the epicuticle of Rhodnius either by abrasion or by the action of surface active substances is accompanied by a marked increase in susceptibility to nicotine, penetrating the integument. Normal nymphs showed only slight effects in 6 hours and severe effects but not collapse in 24 hours from the application of 2 per cent nicotine to the dorsum in a capsule, but when the area was lightly rubbed with alumina before the insecticide was applied, collapse of the insect occurred in 20 minutes.

Table 4--Properties of lipids of epicuticle

Species	Thickness of wax in microns	Wax thickness as per cent of exuvial thickness	Approximate m.p. of wax	Critical temperature of wax*
Rhodnius prolixus fifth instar	0.25	3.8	60.5	61.0.
Tenebrio molitor large larva	0.20	2.6	57-59	57.1
Calliphora erythrocephala pupa	0.18	5.8	50-55	54
puparium	0.27	0.55	indef.	41.5
Nematus ribesii last instar larva	0.095	1.65	36-42	39.3
Pieris brassicae (P. rapae) larva	0.33	4.5	57	46.2
pupa	0.4	2.4	white 67 yellow >100	66.4
Blatta orientalis	0.6	---	liquid	40.0

*Temperature where permeability of wax film 1.0 microns thick reaches 5 mg./cm.^{2}/hr.

Similarly, with 2 per cent nicotine in paraffin oil, no effects occurred in two days, but when 2 per cent nicotine was applied in cetyl ether of polyethylene glycol, the insects were dead in 24 hours. From these data, it appears that the penetration of nicotine and rotenone (p. 33) and probably most other contact insecticides through the cuticle is partially dependent at least on the properties of the surface lipids of the epicuticle. Wigglesworth (1945) and Beament (1945) have extensively investigated these lipids in different insects. Table 4 from Beament provides an indication of the variability in properties as found in several insects. The author points out that Nematus, which has a low resistance to most insecticides and to desiccation has a relatively thin layer of wax.

Physiological studies of the mode of action of nicotine--McIndoo (1916) made a very extensive study of the action of nicotine on several insects. He describes the onset of symptoms in honeybees poisoned with nicotine as (1) stupefaction, (2) hind legs paralyzed usually before other pairs, followed by wings, then other legs, (3) staggering gait, (4) falls on back, (5) tongue, antennae, and mandibles paralyzed, and (6) only occasional twitching of tarsus, antennae, or abdomen. Thus it appears that nicotine causes ascending motor paralysis of the nerve cord. In a later paper McIndoo (1937) has described the characteristic action of nicotine on several insects. Injected nicotine caused almost immediate reaction in the fly Phormia. The abdomen, legs, and wings quivered violently. The legs were usually folded together and the wings bent toward the body, while the proboscis was always extended and then retracted. Silkworm larvae showed violent convulsions followed by paralysis when injected by nicotine. In the case of Phormia, the nicotine was the more effective as the point of injection approached the ventral ganglion. Hockenyos and Lilly (1932) found that the speed of paralysis in larvae of Celerio lineata was directly proportional to the distance of the injection from the head.

Yeager and Gahan (1937) have studied the action of aqueous solutions of nicotine on the heart action of Periplaneta americana and Prodenia eridania. The roach heart was much more responsive to nicotine, showing a marked stimulation at concentrations as low as 0.0005 per cent without subsequent depression. At higher concentrations, this stimulation was followed by partial depression or complete depression and paralysis, the heart stopping in systole. Fibrillation of the heart was also observed in the roach. The larval heart behaved similarly over a higher dosage range, but stopped in diastole. According to the authors, this difference may be explainable by the assumption that the heart in the roach possesses intrinsic ganglionic cells with a low threshold of response to nicotine, while in the larva, the heart is without ganglionic cells and the nicotine must overcome a higher threshold of intrinsic nerve fibers, motor endings, and cardiac musculature. Subsequently, Yeager (1938), using a most ingenious mechanographic recording method, was able to demonstrate the effect of nicotine perfusion on the amplitude and contraction rate of isolated heart preparations of P. americana. Nicotine treatment produced an irregularity of amplitude, the diastolic fall of the heart becoming less and less as relaxation was inhibited, and the heart was eventually brought to systolic arrest. There was, however, little corresponding effect on contraction rate, and if nicotinization was not too intense or prolonged, continued washing of the preparation with fresh saline fully restored the normal heart action.

Similarly, Hamilton (1939) working with isolated heart preparations of Melanoplus differentialis, studied the effect of the application of purified nicotine alkaloid. A concentration of 0.01 per cent nicotine resulted in no loss in contractility, but a concentration of 0.1 per cent paralyzed the entire body with the exception of the heart and reduced the alary muscle response. Several applications of fresh 1.0 per cent nicotine stopped the heart and paralyzed the alary muscles. Recovery from this paralysis, however, could be effected by repeated washings in saline, even after 5 hours. The response of the intact heart to nicotine was typically a decrease in rate of beat, accompanied by pronounced irregularities due to alary muscle reaction. In preparations in which the alary muscles were clipped, it was shown that typical nicotinic action was increased amplitude, but little change in rate resulted except at high dosages which caused a slight decrease.

Yeager and Munson (1942) have investigated the effects of nicotine on the blood elements of Prodenia eridania. Exposure to nicotine vapor and the injection of nicotine peat and nicotine bentonite did not produce any obvious alterations in the normal blood cell picture, although prolonged exposure to nicotine vapor produced an abnormal vacuolization of the blood cells. Babers (1941) found that a 24-hour exposure to saturated nicotine vapor was without effect on the normal blood pH, 6.65, of P. eridania although chemical analysis indicated a concentration of about 7.15 mg. of nicotine per 100 ml. of blood. Yeager et al. (1942) attempted to investigate possible detoxification sites in Periplaneta americana by blocking hemocytes with injected carbon granules and staining pericardial nephrocytes with the vital dye Trypan blue, and then comparing the resistance of treated and untreated insects to application of aqueous solutions of nicotine at 0.55 mg. per kg. of body weight. The blocking of the hemocytes resulted in a slightly increased susceptibility to nicotine, but the staining of the nephrocytes was without effect. The investigators consider that these two groups of cells in the insect may have a functional analogy to the reticulo-endothelial system of the vertebrate.

Coon (1944), using sodium fluoresceinate injected into the cercus of P. americana as a fluorescent tracer, found nicotinization to cause an irregular decline in the rate of heart beat which continued long after paralysis of the appendages, and apparently impeded normal circulation.

Site of action of nicotine--Roeder and Roeder (1939) have studied the effects of nicotine and other drugs on the spontaneous electrical activity of the isolated nerve cord of P. americana. Perfusion of such a preparation with nicotine at 1×10^{-5} M

produced an appreciable increase in activity, causing spikes of recorded waves to increase two to three times in amplitude. With the application of nicotine at 1×10^{-4} M an enormous burst of activity occurred, and spikes of seven to ten times normal resulted; but within two minutes complete inactivity ensued. Nicotine at 1×10^{-3} M caused complete inactivity, but the normal activity usually returned upon washing the preparation in fresh saline. Nicotine exerted a more pronounced blocking effect on the nerve impulse transmission than did eserine.

Yeager and Munson (1945), in connection with a study of DDT, performed several experiments by injecting nicotine solutions into various sites of the body of Periplaneta americana. Injection into isolated legs or the attached leg of a roach with a cauterized heart produced no effects, but injection of nicotine into the ganglionic region of a normal roach, a cauterized roach, or a leg ganglion preparation produced violent tremors in the legs, which ceased when legs were severed from the body. It was concluded that nicotine could produce violent tremors by action on a single ganglion but seemed not to excite motor fibers. Injection of nicotine in a pinhole in the insect eye of a cauterized roach caused violent tremors in the whole body, which were abolished posterior to the neck by decapitation. Pretreatment with DDT did not prevent the appearance of symptoms of nicotine poisoning.

Richards and Cutkomp (1945) found that nicotine had no in vitro inhibitory effect on cholinesterase activity in bee brains.

Welsh and Gordon (1947) found that nicotine applied to the nerve axons of crustacea and insects (P. americana) caused a characteristic multiplication of nerve impulses, a single electrical stimulus resulting in a train of many impulses. Similar action was reported for DDT, the pyrethrins, and a variety of other compounds. The authors suggest that this effect is a nonspecific action resulting from surface effects on the nerve axon and is characteristic of substances having a high lipid/water solubility ratio. It is difficult to see that nicotine meets this latter qualification as the oil/water distribution ratios of 5 per cent nicotine solutions were determined by Norton (1941) and found to be 1.27 for fish oil, 1.09 for corn oil, 1.1 for olive oil, and 1.01 for peanut oil at 25°. For the purposes of comparison, the oil/water distribution coefficient of a 5 per cent DDT solution, using olive oil, must be in the vicinity of 999,000 using the values of 10.5 g./100 ml. for olive oil solubility at 37° (Van Oettigen and Sharpless, 1946), and 0.1 p.p.m. for water solubility at 18° (Gavaudan and Poussel, 1947).

The evidence given above suggests that nicotine acts primarily on the ganglia of the insect central nervous system, possibly at the synapses, causing excitation at low concentrations and depression or paralysis at high concentrations. It apparently has little or no action on the nerve fibers or myoneural junctions.

Gause and Smaragdova (1939) developed a theory that nicotine acted by interfering with the acetylcholine receptors in the synapses. They studied the effects of dextro- and levo-nicotines on a number of vertebrates and invertebrates. In vertebrates, levo-nicotine was, on the average, 2.8 times as effective as dextro-nicotine. In invertebrates known to possess the acetylcholine-cholinesterase mechanism, the ratio was 2.6 to 1. The authors stated that there was a complete coincidence between the presence of acetylcholine mediation and the higher toxicity of levo-nicotine. It is of interest here that no difference in toxicity of the two isomers was shown by the single insect studied, the larva of Drosophila, and the authors concluded that this was evidence for the nonexistence of acetylcholine mediation in insects. This appears to be an erroneous idea in view of the large amount of data on the presence of acetylcholine in insect nervous systems (see page 61), and the fact that Hansberry and Norton (1940) found levo-nicotine seven times as effective as dextra-nicotine to Aphis rumicis.

Physiological action of anabasine--Rotman (1936) studied the action of 0.5 per cent

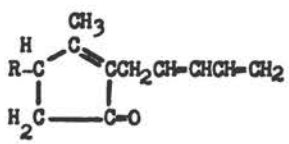
anabasine solution on larvae of Pteronus ribesii. The insects were paralyzed in 2 to 15 minutes after dipping in this solution, and it was concluded that the action was largely on the neuro-muscular system. When a 1 per cent anabasine sulfate solution was used, the respiratory rate was also strongly increased, but soon returned to normal. Tarasova (1936), studying the same insect, found that application of 1 per cent anabasine sulfate resulted in strong movements, the emission of fluid from mouth and anus, and paralysis in about 7 to 10 minutes. The rate of pulsation of the dorsal vessel increased to four times normal. This increase was much less noticeable when the ventral nerve cord was severed. The application of solutions of anabasine directly to the nerve cord of larval Pieris brassicae produced immediate stoppage at concentrations as low as 0.00001 per cent. Ivanova (1936) suggested that anabasine produced narcosis of the hypodermal cells since it facilitated the penetration of fluids through the integuments of Pieris brassicae and Pteronus ribesii.

The penetration of anabasine and anabasine sulfate from aqueous solutions through empty larval skins of Chironomus plumosus using enclosed Paramecium as an indicator has been investigated by Iljinskaya (1946). The ability of the materials to penetrate was expressed as a ratio, $K = C_1/C_2$, where C_1 is the minimum lethal concentration using skins treated with alkali, which were completely permeable, and C_2 is the minimum lethal concentration using untreated skins. With anabasine alkaloid $K = 0.63$ and $C_1 = 0.125$, and with anabasine sulfate $K = 0$ and $C_1 = 7.5$. These degrees of effectiveness were correlated with tests on Aphis pomi where anabasine at 0.1 per cent gave 94 per cent mortality, and anabasine sulfate at 0.5 per cent gave 67 per cent mortality. It was concluded that undissociated molecules of anabasine readily penetrate insect cuticle while the free cations of the anabasine sulfate solution do not penetrate appreciably.

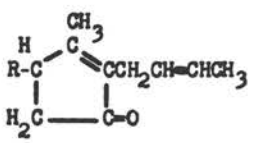
PYRETHRUM

Introduction--The use of pyrethrum powder as an insecticide apparently originated in Persia several hundred years ago. The material was introduced into Europe at an unknown date and its manufacture begun about 1828. The researches of Staudinger and Ruzicka resulted in the partial identification of the active chemical constituents in 1924, but new discoveries as to the exact compounds concerned are yet being made due principally to the work of La Forge and Barthel. The constituents of pyrethrum flowers are unique among insecticides in the rapidity with which they paralyze insects affected, and they are very widely employed in fly sprays, household insecticides, and for certain agricultural pests. About 12,000,000 pounds of pyrethrum flowers were imported into the United States in 1941, principally from Japan and Kenya.

Chemistry--Following the researches of Staudinger and Ruzicka (1924), it was generally considered that the active constituents of the pyrethrum flowers were two esters, pyrethrins I and II, formed from an alcohol pyrethrolone, b.p. 110-112°/0.1 mm., and two acids, chrysanthemum monocarboxylic acid, b.p. 135°/12 mm., and chrysanthemum dicarboxylic acid-monomethyl ester, b.p. 140°/0.5 mm. Recently, however, the researches of La Forge and Barthel (1945a,b,c) have disclosed the presence of another alcohol, cinerolone, b.p. 120-124°/1-2 mm. Both pyrethrolone and cinerolone exist in optically active and racemic forms. La Forge and Barthel state that the classically defined terms pyrethrin I and pyrethrin II should be regarded as defining only groups of esters characterized by the acid component, the former containing a mixture of pyrethrin I and cinerin I, and the latter pyrethrin II and cinerin II. La Forge and Barthel (1947) have prepared these four constituents from both optically active and racemic pyrethrolone and cinerolone. They remark that the cinerins are more stable than the corresponding pyrethrins. The four dihydro-esters, in which the double bonds in the acid constituent have been saturated, and a tetrahydropyrethrin I, in which both double bonds in the pyrethrolone side chain have been saturated, were also prepared. Soloway and La Forge (1947) and Dauben and Wenkert (1947) have synthesized 2-butyl-4-hydroxy-3-methyl-2-cyclopenten-1-one and 2-amyl-4-hydroxy-3-methyl-2-cyclopenten-1-one respectively, which were found to be identical with dihydrocinerolone and tetrahydro-pyrethrolone prepared from natural sources. Thus the structural formulae for the four active constituents are apparently as follows:

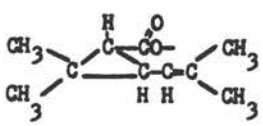


pyrethrins I and II

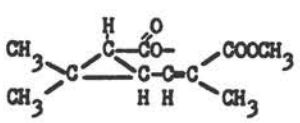


cinerins I and II

For pyrethrin I and cinerin I, R =



For pyrethrin II and cinerin II, R =



Acree and La Forge (1937) found that esters of pyrethrolone (and presumably cinerolone) with palmitic and linoleic acids also occur in oleoresin of pyrethrum. These acids also occur in the free state. Campbell and Harper (1945) succeeded in synthesizing chrysanthemum monocarboxylic acid in three crystalline isomers, dl-trans, m.p. 54°, dl-cis, m.p. 116°, and l-trans, m.p. 17-21°.

The commercial source of the pyrethrins and cinerins is in the flowers of Chrysanthemum cinerariaefolium, where they occur in amounts ranging from 0.7 to 3 per cent, of which more than 90 per cent is in the achenes (Gnadinger, 1936).

The pyrethrins are highly unstable in the presence of light, moisture, and air. Whole flowers decompose more slowly than ground flowers or dusts, and the potency of the material can best be preserved in sealed, light-proof containers at low temperature. Various antioxidants, such as hydroquinone, pyrogallol, and pyrocatechol, have been shown to greatly retard the destruction of the pyrethrins in storage, but have not proven of value in preserving insecticidal residues (Gnadinger, 1945). West (1943) studied the changes in the pyrethrins occurring in storage and suggests that a polymerization probably occurs involving the pentadienyl sidechain.

Relation of chemical structure to toxicity--Staudinger and Ruzicka (1924) showed that, while the pyrethrin esters were highly toxic to certain insects, the alcohol pyrethrolone and the two carboxylic acids in the uncombined form were nontoxic. They also prepared many esters of pyrethrolone with various acids, and of chrysanthemum monocarboxylic acid with various alcohols and phenols, but found none of the products to compare with the pyrethrins in activity to insects. The specific compounds, however, were not isolated and identified. Harvill (1939) prepared a series of aliphatic esters of chrysanthemum monocarboxylic acid and tested these compounds as sprays against Aphis rumicis. The cetyl, lauryl, myristyl, and diethanol amine esters were nearly as toxic as the pyrethrins to the aphid, but when applied to the bodies of Periplaneta americana the compounds failed to produce the characteristic symptoms of pyrethrum poisoning. Hydrogenation was found to largely destroy the knockdown of pyrethrins I and II to the housefly and decidedly lowered the kill obtained (Haller and Sullivan, 1938).

Considerable effort has been expended in determinations of the relative toxicities of pyrethrins I and II, although the unsuspected presence of the cinerins makes the value of the data somewhat uncertain. Gnadinger and Corl (1929) found the 24-hour median lethal concentration for suspensions in water sprayed on Blatella germanica to be 10 mg. per liter for pyrethrins I, and 12.5 mg. per liter for pyrethrins II. The same authors (1930) tested these materials as kerosene sprays against the housefly. They concluded that pyrethrin II was about 80 per cent as toxic as pyrethrin I, the respective LD_{50}'s being I - 65 mg./100 ml., and II - 85 mg./100 ml. Tattersfield et al. (1929), using pyrethrins sprayed in aqueous saponin solutions on Aphis rumicis, found the 24-hour median lethal concentration of pyrethrin I to be 0.001 g. per 100 ml., and pyrethrin II to be 0.01 g. per 100 ml. Pyrethrolone, chrysanthemum monocarboxylic acid, and chrysanthemum dicarboxylic acid, monomethyl ester, were nontoxic at 0.2 g. per 100 ml. Hartzell and Wilcoxon (1936), using acetone solutions diluted with water as direct sprays against A. rumicis, found concentrates of pyrethrin I considerably more toxic than concentrates of pyrethrin II, while when miscible oil was used as a solvent, the extracts were of nearly equal toxicity. The same extracts in kerosene sprayed on houseflies, or applied directly to the flies from a pipette, were about equally toxic. Comparisons of the toxicity of pyrethrins concentrates in heavy mineral oil sprays using adult Tribolium castaneum (Martin, 1943) showed that the two pyrethrins were about equally effective, but when alcoholic solutions were diluted with water and sprayed, the pyrethrin I concentrate was many times as effective as the pyrethrin II concentrate. Sullivan et al. (1938) found in spray tests on houseflies that kerosene solutions of pyrethrin I concentrate produced two times the mortality in 24 hours as did concentrates high in pyrethrin II, but the 10-minute knockdown with pyrethrin II was

about 3.5 times that of pyrethrin I. They concluded that the more rapid knockdown of pyrethrin II might be the cause of its lower kill, as the flies did not have as long a time of flight in which to accumulate a greater dosage of the material. McGovran et al. (1941), using a micropipette to apply measured drops of pyrethrins in kerosene to Periplaneta americana, determined that pyrethrin II concentrates caused a more rapid knockdown than did pyrethrin I concentrates, while the latter produced a slightly higher mortality. For example, the concentrations for 50 per cent knockdown in 30 minutes were 1.0 mg./liter for II, and 1.5 mg./liter for I, while for 50 per cent kill in 24 hours, the concentrations were 1.5 mg./liter for II and 1.0 mg./liter for I.

The most significant work in the determination of the relative toxicity of the various components of pyrethrum is that of Gersdorff (1947) who has obtained toxicity data on the true pyrethrins I and II, and the cinerins I and II, as well as their hydrogenated products. The compounds were tested against houseflies using kerosene extracts. No differences could be demonstrated between optically active and racemic compounds prepared from optically active and racemic pyrethrolone and cinerolone. A summary of the results obtained is presented in the following table:

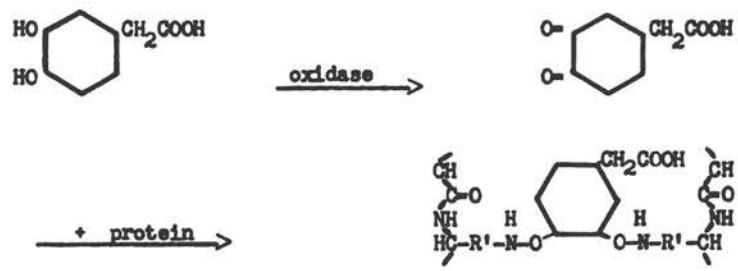
Table 5--Toxicity of components of pyrethrum

Compound	Relative median lethal concentration to housefly pyrethrin I = 1
pyrethrin I	1.0
pyrethrin II	4.3
cinerin I	1.4
cinerin II	5.8
isodihydropyrethrin I	2.0
isodihydropyrethrin II	nontoxic at level of std.
isodihydrocinerin I	3.6
isodihydrocinerin II	nontoxic at level of std.
tetrahydropyrethrin I	>16

Theories of toxic action--Lauger et al. (1944) consider that a highly effective contact insecticide must possess a toxic component (toxaphore) and must have groups attached which absolutely insure pronounced lipid solubility (see under DDT). With reference to the pyrethrins, these authors consider that the cyclopropane ring, the methyl, dimethylethylene, and allene groups are responsible for the lipid solubility of the molecule. The question of the toxaphore cannot be so simply defined, but Lauger considers it to be $-\underset{L}{C}=\underset{L}{C}-CO-O-L$, where L is the lipid solubilizing group. Here again it should be emphasized that this is a highly theoretical viewpoint and is not adequate to explain the loss in effectiveness associated with minor alterations in the molecule.

Hurst (1945) discusses the similarity in action of the pyrethrins and of DDT as indicated by a dispersant action on the lipids of insect cuticle and internal tissue. He has developed an elaborate theory of contact insecticidal action although experimental data are not provided. Hurst believes that susceptibility to insecticides depends partially on cuticular permeability but more fundamentally on the effects on internal tissue "receptors" which control oxidative metabolism, i.e., oxidative enzyme systems. The

access of the pyrethrins to insects, for example, is facilitated by adsorption and storage in the lipophilic layers of the epicuticle. The epicuticle is to be regarded as a lipo-protein mosaic (Hurst, 1943) consisting of alternating patches of lipid and protein receptors which are sites of oxidase activity. Such a condition exists in both the hydrophilic type of cuticle found in larvae of Calliphora and Phormia and in the waxy cuticle of Tenebrio larvae. Dennell (1946) has also found a similar lipo-protein complex in the epicuticle of Sarcophaga. Wigglesworth (1946) has discussed the presence of polyhydric phenols, such as dihydroxyphenyl acetic acid, in the second layer of the epicuticle of Rhodnius, for example. According to Pryor (1940a,b), phenols (probably 3,4-dihydroxyphenyl acetic acid) are oxidized to quinones by the action of oxidases present in the cuticle, and these quinones react with proteins linking together protein molecules and converting the whole of this epicuticular layer (the innermost layer (1) of the epicuticle in Rhodnius) into a polymerized lipo-protein tanned with quinones.



The result has been described as being analogous to the tanning of gelatin films with para-benzoquinone. The effect of such a process is to convert a soft colorless substance into a horny, brown, insoluble, and hydrophobic product characteristic of most insect epicuticle. Fat solvents which have been shown to increase the permeability of the pyrethrins (p. 21) also increase the rate of tanning by quinones, so that the site of oxidase action appears more reactive to an oil solution. Such lipid solvents penetrate the cuticle primarily through the lipid patches and have the net result of exposing the enzyme receptor patches to the action of the insecticide. Thus, Hurst would explain pyrethrinization as a preliminary narcosis or "knockdown" phase in which oxidase action is blocked by adsorption of the insecticide on lipo-protein tissue components, followed by death when further dispersant action of the insecticide results in an irreversible increase in phenoloxidase activity as a result of displacement of protective lipids. This increase in phenoloxidase activity is accompanied by the accumulation of toxic quinoid metabolites in the blood and tissues; as, for example, reactive ortho-quinones which would block substrate access to normal enzyme systems. The varying degrees of susceptibility shown by different insect species to an insecticide may be explainable not only in terms of differences in cuticle makeup but also as internal factors associated with the stability of oxidase systems. Thus, in Musca larvae concentrations of ethyl alcohol in the hemolymph which decreased tissue phenolase activity and induced narcosis, increased phenoloxidase activity in Tenebrio larvae and were lethal.

Entrance of pyrethrins into the insect body--The pyrethrins are generally considered to be strictly contact insecticides and to have little stomach poison action. Voskresenskaya (1938) fed pyrethrum on leaves to Agrotis segetum, Pieris brassicae (P. rapae), Porthetria dispar, and Locusta migratoria. This resulted in regurgitation, spasmodic contractions, and in quiescence but the insects recovered. Hockenyos (1936) found that the ingestion of pyrethrum powder by Blatta orientalis produced symptoms of discomfort but no effects after 12 hours. Woke (1939) found, upon feeding the pyrethrins in leaf sandwiches to sixth instar Prodenia eridania, that the larvae were unaffected, although they were susceptible to pyrethrins poisoning by contact. By using mosquito larvae for a bio-assay, it was shown that little or no pyrethrins remained in the tissues, digestive tract, or feces, in 6 to 24 hours after ingestion. On the other hand, Bottcher

Table 6--Determinations of LD_{50} values for pyrethrins to insects

Species		LD_{50} in micrograms per gram body weight	Method and site of administration	Reference
Cimex lectularius		5	Direct spray-contact (calculated)	Busvine (1946)
Pediculus humanus		42	Direct spray-contact (calculated)	"
Musca domestica	male	31	Direct spray (calculated)	David (1946)
	female	38	Direct spray (calculated)	"
Aedes aegypti	male	0.5	Direct spray (calculated)	"
	female	1.0	Direct spray (calculated)	"
Periplaneta americana	male	1.25	4-day mortality from measured drop of kerosene solution on dorsum	Calculated from McGovran et al. (1941)
	female	2.5		
Apis mellifera	20°C	0.5	Stomach poison from alcohol 3 days	Calculated from Bottcher (1938) using 0.1 g. for wt. of bee
	34.5°C	5		

(1938) showed that the pyrethrins were highly toxic as stomach poisons to the honeybee.

The pronounced contact action of the pyrethrins has been demonstrated by O'Kane et al. (1933) who applied droplets of 15 per cent pyrethrins in kerosene to various areas of the integument of Tenebrio molitor larvae and Periplaneta americana adults. Typical toxic symptoms developed from the application of the insecticide to such diversified areas as antennae, cerci, legs, head, spiracles, abdomen, and thorax. Applications made to the cervical region and to other intersegmental areas resulted in the development of toxic symptoms in about one-half the time required when applications were made on highly sclerotized regions. Hartzell and Wilcoxon (1932) noted that a drop of pyrethrins applied to the tarsus of the rose chafer, Macrodactylus subspinosus, was rapidly fatal, and Potts and Vanderplank (1945) found that residues of pyrethrins paralyzed Glossina adults in a few seconds when applied to the puivilli for periods as short as two seconds.

The application of 25 per cent dry pyrethrum powder to Blatta orientalis produced the following symptoms (Hockenyos, 1936): no reaction for 1 1/2 minutes, then sudden and intense excitement for 2 minutes, followed by paralysis beginning with the metathoracic legs and spreading to others; and the insect was completely helpless in 8 minutes. Applications of the powder to the tracheae were not effective. The action of

the pyrethrum powder seemed to be localized, as application to the junction of the head and thorax paralyzed only the antennae, while applications on the thoracic sternum caused only partial paralysis followed by recovery. It was found that, unless one-half or more of the roach body were covered, total paralysis within 12 hours could not be ensured. Roy et al. (1943) found that dry pyrethrum powder introduced into the spiracles of Periplaneta americana produced characteristic symptoms of the legs in the same order as those following the injection of kerosene-pyrethrins solutions. They postulate that the pyrethrins are solubilized by moisture in the lumen of the trachea, and then pass by diffusion through the tracheal walls into the hemolymph. This was confirmed by the diffusion of dye solution injected into the spiracles through the tracheae and into the nearest ganglion.

The most careful study of the mode of entry of the pyrethrins into the insect body is that of Wigglesworth (1942) using Rhodnius prolixus. It was found that the onset of toxic symptoms produced by pyrethrins in oils could be correlated with the boiling point of the oil, lighter fractions such as hexane and heptane acting more rapidly than higher boiling mineral oils. Vegetable oil solutions produced a very slow reaction. Oleic and other fatty acids accelerated the penetration of pyrethrins dissolved in mineral oils. This is shown in the following table:

Table 7--Effect of pyrethrin solvents

Solvent with 2 per cent pyrethrins		Approximate time for paralysis to occur in fifth instar Rhodnius prolixus
hexane		1.25 hours
heptane		1.5 hours
white spirit	b.p. 150-190°C	2.0 hours
white oil	b.p. 265-365°C	5.0 hours
" "	b.p. 310-390°C	10.0 hours
oleic acid		4.5 hours
olive oil		more than 24 hours

Pretreatment of the cuticle with petroleum ether greatly accelerated the reaction time, presumably by dissolving away interfering surface lipids. It was found that the individual response varied considerably and this could be correlated with the thickness of the insect cuticle. For example, the following cuticular thicknesses and paralysis times were noted: 8 to 9 microns, 1 1/2 hours; 10 microns, 2 hours; 18 microns, 8 hours. Further exploration showed that cuticle thickness was correlated with the number of times the insect had fed; the paralysis times averaging 4 hours in unfed nymphs and 14 hours for nymphs fed repeatedly. The only histological difference appeared to be in the endocuticle, and Wigglesworth believes that the pore canals are important in passage of the pyrethrins through the cuticle. In the nymphs, pyrethrins in oil were first taken up by the epidermal cells in the zones around the bristles, and later by the general epidermis, and in older insects by the dermal glands. Kruger (1931), in observations of Corethra plumicornis immersed in a suspension of pyrethrins in water, found that after one day's exposure the hypodermal cells of the body wall began to degenerate, vacuolize, and separate.

In connection with the age of the insect as related to the penetration or suscepti-

bility to the pyrethrins, it has been shown that the older larvae of the tick, Ornithodorus moubata, respond more slowly to immersion in pyrethrins-containing solutions than do the young larvae (Robinson, 1942). This is explained as a result of greater cuticle thickness in the older animal. In contrast, however, Simanton and Miller (1937) and Anderson and Hook (1941) found young housefly adults to be more quickly paralyzed but less readily killed by pyrethrins sprays than older flies. Pepper and Hastings (1943) found that pyrethrum dusts and sprays were highly effective against first, second, and third instar larvae of the sugar beet webworm, Loxostege sticticalis, but that the material was practically ineffective against fifth instar larvae. They showed that fat content of the larval exo-skeleton decreased from 11.7 per cent in the third instar to 0.2 per cent in the fifth instar. They feel that a membrane with a very small percentage of fatty materials would present a much greater barrier to oil-soluble substances, such as pyrethrins, than one containing a considerable amount of fat as in the younger larval instars.

Hurst (1943) discusses factors involved in the penetration of the pyrethrins through the "lipo-protein mosaic" which composes the insect cuticle, although his conclusions are largely unsupported by experimental evidence. It is stated that the concentration of pyrethrins necessary to produce primary narcosis of the insect decreases as the proportion of water in the insecticide mixture increases. This phenomenon is attributed to the establishment of scattered functional lipoid/water interfaces on the surface of the cuticle at which adsorption occurs. The increase in concentration presented at this two-dimensional interface is thus greater than the corresponding decrease in concentration in the insecticide solution. Hurst states that the penetration of pyrethrins (and other drugs) into lipo-protein systems is facilitated by nonpolar fat solvents, such as cyclohexane and carbon bisulfide. Saturated aromatic compounds have a lower degree of action, and unsaturated aromatics are least effective. This decrease in effectiveness is associated with a progressive increase in capillary activity. It is concluded that the active concentration of capillary active drugs in an aqueous carrier does not necessarily indicate the active concentration at the interface between the carrier and the insect cuticle, owing to the possibility of selective adsorption at functional lipid/water interfaces. In this connection, Bredenkamp (1942) observed that larvae of Lymantria monacha dusted with pyrethrum at 21°, showed symptoms of poisoning in an average time of 518 seconds at 30 per cent, and 364 seconds at 100 per cent relative humidity. When the larvae were sprayed with water before dusting, symptoms occurred at 30 per cent relative humidity in 378 seconds for the wetted larvae, and 419 seconds for unwetted controls; and at 100 per cent relative humidity in 371 seconds for wetted larvae, and 392 seconds for unwetted controls. In experiments using cuticle removed from living larvae and tested for permeability, Bredenkamp found that solvents having great surface activity, such as alcohols, aldehydes, ethers, and esters, penetrated readily, but those with little surface activity, such as amino acids and disaccharides, passed on slightly or not at all. Fatty acids and paraffins also failed to penetrate. The author concluded that permeability is a purely chemical process.

Hurst (1940) reports experiments on Calliphora erythrocephala larvae immersed in mixtures of polar and apolar solvents, such as ethyl alcohol and paraffin oil, in which penetration of the alcohol was so rapid that the larva dies in a few seconds, and swells and bursts within 4 to 6 minutes, although neither alcohol nor paraffin oil alone was effective within one hour. Thus Hurst believes that the thin outer lipoid layer of the cuticle is relatively impermeable to polar compounds, and the inner chitinous layer is permeable to both polar and apolar compounds, and that the permeability of the outer lipid layer to polar compounds is greatly increased by the presence of apolar substances. Wigglesworth (1941a) cites further effects of polar and apolar mixtures. Xylene and mineral oils were observed to draw water droplets out of the cuticle of immersed insects. The presence of ethyl alcohol and kerosene caused a very rapid separation of water from the insect. The author considers the partition coefficient of a

toxic material between oil/water to determine the rate at which it will leave its oily carrier and enter the insect.

General toxic effects of pyrethrins--Hartzell and Wilcoxon (1932) have described the reaction resulting from placing a drop of pyrethrins solution on the dorsum of the tomato worm, Protoparce. The larva acts normally for about 30 minutes, when the last pair of prolegs is affected, the larva lifting the segment without the use of the legs. This segment then becomes paralyzed. The insect regurgitates, rolls over and over for a period of about 15 minutes, then movements become very uncoordinated and violent for 30 minutes when the larva can no longer crawl, and it dies in about 24 hours. An axial gradient in reactivity was found to exist as a drop placed on the head caused a response in about 8 minutes. Injection of pyrethrins into the last abdominal segment produced intoxication in 2 minutes. In Rhodnius prolixus (Wigglesworth, 1942), pyrethrinization produced the following sequence of symptoms: (1) incoordination of hind legs, (2) all legs incoordinated but still can walk, (3) insect unable to walk, proboscis progressively extended, (4) final paralysis which may last 10 to 20 days during which time the heart continues to beat and gut and leg motions are observable. Hutzel (1942) measured the activation rate of the pyrethrins on Blattella germanica by an entomographic method. After a short latent period averaging 2 seconds with oil solutions, and 5.5 seconds with dusts, there followed a period of intense excitement during which the running rate of the insect increased from 3 to 11 cm. per second. The second phase of poisoning was submaximal activity in which the leg muscles showed signs of incomplete relaxation.

Latent or secondary effects of pyrethrinization have been described by Klinger (1936) and by Sweetman and Gyrisko (1944). These latter workers found in Thermobia domestica, the firebrat, recovering from sublethal doses of the pyrethrins, the development of discolored areas and the sloughing off of appendages, such as legs, antennae, cerci, palpi, and ovipositor. Such injury occurred as long as 19 weeks after exposure.

Action on physiological systems--In Corethra larvae, pyrethrinization in aqueous medium slowed the heart rate from a normal of 21 to 24 beats per minute to 15 the first day, and 4 to 6 after several days (Kruger, 1931). Belleuvre (1938, as quoted by Hoskins, 1940) found the heart beat in larval Galleria mellonella to be slowed by prolongation of diastole, while Yeager et al. (1935) found that the pyrethrins extracts stopped the heart of Blatta orientalis in systole. Inasmuch as the heart action of pyrethrinized insects may continue for 10 to 20 days after paralysis (Wigglesworth, 1941), it seems very unlikely that this effect is of importance as a cause of death. Wigglesworth (1941) investigated the effect of the pyrethrins on the spiracular mechanism of Cimex lectularius and Rhodnius prolixus. He found that the spiracles in poisoned Cimex remained closed and opened normally in the presence of carbon dioxide. Pyrethrins paralyzed Rhodnius did not lose weight at a significantly greater rate than did normal insects deprived of food and water, with the exception of the first day where the loss was slightly greater due probably to the spasmodic hyperactivity. Poisoned insects weighing 100 to 200 mg. lost an average of 2 to 4 mg. per day, while insects with their spiracles opened by exposure to 10 per cent carbon dioxide lost 9 to 12 mg. per day. It was concluded that death was not caused either by desiccation from open spiracles or starvation (compare with DDT, p. 63).

McGovran et al. (1944) were unable to demonstrate that blocking of the hemocytes with carbon granules or the nephrocytes with Trypan blue could alter the effects of the pyrethrins on Periplaneta americana. Woke (1939) using mosquito larvae as a bioassay found that the incubation of certain body tissues of larval Prodenia eridania for 18 hours at 29° with pyrethrum extracts would greatly reduce the toxicity. The fat body was most effective, with the skin and muscle less so, and the blood, digestive tract, and gut contents were essentially ineffective.

Locus of action of pyrethrins--The characteristic paralytic effects of the pyrethrins and their very rapid action clearly indicates a primary action on the insect central nervous system. Hutzel (1942a) applied surgical technics to a study of pyrethrinized P. americana. When pyrethrins were applied to the abdomen, and the nerve cord subsequently severed at the third abdominal segment, no effects were observed in the legs, but the abdomen twitched in a characteristic manner. Applications to the thorax, however, caused twitches in the isolated legs. Complete isolation of the abdomen behind the third segment with the exception of the nerve cord did not prevent the onset of pyrethrins action, and even with the nerve cord severed, applications to the abdomen resulted in death. Pyrethrins applied to the cut end of an isolated leg resulted in fibrillation of the leg muscles and slow contraction when the solution crept up the main tracheal trunk. Roy et al. (1943) found that pyrethrins injected into abdomen or into a spiracle produced a progressive weakening or pseudoparalysis in the legs, starting with the leg innervated by the ganglion nearest the point of injection, passing to its opposite leg, and so on, either cephalically or caudally, depending on the point of injection. If a leg was severed during the stage of paralysis, it continued to contract or relax for one-half hour or more. However, if pyrethrins were applied directly to an exposed thoracic ganglion, the legs innervated by it were immediately paralyzed. If the nerve cord was severed in the upper part of the ganglion, pyrethrins applied to the abdomen, posterior to the cut, still resulted in leg paralysis. These data indicate that the pyrethrins, whether injected into the body cavity or into a spiracle, find their way into the hemolymph and then are carried to the ganglia. The particularly localized action is shown by the effect on one-half of a ganglion before the other. Lowenstein (1942) has developed an interesting application of the measurement of nerve action potentials in the abdominal nerve cord of Blatta orientalis, as a physiological assay for pyrethrum extracts. He found that there was an approximate correlation between the potential produced by the application of 1.6 per cent pyrethrins externally and by 0.3 per cent pyrethrins applied directly to the nerve cord.

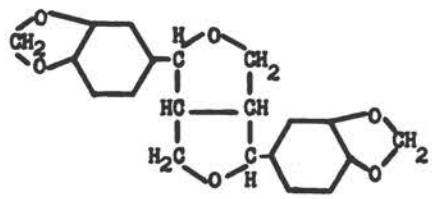
Welsh and Gordon (1947) have described the action of the pyrethrins on nerve-muscle preparations in crustaceans and Periplaneta americana. They note a similarity of action to that of DDT. The pyrethrins at 0.01 to 0.1 p.p.m. were found to act on the nerve axon where they produced a regular, rhythmic, spontaneous nerve discharge, but the effect was less persistent than that of DDT, which may be due to the chemical instability of the pyrethrins molecules. The pyrethrins acted very quickly on the nerve trunks, spontaneous discharges resulting in one minute after the application at 1 p.p.m. as compared to 15 to 60 minutes after the application of 10 p.p.m. of DDT. Concentrations greater than 1 p.p.m. of the pyrethrins usually resulted in a blocking of nerve conduction which could be quickly reversed by perfusion with saline. The authors believe that this spontaneous activity is the result of a nonspecific surface effect on the nerve axon (see DDT, p. 59).

The primary effects of pyrethrinization on the ganglia have been confirmed by a number of workers. Kruger (1931) was able to observe in living transparent Corethra larvae, the appearance of vacuoles in the ganglia and connectives of the nerve cord within 10 to 20 minutes after the onset of convulsions. The vacuoles made their appearance in the nerve fibers rather than in the cells. It is significant that no vacuolization was observed even after one day following convulsions from sublethal doses, so that this phenomenon probably represents only the extreme manifestation of toxicity. Etherization of poisoned larvae prevented the convulsions and yet had no effect on the appearance of vacuoles. Histological studies confirmed the greatly altered appearance of the normal nerve tissue. Hartzell (1934) made a detailed histological study of the nervous systems of Melanoplus femur-rubrum and Tenebrio molitor larvae poisoned by external applications of pyrethrins. After 16 hours, lesions were observed in the brain, subesophageal ganglion, thoracic ganglia and connectives. These showed marked vacuolization and disintegration of tissue and tigrolysis, which was greatest in the brain and less in the connectives than in the ventral ganglia. The lesions were remarkably

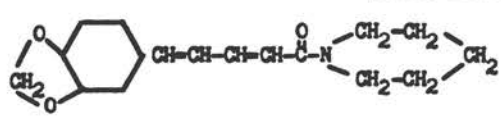
similar to those produced by tri-ortho-cresylphosphate in mammals (Lillie and Smith, 1932), and by the venom of the wasp, Sphecius speciosus, on the cicada, Tibicen pruinosa (Hartzell, 1935). Hartzell concluded that death from pyrethrinization was caused by destruction of cells in the central nervous system. Wigglesworth (1941) examined the central nervous system of Rhodnius and Cimex paralyzed by pyrethrins for 10 days but still with movements of the heart, gut, and legs, and found the thoracic and abdominal ganglia greatly shrunken with few cells recognizable and the greater part in an amorphous granular state with scattered vacuoles. Hartzell (1945) continued his histological studies of the effects of pyrethrins and various activators on the central nervous system and muscles of the housefly (see under activation and synergism). Richards and Cutkomp (1945a) injected the pyrethrins into the first thoracic spiracle of Periplaneta americana and studied the effects on the central nervous system using polarized light. Electrical stimulation of the nerve cord of roaches paralyzed up to 52 hours yielded no response, although muscular movements were still evident. This is cited as good evidence for the selective nerve action of pyrethrins. The authors interpreted their data using polarized light analysis to indicate the following progressive action of the insecticide: One-half to 2 1/2 hours, degeneration of the proteins of the axis cylinder of the nerve, but the lipo-protein nerve sheath still normal; 3 1/2 to 7 hours, sheaths still normal, but proteins of axis cylinder have degenerated to about half normal value; 12 to 14 hours, the sheaths beginning to degenerate; 24 hours, advanced degeneration of the axoplasmic colloid; 52 to 55 hours, typical pyrethrins degeneration including vacuolization, and chromatolysis. The degeneration proceeded away from the region of application and had no fixed relation to death. These authors point out that all the histological changes observed appeared subsequently to irreversible paralysis and should be classed as post-mortem changes, and they comment on their similarity to changes seen in autolytic degeneration of nerves in saline solutions, and in insects killed by suffocation from petroleum oils. Richards and Cutkomp conclude that it is questionable as to whether the pyrethrins poisoning has any causal relationship to this degeneration other than killing the nerves.

Klinger (1936), using a galvanometer, found that the flow of nerve impulses in a pyrethrum injured gypsy moth, Porthetria dispar, nerve was about one-fifth that of a normal insect.

Activation or synergism of the pyrethrins--The utilization of supplementary substances which may be nontoxic in themselves, as activators or synergists for insecticides, has been most fruitful in the case of the pyrethrins. There appear to be many hundreds of diversified materials which possess this property in some degree. Among those which have attained commercial importance are: N-isobutylundecyleneamide, $CH_2{=}CH(CH_2)_8CONHCH_2CH(CH_3)_2$ (Weed, 1938); sesamin, a naturally occurring crystalline product from sesame oil of Sesamum indicum, which contains about 0.25 per cent sesamin (Haller et al., 1942b).

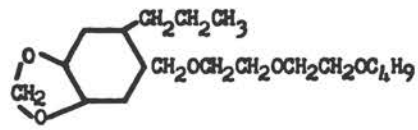


The ethyleneglycol ether of pinene, b.p. 257-273.5°, sp. gr. 0.9846/15.6°, (Pierpont, 1939); piperine alkaloid from black pepper, Piper nigrum, a crystalline solid, m.p. 128-129.5°,



(Harvill et al., 1943);

and (6-propylpiperonyl)-butylcarbityl ether, b.p. 180°/4 mm., sp. gr. 1.06, (Wachs, 1947):



The use of these materials with the pyrethrins has the effect of greatly increasing the potency of a given amount of pyrethrins and consequently is sparing of the expensive constituents. Certain of the activators also have the effect of stabilizing the pyrethrins and producing long-lasting residual applications (Dove, 1947). Examples of these synergistic or activating properties are illustrated in table 8. N-isobutylundecyleneamide, used at 0.5 to 0.7 per cent with pyrethrins at 0.03 per cent, has been reported to produce a fly spray as effective as pyrethrins alone at 0.1 per cent (Weed, 1938). Haller (1947) has pointed out the high degree of effectiveness of this material in synergizing the pyrethrins as a constituent of the Army MYL louse powder. Used at 2 per cent alone the compound was nontoxic to the human body louse, but combined with 0.01 per cent pyrethrins, the mixture was as effective as 1 per cent pyrethrins, and thus increased the efficiency of the pyrethrins about 100 times.

Haller et al. (1942b), Gertler et al. (1943a,b), Gersdorff and Gertler (1944), Synerholm et al. (1945), Synerholm and Hartzell (1945), and Prill et al. (1947) have studied the synergistic action of many compounds related to sesamine and piperine. The presence of the methylene-dioxy- group seems to be an important adjunct to activity in these

Table 8--Some examples of pyrethrins activation to the housefly

Conc. activator per cent w/v	Conc. pyrethrins per cent w/v	Per cent knockdown 10 min.	Per cent kill 24 hrs.	Reference
Ethylene glycol ether pinene				Pierpont (1939)
5		80.8	12.6	
10		87	22	
	0.1	98.6	47.4	
	0.05	96.5	25.2	
5	0.05	98.9	56.9	
10	0.05	99	71	
Piperine				Harvill et al. (1943)
0.5		0	81	
	0.1	98	46	
0.1	0.05	100	99	
0.05	0.03	99	88	
Sesamin (cryst.)				Haller et al. (1942b)
0.25		0	5	
	0.1	100	20	
0.25	0.1	100	85	
(6-propylpiperonyl)-butylcarbitol ether				Wachs (1947)
0.3		8	--	
	0.1	95	46	
	0.04	84	34	
0.2	0.02	96	77	
0.4	0.04	97	90	

compounds, but its presence does not necessarily ensure an active compound. Esters and amides containing this group were the most effective compounds studied, which included fagaramide (N-isobutyl-3,4-methylenedioxycinnamamide), tetrahydrofurfuryl-piperate, and N,N-diethylpiperonylamide. Prill et al. (1946) and Synerholm et al. (1947) extended the work on these methylene-dioxy- compounds to include the thio-ethers of safrole and isosafrole and their oxidation products, the sulfoxides and sulfones. These latter two types of compounds were more effective than the parent thio-ethers.

Lindquist, Madden, and Wilson (1947) made the interesting observation that pretreatment of houseflies with the synergists, "piperonyl cyclonene",[1] sesame oil, and N-isobutylundecyleneamide, followed by pyrethrins resulted in a high knockdown even when the pyrethrins application was delayed as long as 4 hours, or in the case of sesame oil, for as long as 24 hours. When the pyrethrins were applied as the initial treatment, followed by the synergists at 30 seconds, the knockdowns with "piperonyl cyclonene" and sesame oil were inferior to the reverse application, but that with N-isobutylundecyleneamide was about the same. When the interval was one hour or longer, this order of application resulted in zero knockdown in all cases. The authors suggest that the synergists may cause slight injury or disarrangement to nerve or other tissue which facilitates pyrethrins action or that they assist in the absorption of the pyrethrins through the integument. An equivalent series of tests using DDT as the toxicant produced no indication of synergistic effects.

Physiological studies of synergistic action--Although few careful studies of the physiological background for synergism have been made, it is evident that it is a very complex phenomenon which depends on the insect species, insecticide, synergist, and method of testing (Hoskins and Craig, 1946). For example, David and Bracey (1944), working with Aedes aegypti adults, found that sesame oil, N-isobutylundecyleneamide, oleic acid, and lubricating oil all increased the kill of pyrethrins-containing sprays. This was explained on the basis of the low volatility of these substances which decreased the evaporation rate of the insecticide droplets, thus increasing the drop size and resulting in a larger deposit on the insect. It was also found that activators decreased the knockdown, thus giving the insects a longer period to accumulate a lethal dose. In contrast, however, Parkin and Green (1944), using Musca domestica as a test insect, found that nonvolatile materials, such as oleic acid, mineral oil, and lubricating oil, had no activating effect on pyrethrins sprays, but that sesame oil did activate the pyrethrins sprays, and that the activation was dependent on the sesamin content of the oil. The activating action of N-isobutylundecyleneamide on pyrethrins louse powders where flight and droplet size are not factors also shows that other mechanisms of synergism exist besides stabilization of spray droplets.

Hartzell and Scudder (1942) have investigated by histological technics the effects of pyrethrins and N-isobutylundecyleneamide on the central nervous system of the housefly 4 hours after knockdown. Pyrethrins alone produced the characteristic clumping effect on the chromatin of the cell nuclei, while the activator alone produced a chromatolysis or dissolution of the chromatin. When the two materials were combined, both effects could be noticed and the authors concluded that the interaction of these two types of nuclear destruction may be the true basis of activation.

Hartzell (1945), in a continuation of this work, evaluated the histological effects of a number of ingredients of fly sprays, using moribund flies. The effects of toxicants and activators were studied independently and then combined. The principal histopathological changes observed were: (1) dissolution of nerve fiber tracts, (2) dissolution of other cell components resulting in prominence of nerve fibers, (3) vacuolization of

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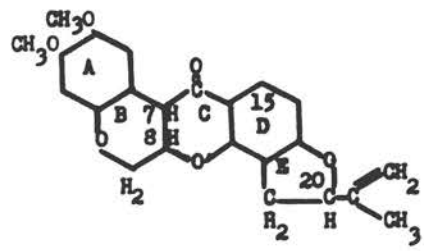
[1] Largely 3,4-methylene-dioxyphenyl-5'-alkyl-4'-cyclohexen-3'-one, (Wachs, 1947) and the related 2'-carbethoxy derivative.

larger nerve cells. Pyrethrins produced effects of the first type, and in addition, vacuolization. Piperine and ethylene glycol ether of pinene produced effects of type two, while sesame oil produced type three changes. Piperine and pyrethrins combined resulted in a partial dissolution of nerve fibers plus a dissolution of certain cellular components, while sesame oil and pyrethrins produced destruction of the nerve fibers and vacuolization of large nerve cells. Three types of effects were also recognizable on muscle tissue: (1) clumping of chromatin of nuclei, (2) accentuation of nodes and Krause's membrane, and (3) destruction of the nuclear membrane. Pyrethrins poisoning produced type one, while sesame oil, piperine, and ethylene glycol ether of pinene caused type two injury. It is pointed out in the discussion that these histological effects may either be of primary importance as a cause of death, or may be of secondary importance and represent the results of abnormal metabolism of the central nervous system as brought about by the effects of the toxicants on enzyme action, the lipid nerve sheaths, etc. (Richards, 1943).

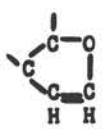
ROTENONE

Introduction--The earliest recorded use of the rotenoids as insecticides was against leaf-eating caterpillars in 1848. The plants containing these materials have been used as fish poisons, however, for many centuries. The active chemical ingredient was isolated in 1895 by Geoffroy and given the name nicouline, and the name rotenone was given in 1902 to an identical compound isolated from Derris by Nagai. The structure of this compound was first determined by La Forge et al. (1933). Rotenone or allied substances are contained in a large number of plant species. Jones (1942) records 21 species of Tephrosia, 12 of Derris, 12 of Lonchocarpus, 10 of Millettia, and 2 of Mundulea, all of the family Leguminosae which have definitely been reported to contain rotenone or rotenoids. Approximately 7,100,000 pounds of derris and cubé roots were utilized as general purpose insecticides in the United States in 1941, the chief sources of supply being Malaya, the Dutch East Indies, and South America (Holman, 1940; Roark, 1941a).

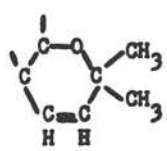
Chemistry--Six rotenoids are known to occur naturally, rotenone, m.p. 163° (a dimorphic form at 181°), having the formula shown (Haller et al., 1942a).



The other naturally occurring rotenoids are elliptone, m.p. 159°, which has a furan ring in place of ring E of rotenone;

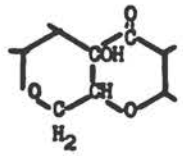


sumatrol, m.p. 188°, which is 15-hydroxy-rotenone; malaccol, m.p. 244°, which is 15-hydroxy-elliptone; levo-alpha-toxicarol, m.p. 101°, which has a hydroxy group at carbon 15, and the following group in place of ring E:



and deguelin, m.p. 165-71°, which has a hydrogen on carbon 15 in place of the hydroxy group of toxicarol. Deguelin has not yet been isolated in the naturally occurring optically active form. A related material, tephrosin, m.p. 197-198°, which has a hydroxy group on either carbon 7 or 8, does not appear to occur naturally in derris resins, but is an oxidation product of deguelin. All of the naturally occurring rotenoids appear to exist as levo-forms. Other non-insecticidal constituents of derris and cubé resins are oils containing sesquiterpenes, two unidentified crystalline compounds, and an acidic material named lonchocarpic acid by Jones (Haller et al., 1942a).

Rotenone is readily oxidized in air, the reaction being catalyzed by light and alkali. According to Cahn et al. (1945) the sequence of reactions is first conversion to a colorless hydroxy- compound I, which passes very readily into a yellow compound II, dehydrorotenone, with the spontaneous loss of water, and under extreme conditions, III is formed.



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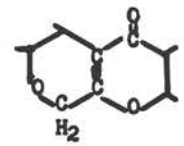
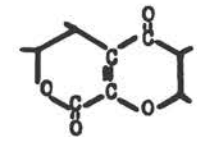


II



III

The indications are that the first oxidation produces a partial but not complete loss in toxicity (see tephrosin, which is 7-hydroxy-deguelin). The rapid second stage, however, produces a complete loss in toxicity (see dehydrorotenone), and rotenonone (III) is probably also nontoxic. The same type of destructive oxidation was undergone by isorotenone which has a double bond at carbon 20, and by dihydrorotenone which has a saturated isopropyl side chain on carbon 20; thus indicating that oxidation does not take place at unsaturation in ring E (as in deguelin) or in the isopropenyl side chain. These reactions probably account for the loss in toxicity of rotenone and rotenoids when exposed to air. Cahn found that antioxidants were ineffective in preventing the destruction of rotenone, but that strong acids, such as phosphoric acid, stabilized rotenone to the destructive action of solvents on powders.

Hydrogenation of rotenone removes the double bond in the isopropenyl side chain of ring E, forming dihydrorotenone which is still highly toxic. More vigorous treatments result in a variety of reactions which are beyond the scope of this review (La Forge et al., 1933).

Commercial rotenone-containing extracts vary considerably in the relative amounts of rotenoids present, depending on the locality where produced and the botanical source. Holman (1940) records the following compositions for three commercial derris resins:

Table 9--Composition of derris resins

Rotenoid	Per cent composition		
	A	B	C
rotenone	40	20	2-5
1-toxicarol	8	25	50-60
1-deguelin	27	27	12
sumatrol	--	trace	5-15
fats, waxes, acids	10	10	10
unaccounted for (elliptone, malaccol ?)	15	18	8-11

Although rotenone is generally considered to be the active ingredient of these resins, the other extractives possess considerable toxicity and much work remains to be done before a proper evaluation of the activity of resins in terms of chemical ingredients can be made (Haller et al., 1942a). Cahn (1936) critically discusses the possibility that the toxicity of resins from which rotenone is absent is largely due to

Table 10--Relative toxicities of rotenone and related materials

Compound	LD_{50} as stomach poison to fourth instar B. mori micrograms per g. Shepard and Campbell (1932)	Median lethal concentration to house fly in mg./ml. in 72 hr. Acetone Sullivan et al. (1939)	Kerosene-cyclohexanone 9:1 Sullivan et al. (1939)	Relative lethal concentration as suspensions to A. rumicis in per cent Davidson (1930)	Relative median lethal concentrations in 48 hours to: A. rumicis Tattersfield and Martin (1938)	Macrosiphoniella san borni Martin (1942)
rotenone	3	0.30	0.30	0.0005	1	1
dihydrorotenone	10					
levo-dihydrorotenone		0.43	0.38			
levo-β-dihydrorotenone		0.71	0.52			
dihydrorotenone	>400					
deguelin	10-12	2.80	0.59	0.005		
levo-deguelin conc.		0.60	0.57			
dihydrodeguelin		10	0.83			
levo-dihydrodeguelin		0.57	0.51			
tephrosin	30-60			0.02		
toxicarol	>1540			0.2		
rotenol	>510					
tubaic acid	>540					
levo-alpha-toxicarol					15	6
summatrol					13.1	
levo-elliptone						5

Table 11--Determinations of LD_{50} values for rotenone to various insects

Species	LD_{50} in micrograms per gram body weight	Method and site of administration	Reference
Periplaneta americana	6-15	Interabdominal as water-oil emulsion	Dresden and Krijgsman (1948)
Bombyx mori fifth instar	7-10	Interabdominal as water-oil emulsion	Dresden and Krijgsman (1948)
Bombyx mori fourth instar	3	Stomach poison	Shepard and Campbell (1932)
Vanessa cardui	30	Stomach poison	Hansberry and Richardson (1936)
Heliothis obsoleta (H. armigera)	>490	Stomach poison	Hansberry and Richardson (1936)
Melanoplus femur-rubrum	4700-7000	Stomach poison	Richardson and Haas (1932)
M. differentialis	>2000	Stomach poison	Richardson and Haas (1932)
Apis mellifera	3	Stomach poison aqueous suspension, 3 days	Calculated from Bottcher (1938a) using 0.1 g. as weight of bee

optically active deguelin. The available toxicological data on the pure chemical constituents are given in table 10.

The rotenone and rotenoid content of various commercial plant species is very different, roots of Derris elliptica averaging from 5 to 9 per cent rotenone and up to 31 per cent ether extractives, while D. malacensis contains from 0 to 4 per cent rotenone, but up to 27 per cent ether extractives. Lonchocarpus utilis averages 8 to 11 per cent rotenone, and up to 25 per cent total extractives (Holman, 1940).

Theories of toxic action of rotenone--Lauger et al. (1944) have discussed the association of the toxicities of various fish poisons and naturally occurring insecticides with the presence of the lactone ring structure which is found in vulpinic and pulvinic acids; the complex coumarins; bergapten, imperatorin, etc., and rotenone and rotenoids (ring C). These investigators believe that the effectiveness of rotenone is due to the presence of the toxaphoric grouping -CO-C=C-O-L, where L represents lipid solubilizing groups
L L
or groups that will enable the compound to reach the site of toxic action, which in rotenone, according to Lauger, are the benzopyran and benzofuran rings (B and E) and the methoxy-groups. Such a theory has the disadvantage of most other general theories of insecticidal action; it does not explain the almost specific toxicity associated with rotenone and the inferior activity of the other rotenoids, all of which embody the same toxophore. Therefore, the effects of lipid solubility (as representing the remainder of the molecule) must be predominant in determining toxicity.

Martin (1946) offers an alternative theory that the hydrogen atoms of the central ring on carbons 7 and 8 are important in determining toxicity. The reasons for this

conclusion are (1) saturation of the isopropenyl side chain of rotenone to produce dihydrorotenone has little effect on toxicity, (2) oxidation to dehydrorotenone, which introduces a double bond in the central ring between carbons 7 and 8, destroys the toxicity, and (3) that the methyl ether of enolized rotenone, having the following structure in ring C is much less effective (Cahn et al., 1938).

Entrance of rotenone into the insect body--From the data in table 11, it is apparent that rotenone can act either as a contact or a stomach poison. Tischler (1935) concluded that derris extracts could enter the insect body through the alimentary canal, the spiracles and tracheal system, or directly through the integument, being extracted by body exudates and body fluids present on the exterior. Webb (1945), in studying the action of rotenone on Melophagus ovinus, found that derris penetrated the insect body chiefly by way of the spiracles. The susceptibility of the insect was decreased by sealing off either the abdominal or thoracic spiracles. The amount of derris entering the spiracles was variable according to the velocity of air currents entering and the structure of the spiracle. When the insect was allowed to breathe air containing 5 per cent carbon dioxide which accelerated the breathing rate, the time for the appearance of symptoms of rotenone poisoning decreased from 2 hours to about 20 minutes. A 10° rise in temperature decreased the average time of kill from 13 to 5.5 hours. By sealing off all the spiracles, Webb was able to show that rotenone would enter directly through the body wall at 30°, but not at 20°. He considered this to be due to a softening of the cuticle wax at the elevated temperature. Wigglesworth (1944, 1945) found that removal of the outer lipid layer of the cuticle by rubbing with alumina or by partial emulsification with detergents greatly accelerated the passage of rotenone through the integument of Rhodnius prolixus nymphs. For example, if 90 per cent powdered rotenone was applied to the normal insect in an attached capsule, no effect was noticed for weeks, while after treatment with alumina, weakness occurred in 8 hours, and death in 24 hours. Similarly, using 0.2 g. of 90 per cent rotenone in 2 ml. of paraffin oil, no effect resulted from application for 7 days; but when applied in the cetyl ether of polyethylene glycol, collapse of the insect occurred in 24 hours.

Webb and Green (1945) studied the effects of various organic solvents on the rate of penetration of rotenone into the sheep tick, Melophagus ovinus. The degree to which a solvent induced a more rapid penetration was referred to as the "carrier efficiency." The following data were obtained using dusts containing 1 per cent solvent and 0.25 per cent rotenone in china clay (table 12). The authors investigated the influence of physical properties of the solvents on the carrier efficiency and found that high efficiency could be correlated with a high rate of penetration through beeswax, a high partition coefficient between beeswax and water and a high solubility of the insecticide in a solution of the solvent in water. It was concluded that certain solvents facilitate the passage of insecticides through the insect cuticle by (1) transporting the insecticide through the lipid elements of the epicuticle to the epi-exocuticular interface, (2) by concentrating the insecticide at this interface, as the solvent passes into the exocuticle and thus increasing the diffusion gradient of the insecticide across the interface, and (3) by increasing the solubility of the insecticide in the water permeating the exo- and endo-cuticles and in effect raising its partition coefficient between the solvent in the epicuticle and the water in the exocuticle. This has the effect of increasing the diffusion rate not only across this interface but through the exo- and endo-cuticles to the hypodermis. Therefore, the incorporation of a solvent in an insecticidal dust enables a far higher proportion of the insecticide to become available to the tissues of the insect and permits the utilization of a lower concentration of active toxicant.

Table 12--Effect of solvent on penetration of rotenone

Solvent	Time for death (hours)	Solubility of rotenone in g./100 g. at 30°	
		In solvent	In 20 per cent saturated solvent-water mixture
ortho-cresol	2	48	0.4
xylenol	2	62	0.75
benzyl alcohol	2	21	0.5
4-methylcyclohexanol	3	1.0	0.2
carbitol	7	2.7	0.75
methylbenzoate	6	18	<0.1
rotenone alone	6		

Burtt (1945) found that the tick, Ixodes ricinus, was especially susceptible to the penetration of rotenone through the tarsi. Pure rotenone applied to the dorsum produced intoxication in an average time of 4 days, but when applied so as to contact only the tarsi, only 4 hours were required. One tenth per cent rotenone in olive oil acted more rapidly than 0.1 per cent in castor oil, in which it is more soluble. Burtt defines a chemical potential as the $\frac{\text{concentration in the medium}}{\text{concentration required to saturate the medium}}$ as being the determining factor in the efficiency of penetration of insecticides in oils. Robinson (1942a) found that petroleum oil solutions of rotenone were much superior in toxicity to vegetable oil solutions to the tick, Ornithodorus moubata, because the more rapid entry allowed less detoxification of the rotenone to occur. Sun and Hansberry (1947) compared the action of rotenone dusts and dusts containing various methyl naphthalenes on third instar Mexican bean beetle larvae, Epilachna varivestis, and on adult pea aphids, Macrosiphum pisi. Rotenone was about equal in effectiveness when dusted on the insects' bodies or when applied as a surface residue, while 2,6-dimethylnaphthalene was more effective when applied as a surface residue.

Physiological action of rotenone on insects--Few studies of the action of rotenone on various physiological systems in insects have been made. Tischler (1935) describes the action of rotenone on the heart action of the silkworm as (1) a period of regular and vigorous beat for about 30 minutes, (2) a rapid decline in rate for 10 minutes, (3) great irregularity of rate and of amplitude with occasional stopping, (4) a second rapid decline for 10 minutes, (5) a long period of feeble activity and irregularity lasting to death. The worms become hyperactive during stage 3, develop incoordination during 4, and fall on sides and remain quiescent in 5. Tischler found that the injection of derris into the metathoracic trochanters of the grasshopper caused almost immediate cessation of respiratory movements, and a decrease in oxygen consumption to about 42 per cent normal. Woke (1938) fed 5 mg. of pure rotenone to individual sixth instar larvae of Prodenia eridania. The feces were highly toxic to mosquito larvae, while the larval tissues and gut contents were not. Nearly 86 per cent of the rotenone fed was recovered in chemical determinations. The incubation of rotenone with tissues of the worms for 18 hours at 29° did not detoxify it in any way. This experiment clearly shows that the rotenone was not absorbed from the gut of this insect and may provide the explanation for the tremendous differences observed (see table 11) in the susceptibility of various insects to rotenone as a stomach poison. Woke (1940) was unable to detect any abnormal appearance in the histology of the mid-gut of larval Prodenia eridania which had previously fed on 5 mg. of crystalline rotenone.

Tischler (1935) states that rotenone poisoning produces no specific effects on motor nerves and attached muscles of insects, and Klinger (1936) was unable to show that rotenone poisoning of the gypsy moth larva, Porthetria dispar, had any effect on the flow of nerve impulses in the nerve cord.

Hartzell (1934) was unable to detect any effects of rotenone poisoning on the histopathology of the nerves of Melanoplus femur-rubrum and Tenebrio molitor. In a further study of the effects of rotenone on the central nervous system of the housefly, Hartzell (1945) found that a spray containing 0.00625 per cent rotenone was lethal without producing a knockdown, and that no lesions could be demonstrated. Flies sprayed with 0.025 per cent rotenone, however, which produced a 94.7 per cent knockdown in 10 minutes, showed dissolution of the fiber tracts of the brain and vacuolization of the larger nerve cells within 10 minutes after spraying.

Sweetman and Gyrisko (1944) found that individual Thermobia domestica, recovering from sublethal doses of rotenone dust, often exhibited latent injury developing many weeks after exposure. This injury developed in appendages of the insect as discolored areas and the appendage usually sloughed off.

ORGANIC THIOCYANATES

Introduction--The use of organic thiocyanates as insecticides was apparently first suggested by Murphy and Peet (1932). Since that time thousands of these compounds have been tested as insecticides but very little information has been published regarding them. Certain of the materials possess rapid knockdown properties, and were used very extensively during the war to replace the pyrethrins in cattle fly sprays and in sprays for the control of flies and other household insects. Approximately 6,000,000 pounds of thiocyanate insecticides were used in the United States in 1944.

Chemistry--Very little has been published on the chemistry of commercially important thiocyanates. "Lorol" thiocyanate is obtained from natural glycerides by reduction to lauryl alcohol which is converted to the chloride by treatment with HCl and then reacted with sodium thiocyanate to give lauryl thiocyanate (Bousquet et al., 1935). A terpene thiocyano ester which consists of a mixture of about 80 per cent isobornyl thiocyanoacetate and 20 per cent related compounds such as bornyl thiocyanoacetate and fenchyl thiocyanoacetate, is produced from the secondary alcohols obtained from pine oil or turpentine by conversion to the alkyl halogen esters and reaction with sodium thiocyanate (Pierpont, 1945).

Relation of chemical structure to toxicity--Some of the more important data regarding the relation of structure to insecticidal action is presented in tables 13 and 14. Many other compounds have been made and tested but the data are either unavailable or unsuited for accurate comparisons.

Theories of toxic action--In common with most other contact insecticides, theories of the mode of action of the thiocyanates are largely dependent on the concept of cuticular permeability, which is generally expressed by the term "lipoid solubility." In the thiocyanates, and thiocyanoacetates, this seems to be controlled by the hydrocarbon radical R- in the type formulae R-SCN and R-$OCOCH_2SCN$. The available data on the

Table 13--Relative toxicities of certain commercial thiocyanate insecticides

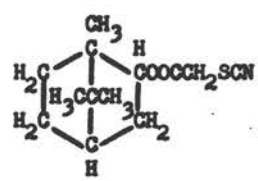
Compound	Formula	Median lethal conc. as a direct spray in per cent (from Busvine, 1946)	
		Human body louse, Pediculus humanus	Bedbug, Cimex lectularius
β-butoxy- β′-thiocyano diethyl ether (Lethane 384)	$C_4H_9O(CH_2)_2O(CH_2)_2SCN$	1.5	4.0
β-thiocyanoethyl laurate (in Lethane 384 Special)	$C_{11}H_{23}COO(CH_2)_2SCN$	8.1	32
lauryl thiocyanate (Loro)	$C_{12}H_{25}SCN$	6.0	19.5
iso-bornyl- thiocyano acetate (Thanite)		3.2	75

Table 14--Comparative toxicities of aliphatic thiocyanates and thiocyanoacetates to certain insects

Compound	No. carbon atoms	M.L.C. to green chrysanthemum aphid (Bousquet et al., 1935)	Time in minutes for 50 per cent knockdown of Musca domestica (Grove and Bovingdon, 1947)
n-hexylthiocyanate	6	1:1200	
n-octylthiocyanate	8	1:2500	
n-decylthiocyanate	10	1:2800	
n-dodecylthiocyanate (lauryl)	12	1:3000	
n-tetradecylthiocyanate (myristyl)	14	1:2700	
n-hexadecylthiocyanate (cetyl)	16	1:1700	
methylthiocyanoacetate	1	---	10
n-hexylthiocyanoacetate	6	---	5
2-ethylhexylthiocyanoacetate	8	---	7.5
caprylthiocyanoacetate	8	---	10
laurylthiocyanoacetate	12	---	2 o/o in 10 min.

relative toxicities of homologous series of compounds of this general nature indicate that a maximum of toxicity is reached by increasing the chain length and that beyond this point the toxicity decreases with further elongation of the chain. This is well illustrated by data obtained by Bousquet et al. (1935) and by Grove and Bovingdon (1947) (table 9). The increase in toxicity with chain length has been shown in many cases to be in a geometric progression with a ratio of about three (Martin, 1946).

The length of carbon chain associated with maximum activity is evidently a function of the characteristics of the molecule as a whole, and probably of the insects under investigation. It is interesting to note that maximum activity in the alkyl thiocyanate series is reached at 12 carbon atoms, while in the alkyl thiocyanoacetate series, the point is about 6 carbon atoms. Ferguson (1939) has pointed out that the distribution coefficients $\frac{\text{aqueous phase}}{\text{non-aqueous phase}}$ of a homologous series of compounds fall in the same geometric progression as do the relative toxicities. In an ascending series, the rate of decrease in solubility is greater than the rate of decrease in distribution coefficient. Hence a point of maximum activity is reached beyond which the effectiveness of the compounds falls off very rapidly with increasing chain length. Therefore, Ferguson believes that the logarithmic increase in toxicity exhibited by a homologous series of compounds, such as the aliphatic thiocyanates, is merely an expression of the phase distribution of the compounds in the external phase of application and the internal phase of site of action, and that when appropriate correction is made for phase distribution, values for the chemical potentials of the compounds will be obtained which lie in a very narrow range for all members of a given series.

Martin (1946) points out in this connection that the n-alkyl thiocyanates exhibit a relatively simple example of the classical approach that a toxic molecule may be separated into a toxophoric group and a conductophoric group whose function is to produce

an effective concentration of the toxicant at the site of activity. It would seem clear that the conductophoric group must function only as a result of its effect on the physical properties of the entire molecule. Martin further states that phase distribution is not usually sufficient to explain the biological properties of related molecules, but that when extended to include the orientation and adsorption of the toxic molecules at the site of action, and the consequent effects on the spatial distribution of the toxophoric groups, some of the problems of sterochemical specificity may become rationalized.

In general, the thiocyanate radical, -SCN, is associated with unpleasant odors which render the compounds somewhat unsuitable for household sprays. Thiocyanoacetates incorporating the group $-OCOCH_2SCN$ are, in general, less unpleasant, have high rates of knockdown, and are highly toxic to the housefly (Grove and Bovingdon, 1947). These substances are more irritant, however, than the corresponding thiocyanates.

Grove and Bovingdon further found that the corresponding alpha-thiocyanoketones, R. CO. CH_2SCN, were more toxic than the thiocyanoacetates R. O. $COCH_2SCN$, as methyl thiocyanoacetate produced 58 per cent knockdown in 10 minutes, while thiocyanoacetone gave 91 per cent. These authors consider that the improved knockdown of the thiocyanoacetates and thiocyanoketones may be due to action on an enzyme system having particular affinity for the $-COCH_2SCN$. Although the thiocyanoketones are readily converted into substituted hydroxythiazoles related to thiamin, two hydroxythiazoles prepared were inactive, casting doubt on the possibility of inhibition of enzyme processes involving thiamin.

Coon (1944) reported that effects of β-butoxy-β'-thiocyanodiethyl ether on the heart action and circulation of blood in Periplaneta americana, viz., a sharp drop in heart beat followed by a rise and leveling off, and a greatly reduced blood circulation, resembled the effects produced by hydrogen cyanide. He suggests that the thiocyanates, like hydrogen cyanide, may act as respiratory poisons. In this connection, von Oettingen et al. (1936) studied the liberation of hydrogen cyanide from alkyl thiocyanates. They found that liver pulp tissue could liberate hydrogen cyanide in considerable quantities from the lower homologues, such as methyl and ethyl, much less from β-butoxy-β'-thiocyanodiethyl ether, while lauryl thiocyanate was stable. This would appear to indicate a more involved mode of action for the insecticidal thiocyanates.

Quantitative toxicology of thiocyanates--Busvine (1946) has calculated the median lethal contact dose of β-butoxy-β'-thiocyanodiethyl ether as 135 micrograms per gram body weight for the human louse, Pediculus humanus, and 450 micrograms per gram body weight for the bedbug, Cimex lectularius.

Physiological studies--Yeager et al. (1935) have studied the effects of 10 aliphatic thiocyanates on the contraction rate and dilation of the isolated heart preparation of the roach, Blatta orientalis. In general, these compounds caused a decrease or cessation of the rate of contraction and an increase in the dilation of the heart. The least effective materials were methyl and ethyl thiocyanates; the intermediately effective materials were iso-propyl-, n-propyl-, and butyl-thiocyanates; and the most effective materials were trimethylene thiocyanate, butyl carbitol thiocyanate, β-β'-dithiocyanodiethyl ether, diethylene glycol thiocyanate, and diethylene glycol dithiocyanoacetate, which at 0.006 per cent concentration caused rapid decrease in heart rate and eventual cessation of contraction. It is suggested by the authors that the thiocyanates may cause an increased tonus in the alary muscles supporting the heart.

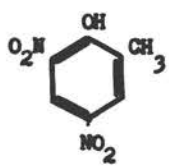
Hartzell and Wilcoxon (1934) found gamma-thiocyano-propylphenyl ether (b.p. 188-191/14 mm.) to be the most toxic of a number of thiocyanates studied. They describe the symptoms resulting from the placing of a drop of this material on the abdomen of Periplaneta americana, as (1) irritation at site of drop in about 15 minutes,

(2) longitudinal convulsive twitchings of body and partial paralysis of posterior pair of legs, (3) within an hour leg paralysis complete, twitchings occurred at about 1 per second, and animal unable to walk, and (4) gradual decrease in convulsive movements and animal dead in 3 hours. Histological study of the ventral nerve cord of mealworm larvae, Tenebrio molitor, killed by applying a drop of this compound to the dorsum showed marked cellular tigrolysis and vacuolization of tissue but not to the degree of pyrethrins poisoning. Hartzell (1945) has investigated the histological effects of β-butoxy-β'-thiocyanodiethyl ether and iso-bornyl-thiocyanoacetate on the central nervous system and muscle tissue of moribund flies. β-butoxy-β'-thiocyanodiethyl ether produced dissolution of the nerve cell components other than the fibers, produced deep staining nuclei, and caused a destruction of the nuclear membrane of muscle tissue. Iso-bornyl-thiocyanoacetate produced vacuolation of the larger nerve cells.

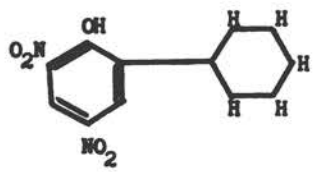
DINITROPHENOLS

Introduction--The earliest recorded use of the dinitrophenols was in 1892 when a mixture containing potassium 3,5-dinitro-o-cresylate was marketed in Germany as an insecticide (Kagy, 1941) and patented under German Patent 72,097, and British Patent 3,301. Tattersfield et al. (1925) made the first quantitative toxicological studies of these materials as insecticides. Subsequent work by Kagy (1936) and Boyce et al. (1939) resulted in the commercial development of these materials, especially for the control of plant feeding mites.

Chemistry--Two dinitro compounds have received considerable attention as insecticides. DNOC is 4,6-dinitro-o-cresol, m.p. 85.8°, a yellow odorless solid, soluble 1 part in 7813 parts of water at 15°. The compound readily forms salts with organic bases, and the ammonium, sodium, potassium, calcium, and barium salts are water soluble (Wain, 1942). DNOCHP is 2,4-dinitro-6-cyclohexylphenol, m.p. 106°, a yellowish-white odorless, crystalline solid produced by nitrating o-cyclohexylphenol in a mixture of sulfuric and nitric acids at temperatures of 60-90° (Britton and Mills, U. S. Patent 1,880,404, October 4, 1932). Like DNOC it forms salts with bases. The water solubility of DNOCHP varies from about 1.8 mg. per liter at pH = 1, to 15 mg. per liter at pH = 6.5 (Boyce et al., 1939). Very recently, 2,4-dinitro-6-sec-butylphenol has been developed for use as an insecticide and fungicide. It is used as the triethanolamine salt (Dow, 1948).



4,6-dinitro-o-cresol



2,4-dinitro-6-cyclohexylphenol

Because of the free phenolic groups of these compounds which behave as pseudo acids, they are often highly toxic to plants. Therefore, several salts have been used in agricultural formulations, notably the dicyclohexylamine salt of 2,4-dinitro-6-cyclohexylphenol, m.p. 197° (Kagy and McCall, 1941). The present compounds and sodium and calcium salts of both DNOC and DNOCHP have been used as toxicants in weed killers and for poison baits and dusts against such pests as grasshoppers and chinch bugs.

The phase distribution of DNOCHP in oil-water emulsions is influenced by the pH of the water. The percentage of toxicant remaining in the oil phase at equilibrium varies from more than 90 per cent under acid conditions (pH 3.5 to 5.0) to less than 5 per cent under alkaline conditions (pH 8.0-11.0), (Boyce, et al., 1939). This phase distribution affects both the ovicidal and phytotoxicity of the emulsion, optimum conditions resulting from strongly acid mixtures (Boyce, et al.). Brian (1945) suggested that the increased insecticidal action of DNOC in dormant oil sprays at low pH can be accounted for by the increased concentration of undissociated compound rather than by increased concentration in the oil phase, the latter being an indirect indication of the former. However, Gimingham et al. (1926) found that the sodium salt of DNOC was only slightly less toxic than the parent compound to eggs of Selenia tetralunaria, the respective LD_{50}'s being 0.0167 per cent and 0.0147 per cent, and Martin et al. (1943) could show no difference in the ovicidal effectiveness of DNOC free phenol and its sodium salt. Reduction of one nitro group of DNOC to form 2-methyl-4-nitro-6-aminophenol, m.p. 173-4°, was readily accomplished by ammonium sulfide. This compound had no ovicidal, contact or stomach poison properties to a number of insect species (Martin et al., 1943; Bennett et al., 1946). Attempts to prepare the diamino-compound by reduction with tin and hydrochloric acid resulted only in amorphous residues from which the dihydrochloride was recovered. The free base was unstable.

Table 15--Relative toxicities of some nitrated phenols to insects

Compound	LD_{50} 5th instar B. mori - stomach poison Kagy (1941)	Median lethal deposit in micrograms per $cm.^2$ to P. citri Kagy (1941)	Approximate median lethal conc. in g. per 100 ml. to Aphis rumicis Tattersfield et al. (1925)
p-nitrophenol			0.25
o-nitrophenol			1-2.5
m-nitrophenol			0.5-1
2,4-dinitrophenol		3.10	0.1
tri-nitrophenol			0.5-1
4-nitro-6-methylphenol			0.25
2-nitro-5-methylphenol			1.0
2-nitro-3-methylphenol			0.5-1
2-nitro-4-methylphenol			0.5-1
2,4-dinitro-6-methylphenol	49	1.80	0.05
2,6-dinitro-4-methylphenol			1.0
2,4,6-trinitro-5-methylphenol			0.5
2,4-dinitro-6-ethylphenol	29	1.20	
2,4-dinitro-6-n-propylphenol	18		
2,4-dinitro-6-n-butylphenol	9		
2,4-dinitro-6-n-pentylphenol	8		
2,4-dinitro-6-n-hexylphenol	4		
2,4-dinitro-6-n-heptylphenol	4		
2,4-dinitro-6-n-octylphenol	10		
2,4-dinitro-6-cyclopentylphenol	9		
2,4-dinitro-6-cyclohexylphenol	7	0.40	
2,4-dinitro-3-methyl-6-isopropylphenol	33		
2,4-dinitro-alpha-naphthol		>3.40	

Relation of chemical structure to toxicity--Table 15 gives a summary of the important relations of chemical structure vs. insecticidal action of the nitro phenols. Tattersfield et al. (1925) showed that maximum activity in the simple phenols was associated with the presence of two nitro groups, and rediscovered the insecticidal properties of dinitro-o-cresol (2,4-dinitro-6-methylphenol). Kagy (1936 and 1941) extended the series to include the effects of various aliphatic side chains. The effectiveness was found to increase with increasing chain length up to a maximum at n-hexyl and n-heptyl

Table 16--Median lethal dosage determinations of dinitrophenols to several insects

Compound	Insect	LD_{50} in micrograms per gram body weight	Reference
2,4-dinitro-6-methyl phenol-sodium salt DNOC	honeybee - Apis mellifera	2.1-2.4 μg. per bee or cal. as about 20 μg. per g. body wt. using 0.1 g. as wt. of bee - oral	Goble and Patton (1946)
2,4-dinitro-6-cyclohexylphenol DNOCHP	corn earworm - Heliothis armigera	87 - oral	Kagy (1936)
"	armyworm - Cirphis unipuncta	15 - "	"
"	imported cabbage worm - Pieris rapae	73 - "	"
"	red-legged grasshopper - Melanoplus femur-rubrum	65 - "	"
"	5th instar Colo. potato beetle - Leptinotarsa decemlineata	16 - "	Kagy (1941)
2,4-dinitro-6-cyclopentylphenol	"	21 - "	"
2,4-dinitro-6-cyclohexylphenol	5th instar painted lady - Cynthia cardui (Vanessa cardui)	20 - "	"
2,4-dinitro-6-cyclopentylphenol	"	50 - "	"
calcium-2,4-dinitro-6-cyclohexylphenate	Heliothis obsoleta (H. armigera)	59 - "	Kagy (1936)
calcium-2,4-dinitro-6-cyclohexylphenate	Cirphis unipuncta	15 - "	"
"	Vanessa cardui	21 - "	"
"	B. mori	20 - "	"
"	P. rapae	73 - "	"
magnesium-2,4-dinitro-6-cyclohexylphenate	H. obsoleta	77 - "	"
lead-2,4-dinitro-6-cyclohexylphenate	"	84 - "	"

Table 16--Median lethal dosage determinations of dinitrophenols to several insects (Continued)

Compound	Insect	LD_{50} in micrograms per gram body weight	Reference
copper-2,4-dinitro-6-cyclohexylphenate	H. obsoleta	97 - oral	Kagy (1936)
2,4-dinitro-6-methyl phenol DNOC	silkworm - Bombyx mori	49 - "	Kagy (1941)
2,4-dinitro-6-cyclo-hexylphenol DNOCHP	"	7 - "	"

and then decreased again. It was found more convenient to produce a cyclohexyl derivative in commercial practice which was slightly less effective than the corresponding n-hexyl compound. The dinitrophenols are highly toxic as contact poisons, stomach poisons, and ovicides to a variety of insects (Gimingham et al., 1926; Kagy, 1941; Viado, 1941; Magy and Hoskins, 1945) and are also effective acaricides. The metallic salts of the dinitrophenols are highly toxic as stomach poisons (Kagy, 1936), (see table 16).

Biochemical effects of dinitrophenols--Bodine and Boell (1936) working with grasshopper embryos found that the application of 0.00025 M 2,4-dinitrophenol (maximum stimulatory value) increased the oxygen uptake to about three times normal, while with dinitro-o-cresol, maximum stimulation occurred at about 0.0001 M, and the oxygen uptake reached 2.5 times normal. Goble and Patton (1946) studied the effects of feeding the sodium salts of dinitro-o-cresol and 2,4-dinitro-6-cyclohexylphenol to the honeybee, Apis mellifera. The feeding of one-sixth the LD_{50} of DNOC increased the oxygen consumption about 52 per cent, and at one-sixtieth the LD_{50} it was increased 28 per cent. A similar effect was obtained with DNOCHP. These results are in accord with the well-known properties of 2,4-dinitrophenol as a metabolic stimulant in mammals (Kagy, 1941).

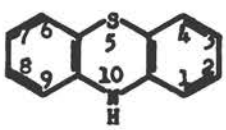
Theories of mechanism of action--Bennett et al. (1946) suggest that the toxic action of DNOC (and presumably DNOCHP) may be due to its in vivo reduction to the nontoxic mono-amino derivative, 2-methyl-4-nitro-6-aminophenol which may lead to a fatal disturbance of oxidative-reductive mechanisms or otherwise produce toxic effects, such as the liberation of toxic products.

Physiological effects--Viado (1941) found that poisoning of the blowfly, Cynomyia cadaverina, with DNOCHP resulted in no effect on the hemolymph cells. Dusting of Periplaneta americana with DNOCHP dust resulted in the development of paralysis proceeding from anterior to posterior of the body.

PHENOTHIAZINE

Introduction--Phenothiazine or thiodiphenylamine was originally prepared by Bernthsen (1883) and its insecticidal properties were apparently first recognized by Campbell et al. (1934) who found it an effective mosquito larvicide. Subsequently, Smith et al. (1935) found it highly effective against the codling moth. Because of its chemical behavior it has given erratic results in many agricultural field tests for codling moth control (Zukel, 1944), but the compound or its oxidation products have been shown to possess considerable fungicidal action (Goldsworthy and Green, 1939) and to be highly effective as an internal parasiticide for mammals (Britton, 1941). About 3,500,000 pounds were used for agricultural purposes in the United States in 1944.

Chemistry--Phenothiazine is a light yellow crystalline material, m.p. 185°, prepared commercially by heating one mole of diphenylamine with two moles of sulfur to 180°, using iodine as a catalyst. The yield is practically quantitative but the commercial product is dark green, owing to the formation of unknown compounds which may be polymers or isomers (Smith, 1938). Phenothiazine is oxidized to phenothiazone, m.p. 162°, which is the 2-keto derivative, by the action of air, (Goldsworthy and Green, 1939), and to thionol (m.p. uncertain), the 2-keto-, 8-hydroxy compound, by the action of acidic hydrogen peroxide (De Eds and Eddy, 1938). Phenothiazine sulfoxide, m.p. 250°,



is a further oxidation product which forms phenothiazone in the presence of air and light (Goldsworthy and Green, 1939). Phenothiazine is the parent compound of dyes of the methylene blue type and like methylene blue, the oxidation products, phenothiazone and thionol, form oxidation-reduction systems with their respective leuco-derivatives (De Eds and Eddy, 1938), leuco-phenothiazone being the 2-hydroxy compound, and leuco-thionol, 2,8-dihydroxyphenothiazine. Guy (1937) found that oxidation of films of phenothiazine in air and light could be largely prevented by the presence of an antioxidant, such as hydroquinone, or a reducing agent, such as mercaptobenzothiazole. Mischler's ketone, which prevented ultra-violet absorption, was also effective.

Insecticidal action of related materials--The relationship of the oxidation products of phenothiazine to the insecticidal action is far from clear. Phenothiazone appeared to be about 1/100 as toxic to Culex larvae as reported by Fink et al. (1938). Zukel (1944) reported that both phenothiazine and phenothiazone were effective, and thionol was ineffective as contact poisons to Periplaneta americana, and that none of the three compounds was effective as stomach poisons to this insect. Gersdorff and Clayborn (1938) mention that phenothiazone and phenothiazone sulfoxide were nontoxic to codling moth larvae and mosquito larvae. It is interesting to note that these same investigators found phenothiazone 10 times as toxic as phenothiazine to goldfish, and that Goldsworthy and Green (1939) found that phenothiazone was fungicidal while phenothiazine was not.

Guy (1937) tested a number of related materials to the Mexican bean beetle adult. Phenothioxin was the only material which showed comparable toxicity to phenothiazine; N-acetylphenothiazine, N-methylphenothiazine, tetranitrophenothiazine sulfoxide, and dinitrophenothiazine sulfoxide were ineffective. Lennox (1940) found that phenothiazine was much more toxic as a stomach poison than N-methylphenothiazine, 3,9-dimethylphenothiazine, 3,5,7,9-tetramethylphenothiazine, N-ethylphenothiazine, N-acetyl-3,9-dimethylphenothiazine, N-acetyl-2,3,9,10-dibenzophenothiazine, or N-acetyl-2,3,9,10-dibenzophenothiazine. Fink et al. (1938) found that phenothioxin was nearly as effective as a culicine larvicide as was phenothiazine, and Knipling (1941) reported that pheno-

thioxin was more effective against the fleece worms, Phormia regina and Lucilia sericata, and equal to phenothiazine against Cochliomyia macellaria.

Smith (1938) found that the ether-soluble fraction of commercial phenothiazine was as toxic as pure phenothiazine to codling moth and mosquito larvae, while the ether-insoluble fraction was nontoxic (see also Siegler et al., 1936).

Entrance into the insect body--Although phenothiazine is generally classed as an organic stomach poison (Frear, 1942), Guy (1937) states that its action on the adult of the Mexican bean beetle is largely, if not entirely, as a contact poison. Zukel (1944) found that phenothiazine was effective against Periplaneta americana when dusted on the insect (with the mouthparts sealed) or when injected into the hemolymph, but that no toxicity occurred when phenothiazine was fed in food or as a suspension. Phenothiazone was toxic under the same conditions as phenothiazine, but thionol was not effective. The inactivity of phenothiazine as a stomach poison was attributed to the impermeability of the intestinal wall to the material.

Physiological effects on insect--Zukel (1944) made a very careful study of the action of phenothiazine on P. americana. The symptoms of contact poisoning observed were lack of coordination in walking about 5 hours after exposure, which became more pronounced until the insect falls upon its back in about 18 hours and responds to tactile stimulation only by movements of the appendages. Deonier and Lindquist (1942) observed the effects of phenothiazine on the overwintering larvae of the Clear Lake gnat, Chaoborus astictopus. The compound apparently affected the nervous system of the insect; the larvae sank to the bottom and did not respond to prodding. The gut action was weak and irregular and muscles of the body wall twitched at irregular intervals; however, the heart action continued. Larvae were observed to remain in this state of paralysis for as long as 3 weeks and recovery sometimes occurred from 4 days of immobility. Guy (1937) also commented on the occurrence of paralysis and recovery in Japanese beetle adults poisoned with phenothiazine. Woke (1940) was unable to determine that phenothiazine had any effect on the normal histology of the midgut in moribund larvae of Prodenia eridania.

Biochemistry of phenothiazine action--Zukel (1944) studied, by colorimetric tests, the chemical reactions of phenothiazine as correlated with various phases of its toxicology. He determined that phenothiazine was lethal to P. americana as a contact poison by the production of a definite threshold concentration of leucothionol conjugate in the hemolymph. Below this threshold concentration, the insect often exhibited uncoordinated walking movements but recovered as the malpighian tubules eliminated the conjugate. The conjugate apparently was formed by conversion of phenothiazine in contact with or in penetrating through the cuticle and its concentration was reduced by elimination through the malpighian tubules. No phenothiazine could be detected in the hemolymph. Phenothiazine was not toxic as a stomach poison because of the impermeable wall of the alimentary canal, but was stored in the crop without alteration except in a few cases where a blue-green compound was formed. The midgut, however, was the site of active conversion of phenothiazine to leucothionol. Similar conversion was observed for phenothiazone and thionol.

Collier (1940) studied the effects of phenothiazine and related compounds on mammalian enzymes in order to obtain evidence of the mode of vermicidal and insecticidal action. The following inhibition of guinea pig liver catalase and beef heart cytochrome oxidase was obtained under the conditions of the experiments:

Table 17--Effect on enzymes

Compound	Per cent inhibition	
	Liver catalase	Cytochrome oxidase
phenothiazine	0	--
leucophenothiazone	100	73
phenothiazone	0	0
leucothionol	100	--
thionol	100	76

Collier comments that only the compounds containing hydroxyl groups were strong inhibitors. Collier and Allen (1942) continued this study on other enzyme systems and found that phenothiazone inhibited horse serum cholinesterase, 50 per cent at 6.7×10^{-5} M as compared with eserine, 50 per cent at 2.5×10^{-8} M. It was speculated that this action might explain the toxicity of phenothiazine to insects. Collier and Allen (1941) found that the system leucophenothiazone-phenothiazone also was a strong inhibitor of oxidase and dehydrogenase components of beef heart.

Theories of mode of action--Zukel (1944) concludes that the toxicity of phenothiazine may be due to the inhibition of the respiratory enzyme, cytochrome oxidase, by the leucothionol conjugate in the hemolymph of the American roach. De Eds and Thomas (1941) have suggested that phenothiazine might act insecticidally by permanent oxidation of the respiratory enzymes by the leucothionol-thionol system, while Collier and Allen (1942) comment on the action of phenothiazone on cholinesterase as a possible site of insecticidal action.

DICHLORODIPHENYLTRICHLOROETHANE

Introduction--2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane, known as DDT, from the initial letters of the generic name, Dichloro Diphenyl Trichloroethane, was first synthesized by Ziedler (1874). The insecticidal properties of the compound were discovered by Paul Müller of J. R. Geigy A. G. Switzerland in 1936-37 (Cristol and Haller, 1945) and patented by him (1940, 1943). DDT had unprecedented development as a synthetic insecticide because of its unusual properties of wide range of insecticidal action, simple structure promoting ready synthesis, stability to light and air resulting in enduring residual toxicity and low mammalian toxicity. Thus the material ideally fulfilled a wartime demand for the control of insects of medical importance, and soon found important agricultural uses. Approximately 45,000,000 pounds were produced in the United States in 1946.

Chemistry of DDT and related materials--Technical DDT is a white to cream colored amorphous powder produced by reacting chloral (or its alcoholate or hydrate) with mono-chlorobenzene in the presence of concentrated sulfuric acid. The technical material has a rather variable composition and is composed of up to fourteen chemical compounds. The range in percentage composition of the major constituents as analyzed by various workers is given in table 18. These materials all possess some slight degree of toxicity to insects as shown in table 19. However, p-p' DDT is the important active constituent and this compound, isolated from technical DDT by several recrystallizations from ethyl alcohol, is used in most of the physiological studies to be reported.

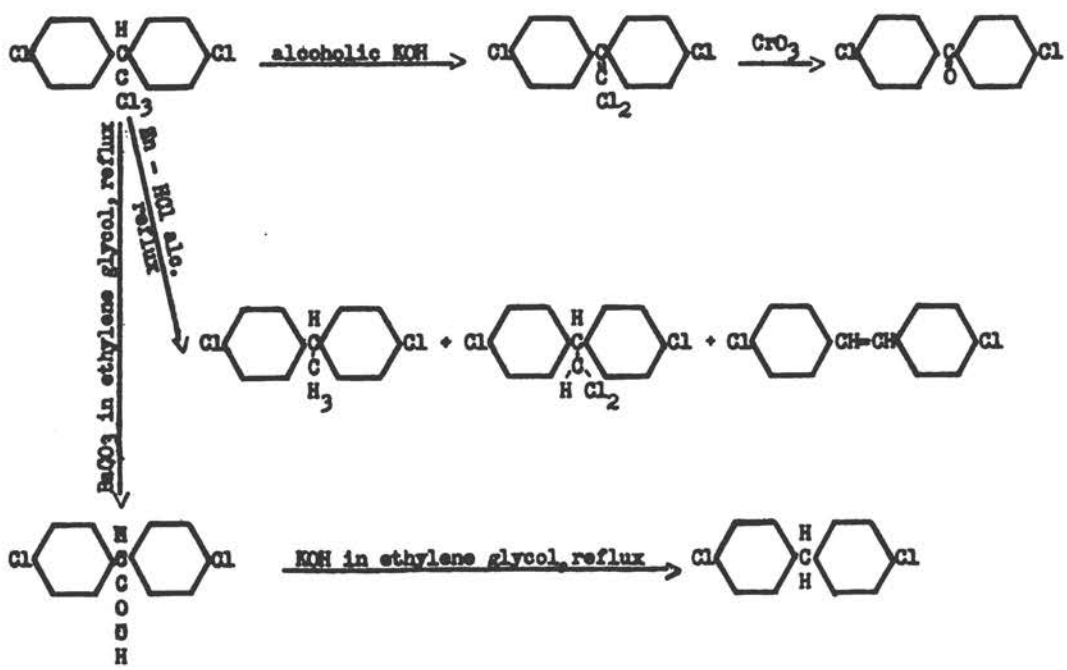
Table 18--Composition of technical DDT

Compound	m.p. °C.	Per cent composition in various samples		
		Haller et al. (1945)	Mosher et al. (1946)	Forrest et al. (1946)
2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane (p-p' DDT)	108-109	65-73	75-80	48-76
2-(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1-trichloroethane (o-p' DDT)	73-74	19-21	15	11-29 (includes o-p DDD)
2-(p-chlorophenyl)-1,1,1-trichloroethanol	b.p.-114-116/0.5 mm.	0.2	1.5	2.4-3.2 (includes other half condensation products)
2,2-bis-(p-chlorophenyl)-1,1-dichloroethane (p-p' DDD)	109-110	0.17-4.0	0	2-37
2,2-bis-(o-chlorophenyl)-1,1,1-trichloroethane (o-o' DDT)	92-93	0.1 (Cristol et al., 1946b)		
bis-(p-chlorophenyl)-sulfone	148	0.034-0.6		

Table 19--Relative toxicities of components of technical DDT

Material	Approximate median lethal concentrations to				
	Anopheles quadrimaculatus larvae - 24 hrs. Cristol et al. (1946a) Deonier et al. (1946)	Pediculus humanus - spray Busvine (1946a)	Cimex lectularius - spray Busvine (1946a)	Drosophila melanogaster - residue Proverbs and Morrison (1947)	Heliothrips haemorrhoidalis - residue Metcalf (1948)
2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane	0.0025	0.3	0.53	1	0.001
2-(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1-trichloroethane	0.012	5.5	>14	145	>0.1
2,2-bis-(o-chlorophenyl)-1,1,1-trichloroethane	>5.0	--	--	--	--
2,2-bis-(p-chlorophenyl)-1,1-dichloroethane	0.0025	0.9	1.2	17	0.006
2-(p-chlorophenyl)-1,1,1-trichloroethanol	>10	6.5	10	25	1.0
bis-(p-chlorophenyl)-sulfone	1.0	--	--	>50	>1.0

Figure 1--Some chemical reactions of DDT



Pure p-p' DDT has a vapor pressure of 1.5×10^{-7} mm. Hg. at 20° C. (Balson, 1947). It readily undergoes certain chemical reactions which are shown in the flow-sheet in figure 1. The relative toxicities of these materials to insects is given in table 20.

Several compounds closely related to DDT have attained some importance as insecticides. DDD or 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane is produced by an analogous reaction between chlorobenzene and dichloroacetal or dichloroacetaldehyde and is thus present in small amounts in technical DDT produced from chloral containing these substances as impurities (Forrest et al., 1946). The pure p-p'-isomer has a m.p. of 109-110°. Methoxychlor or 2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane is produced from anisole and chloral (Stephenson and Waters, 1946) and the pure p-p'-isomer melts at 88°. DFDT or 2,2-bis-(p-fluorophenyl)-1,1,1-trichloroethane was produced commercially in Germany during the war as "Gix" (Kilgore, 1945). The technical product is a liquid and contains about 10 per cent o-p'-isomer (Bradlow and Vander Werf, 1947), and the purified p-p'-isomer has a melting point of 45°. This compound has a higher vapor pressure than DDT (b.p. 177-8°/9 mm.) and is consequently less long lasting as a residual treatment. The dimethyl-analogue of DDT, 2,2-bis-(p-tolyl)-1,1,1-trichloroethane, m.p. 90°, is also highly effective to insects, and has been tested on a semi-commercial scale.

Table 20--Relative toxicities of degradation products of p-p'-DDT

Compound	Approximate median lethal concentrations			
	Pediculus humanus Busvine (1946)	Cimex lectularius Busvine (1946)	Anopheles quadrimaculatus larvae in 24 hrs. Deonier et al. (1946)	Heliothrips haemorrhoidalis Metcalf (1948)
2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane	0.3%	0.53%	0.0025 p.p.m.	0.001%
2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene	>10	>10		>1.0
2,2-bis-(p-chlorophenyl)-ethane	8.5	>20		>1.0
4,4'-dichloro-diphenylmethane			ca. 1.0	0.16
4,4'-dichlorobenzophenone			ca. 10.0	1.0
4,4'-dichlorodiphenyl-acetic acid			ca. 1.0	>1.0
4,4'-dichlorostilbene				>1.0

Table 21--Relative toxicities of some analogues of DDT

Compound	Approximate median lethal concentration to				
	Pediculus humanus Busvine (1946)	Cimex lectularius Busvine (1946)	Anopheles quadrimaculatus larvae Deonier et al. (1946)	Drosophila melanogaster Proverbs and Morrison (1947)	Heliothrips haemorrhoidalis Metcalf (1948)
(a) Symmetrical analogues with trichloromethyl group					
2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane	0.3%	0.53%	0.0025 p.p.m.	1.0	0.001%
2,2-bis-(p-fluorophenyl)-1,1,1-trichloroethane	1.4	5	0.0075 cal.	0.11 2.5 (Kirkwood and Dacey, 1946)	0.006
2,2-bis-(p-bromophenyl)-1,1,1-trichloroethane	0.6	1.4	0.0025	26	0.06
2,2-bis-(p-iodophenyl)-1,1,1-trichloroethane				ca. 500	
2,2-bis-(p-methylphenyl)-1,1,1-trichloroethane	1.7	3.6	0.01	ca. 650	0.03
2,2-bis-(p-ethylphenyl)-1,1,1-trichloroethane	5.0	4.0			0.04
2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane	0.9	0.55	0.01		0.03
2,2-bis-(p-ethoxyphenyl)-1,1,1-trichloroethane	1.8	0.8			
2,2-bis-(phenyl)-1,1,1-trichloroethane	7.5	>12	1.0	ca. 500	1.0
2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane	non-toxic 100%	non-toxic 100%	>10		>1.0
2,2-bis-(p-nitrophenyl)-1,1,1-trichloroethane					>1.0
(b) Analogues with more than one ring substituent					
2,2-bis-(3-nitro-4-chlorophenyl)-1,1,1-trichloroethane	non-toxic 100%		>10		>1.0
2,2-bis-(3,4-dimethylphenyl)-1,1,1-trichloroethane					0.05
2,2-bis-(2,4-dimethylphenyl)-1,1,1-trichloroethane	>10	>10			>0.1

Table 21--Relative toxicities of some analogues of DDT (Continued)

Compound	Approximate median lethal concentration to				
	Pediculus humanus Busvine (1946)	Cimax lectularius Busvine (1946)	Anopheles quadrimaculatus larvae Deonier et al. (1946)	Drosophila melanogaster Proverbs and Morrison (1947)	Heliothrips haemorrhoidalis Metcalf (1948)
(b) Analogues with more than one ring substituent (Continued)					
2,2-bis-(2,5-dimethylphenyl)-1,1,1-trichloroethane					>0.1
2,2-bis-(3,4-dimethoxyphenyl)-1,1,1-trichloroethane	non-toxic 100%	non-toxic 100%	>10		
2,2-bis-(2,5-dimethoxyphenyl)-1,1,1-trichloroethane	non-toxic 100%	non-toxic 100%	>10		
2,2-bis-(2-methyl-4-chlorophenyl)-1,1,1-trichloroethane	non-toxic 100%				
2,2-bis-(3,5-dinitro-4-chlorophenyl)-1,1,1-trichloroethane			>10		>1.0
(c) Unsymmetrical analogue					
2-(p-chlorophenyl)-2-phenyl-1,1,1-trichloroethane	2.1	4.5	0.025	90	0.25
(d) Symmetrical analogues with altered aliphatic portion					
2,2-bis-(p-chlorophenyl)-2,1,1,1-tetrachloroethane			0.1	>1000 (original value)	>1
2,2-bis-(p-chlorophenyl)-1,1,1-trifluoroethane				100 (Kirkwood and Dacey, 1946)	0.9
2,2-bis-(p-chlorophenyl)-1,1,1-tribromoethane			0.1		
2,2-bis-(p-chlorophenyl)-1-chloroethane			0.1		
Bis-(p-chlorophenyl)-methanol			10		>1
1,1-bis-(p-chlorophenyl)-ethanol					>1

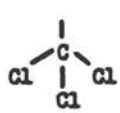
Toxicology of DDT analogues--A very large number of analogues of DDT have been studied with regard to their toxicity to insects. Although there appears to be a considerable difference in the effects of these materials to varying species of insects, the data presented in tables 19, 20, and 21 represent the general trends which exist. It should be emphasized that the relative toxicities of all the compounds vary with the test insect and the test method.

Martin and Wain (1944a) have commented on the results of a study of the relative toxicities of a series of DDT-analogues. They state that the nature of the nuclear (ring) substituent affects toxicity either because of the electronic effect of the group or solubility factors. When the substituent is either chlorine or methoxy, both inductive and electromeric effects are operative but are opposite in influence on the alpha-carbon of the ethane. Since the compounds incorporating these two groups and the methyl-substituted compound as well are highly toxic, the evidence appears to favor the solubility factor as being the controlling influence. The low activity shown by polar groups, such as hydroxyl- and nitro-ring substituents, indicates the importance of nonpolar groups in this position. Martin and Wain point out that with asymmetrical analogues, solubility may likewise be the controlling factor as the surface activity of the molecule and its adsorption may be affected by symmetry. Busvine (1946a) notes that the molecules of the most toxic DDT-analogues are most similar to DDT in shape and weight, and he considers it possible that the entire molecule is involved in a steric association with a vital enzyme. Further theories of the influence of structure on activity are presented in the next several sections.

The order of effectiveness of DDT-analogues in mice and rats is very different from the effectiveness in insects (Smith et al., 1946; von Oettingen and Sharpless, 1946), and suggests an entirely different locus of action. For example, compounds such as DDD, methoxychlor and DFDT, which are the most toxic DDT-analogues to insects, were respectively about 1/10 to 1/20, 1/40, and 1/4 as toxic as DDT to rats and mice.

Theories of mode of toxic action--Two general theories of the mode of action of DDT and related materials have been suggested, both depending on the classical Overton-Meyer theory (Meyer and Hemmi, 1935; Ferguson, 1939, pp. 74, 112), which relates the lipoid solubility, i.e., the oil/water distribution coefficient of physiologically active materials and their activity. Läuger, Martin and Müller (1944), in an ingenious theory, have speculated that the effectiveness of DDT is due to combination in one molecule of a lipid solubilizing group, viz., the "trichloromethyl group," and a toxic component, the bis-(p-chlorophenyl)-methylene group. These investigators, using data from a study of mothproofing agents, point out that many substances having two p-chlorophenyl groups attached to a central nucleus, such as di-(p-chlorophenyl)-sulfone, di-(p-chlorophenyl)-sulfide, and di-(p-chlorophenyl)-ether, proved to be effective stomach poisons. When a lipid solubilizing group, such as the trichloromethyl group, was added to the molecule, the very effective contact action of DDT resulted. Therefore, they theorize that the

group is the toxic portion of the molecule and that the



group is the lipid-solubilizing group. Martin (1946) has commented that the effectiveness of various alkoxy analogues of DDT, such as the bis-(p-methoxy)- and bis-(p-ethoxy)- make it evident that the toxicity of DDT is not uniquely explained by the presence of the dichlorodiphenyl system.

Table 22--Solubility and dehydrohalogenation values for DDT and certain related compounds

Compound	Hydrolysis rate constant 10^5 k, liters per sec. per mole		Solubility in olive oil g./100 ml.
	20°C (Cristol, 1945)	37°C (von Oettingen and Sharpless, 1946)	37°C (von Oettingen and Sharpless, 1946)
2,2-bis(p-iodophenyl)-1,1,1-trichloroethane	---	19,800	0.5
2,2-bis(p-bromophenyl)-1,1,1-trichloroethane	3,470	18,760	2.0
2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane	2,480	12,515	10.5
2,2-bis(p-chlorophenyl)-1,1-dichloroethane	567	4,035	8.0
2,2-bis(p-fluorophenyl)-1,1,1-trichloroethane	303	2,319	>45.0
2-phenyl-2-(p-chlorophenyl)-1,1,1-trichloroethane	301	2,200	27.8
2,2-bis(p-chlorophenyl)-1-chloroethane	91	563	---
2,2-bis(phenyl)-1,1,1-trichloroethane	37	272	33.7
2-(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1-trichloroethane	37	255	---
2,2-bis(p-tolyl)-1,1,1-trichloroethane	11	75.6	12.1
2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane	9	76.8	9.7
2,2-bis(p-chlorophenyl)-2,1,1,1-tetrachloroethane	0	(See Müller, 1946)	---
2,2-bis(p-chlorophenyl)-1,1-dichloroethylene	---	0	21.1

Läuger's theory has received support from the work of Kirkwood and Phillips (1946) who synthesized the compound 2,2-bis-(p-chlorophenyl)-1,1,1-trifluoroethane, which was found to be only feebly insecticidal. This compound which contains a trifluoromethyl group was fed to rats and was found not to be stored in the perirenal fat, although the insecticidally active compound 2,2-bis-(p-fluorophenyl)-1,1,1-trichloroethane fed in a similar manner accumulated to the extent of 3.2 mg./g. fat. In this connection it was pointed out that fluoroform, HCF_3, has no anesthetic properties (Henne, 1937). The compound, 2,2-bis-(p-chlorophenyl)-1,1,1-trifluoroethane, is stated to be very fat soluble even though it was not accumulated in the body fat, a conclusion which casts some doubt on the validity of experiments such as von Oettingen and Sharpless (1946), Busvine (1946a), and Domenjoz (1946), who measured the solubility of various DDT-analogues in fats, such as olive oil, and found no correlation between lipoid solubility and toxicity to mammals and insects (table 22).

Pouterman and Giradet (1946) report that they have synthesized p,p'-dichlorodiphenyldifluorodichloroethane,[1] m.p. 89-90°. This compound tested insecticidally on Phylloperta horticola had a diminished paralytic action as compared to DDT but the toxicity was not altered.

Martin and Wain (1944b) have developed a theory of the toxic action of DDT which is almost directly opposed to the theory of Lãuger. This theory suggests that the p-chlorophenyl groups are responsible for the lipid solubility of the compound, and the trichloromethyl group for the toxic action, which is produced by the liberation of hydrogen chloride in vivo at vital centers. Martin (1946) sums up his conclusions that DDT is insecticidal because it has (1) ability to penetrate and concentrate at the site of action, (2) adequate stability to reach site of action, and (3) ability to release hydrogen chloride when absorbed at site of action. As a consequence of the validity of such a theory one might expect a positive correlation between rate of dehydrochlorination and degree of the toxic action in the DDT-analogues. The dehydrochlorination rates have been studied by Cristol (1945) and von Oettingen and Sharpless (1946) who obtained the data presented in table 22. As has been pointed out by Busvine (1945), Müller (1946), Domenjoz (1946), and Cristol (1945), there is no correlation between the rate of dehydrochlorination and degree of toxicity to insects (tables 19, 20, 21). This is especially noticeable in the case of 2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane which is comparable to DDT in insecticidal potency to many insects and yet has a dehydrochlorination rate constant of about 1/275 that of DDT. Martin replies to this criticism that the factors of absorbability and permeability may also outweigh dehydrochlorination in determining the relative potency of the compound. Furthermore, in vitro dehydrochlorination might be produced by enzyme action or other conditions completely dissimilar to exposure to alkali. It is especially interesting that the compound 2,2-bis-(p-chlorophenyl)-2,1,1,1-tetrachloroethane which does not dehydrochlorinate even upon prolonged refluxing with alcoholic alkali is almost completely inert insecticidally despite its close resemblance to DDT and its conformity with the theoretical requirements for toxicity according to Lãuger's hypothesis.

As a final comment upon these two theories, the data obtained by Metcalf (1948) regarding the activity of DDT-analogues as acaricides to the citrus red mite, Paratetranychus citri, are of interest. DDT was completely inactive but progressive increases in toxicity were obtained by successive eliminations of aliphatic chlorine atoms, the order of effectiveness increasing from:

$$\begin{matrix} R \\ R \end{matrix}\!\!>\!C\!<\!\begin{matrix} R \\ R \end{matrix} = CCl_2\!<\!\begin{matrix} R \\ R \end{matrix}\!\!>\!C\!<\!\begin{matrix} R \\ R \end{matrix} = CHCl\!<\!\begin{matrix} R \\ R \end{matrix}\!\!>\!C\!<\!\begin{matrix} R \\ R \end{matrix} = CH_2\!<\!\begin{matrix} R \\ R \end{matrix}\!\!>\!CF_3 = \begin{matrix} R \\ R \end{matrix}\!\!>\!CCH_3\!<\!\begin{matrix} R \\ R \end{matrix} = CH_2\!<\!\begin{matrix} R \\ R \end{matrix}\!\!>\!COCH_3;$$ where R = p-chlorophenyl-. The most effective acaricides such as 1,1-bis-(p-chlorophenyl)-ethanol, 4,4'-dichlorodiphenylmethane, 2,2-bis-(p-chlorophenyl)-ethane, and 2,2-bis-(p-chlorophenyl)-1,1,1-trifluoroethane are much less effective insecticides than DDT (see tables 20 and 21) and contain neither the lipid solubilizing groups (as defined by Lãuger) nor are capable of eliminating hydrogen chloride.

Gavaudan and Poussel (1947) have explained the action of DDT as that of an indifferent narcotic acting on a lipoidic substrate. Using a nephelometric method they determined the solubility of DDT in water as about 0.1 mg. per liter at 18°. (Roeder and Weiant, 1946, suggest that the solubility of DDT in water is from 0.1 to 0.01 p.p.m.) They have calculated the thermodynamic activity for DDT using the expression C/C_o, where C is essentially the minimum lethal concentration of DDT to the organism and C_o is the saturated concentration in water. Using values obtained from the literature for the minimum lethal concentration in water for several species of insects, the following values for the thermodynamic activity were obtained:

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[1] In the summary of this paper and in Chemical Abstracts the compound is termed p-p'-dichlorodiphenyldifluorochloroethane, apparently an error.

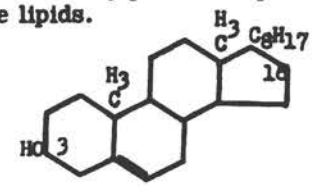
Table 23--Thermodynamic activity of DDT

Species	C g./liter	C/C_o	Reference
Chaoborus punctipennis larvae	0.000013	0.13	Lindquist and Bushland (1944)
Chaoborus punctipennis pupae	0.000026	0.26	
Culex pyrenalcus larvae	0.0001	1.0	Sautet et al. (1947)
Theobaldia longeareolata larvae	0.00002	0.20	
Periplaneta americana adult	0.00001	0.10	Roeder and Weiant (1946)

The thermodynamic activity is observed to fall in the range of 0.1 to 1.0 for the insects listed. This value is in good agreement with the theory of indifferent narcotics of Meyer and Hemmi (1935), who have postulated that narcosis occurs when a chemically indifferent substance reaches a specific threshold concentration in the cell lipids, regardless of the concentration in the external environment. This theory has been elaborated by Ferguson (1939) into his concept of chemical potentials, which expresses the true concentration of the agent at the site of action. Using Busvine's (1945) value for the solubility of DDT in olive oil, 10 per cent or 0.28 moles per liter, as a measure of the lipid solubility of DDT, and Meyer and Hemmi's average threshold value for various narcotics, 0.06 moles per liter, the authors obtained a ratio of 0.06/0.28 or 0.21 for the theoretical thermodynamic activity of DDT in the cellular phase. The close agreement of this value with the range of experimental values in the table is suggested as an indication that DDT acts as an indifferent narcotic.

Hurst (1947) has suggested that DDT and analogous compounds act by indirect blocking of cytochrome oxidase and succinic dehydrogenase, which may be accomplished by uptake and storage of the insecticide in the phospholipids of cell membranes. He postulates a transition from a primary or narcotic stage to a lethal stage which is associated with an irreversible decrease in the stability of the lipoproteins. The evidence for such a theory is apparently derived from the behavior of chloroform in dispersing the lipids of the insect cuticle. For example, Wigglesworth (1945) found that exposure of insects to chloroform vapor for one hour greatly increased the permeability of the insect cuticle to water. The percentage weight losses within 4 hours after exposure were Nematus larvae 49, Blatta adults 6.8, and Rhodnius nymphs 3.4. The loss was least in insects with relatively hard cuticle waxes (see p. 12). Hurst suggests that such variations account for the differences in susceptibility of insects to DDT.

Stereochemistry of DDT molecule--Läuger et al. (1946) state that the distance between the para-chlorine atoms is 11.0 Angstroms and that the valence angle between the phenyl rings is 123°, as determined by X-ray diffraction analysis. Wilde (1946) has presented determinations of the dipole moments of a number of compounds related to DDT, and from these values has calculated that the valence angle lies between 110° and 120° for DDT and most of the other trichloroethanes studied. Läuger (1946) believes that the dimensions of the DDT molecule from para-Cl to para'-Cl atom are similar to those of cholesterol (as measured from position 3 to position 16), a substance which is an important constituent of nerve tissues and which is a fat-soluble vitamin for many insects (Träger, 1941). Läuger suggests that DDT may possess a particular affinity for cholesterol and other sterols present in nerve lipids.



Cholesterol

Campbell and West (1945) have suggested that the three beta-chlorine atoms are necessary for optimal toxicity, owing to a certain steric orientation requirement which reaches a maximum when the remaining chlorine atoms are in the para-positions. Thiophene analogues of a number of biologically active compounds containing benzene rings have been reported to possess activities similar to those of the parent compound. In this connection, Metcalf and Gunther (1947) prepared 2,2-bis-(2-chlorothienyl)-1,1,1-trichloroethane. This compound, which is an isoster of DDT, was generally much less effective against several species of insects despite the similarity in size and shape of the benzene and thiophene rings, yet it dehydrochlorinated readily in dilute alkali. However, at high dosages the compound produced characteristic DDT-like tremors in insects and in isolated legs of Periplaneta americana so that it appears to resemble DDT in its mode of action (Metcalf, 1948a).

Erlenmeyer et al. (1948) have studied the insecticidal properties of bis-(p-chlorophenyl)-acetic acid and 1,1-bis-(p-chlorophenyl)-acetone which they consider to be isosters of DDT by virtue of their ability to form hydrates as shown below. The hy-

drated products are isosteric with DDT because of the similar dimensions of the hydroxyl group (radius about 1.7 Ångströms), methyl group (radius 2.0 Ångströms), and chlorine atom (radius 1.8 Ångströms), (Pauling, 1940). This degree of structural conformity has been shown by Pauling and Pressman (1945) to be well within the limits required for isosteric activity in serological reactions. However, the DDT-isosters mentioned were insecticidally inactive when tested against Musca domestica, Tinea granella, and Dermestes frischii. Thus the authors conclude that the similarity in inductive action on the remainder of the molecule which they ascribe to the trichloromethyl-, carboxy-, and acetyl- groups, is not a factor in the toxic action of DDT.

Quantitative toxicology of DDT to insects--Table 24 gives data obtained on the quantitative toxicity of DDT to a number of species of insects.

Entrance of DDT into the insect body--The data of Tobias et al. (1946) is of especial interest in that it points out that the toxicity of DDT in acetone to the roach is almost identical whether applied externally or intra-abdominally, indicating a very efficient absorption of the toxicant through the insect body wall. The actual toxicity of DDT to the roach is about equal to the intravenous dosage for mammals and is not especially great as compared to many poisons. However, in mammals the surface lethal dosage is very high, up to 4000 mg. per kg. This subject has also been discussed by Dresden and Krijgsman (1948) who feel that insecticides, such as DDT and gamma-hexachlorocyclohexane, are not specific insect poisons but only appear so by their effective penetration of the insect cuticle. In this connection, Richards and Cutkomp (1946) have pointed out the correlation between the possession of a chitinous exoskeleton and susceptibility to DDT shown by various phyla. These investigators have shown that DDT is adsorbed from solutions by cuticle containing chitin and by purified chitin, and they suggest that the chitinous cuticle facilitates the entry of DDT into the insect body by selective concentration through adsorption. It is difficult to explain the relative insusceptibility of certain insects, mites, and ticks to DDT on this basis, however. In a subsequent paper, Fan, Cheng, and Richards (1948) demonstrate the existence of a negative temperature coefficient for Aedes aegypti and Chaoborus larvae immersed in dilute suspensions of DDT. At a DDT concentration of 1:10,000,000, 50 per cent mortality of Aedes larvae resulted in about 3 days at 10°, 1.75 days at 15°, 1.25 days at 20°, 0.75 days at 25°, and 0.5 days at 30° (a positive coefficient); while at 1:500,000,000, 4 days were required at 10°, 4.5 days at 15°, and about 5 days at 20° (a negative coefficient). When an emulsion of DDT was injected in Aedes larvae the mortalities in 5 days were:

Table 24--LD_{50} determinations of DDT to several insects

Species	LD_{50} in micrograms per gram body weight	Method and site of administration	Reference
American roach Periplaneta americana	5-8	intra-abdominal acetone	Tobias et al. (1946)
"	10	surface, acetone	"
"	18	intra-abdominal lecithin-peanut oil emulsion	"
"	82	intra-abdominal peanut oil	"
"	20	intra-abdominal water-oil emulsion	Dresden and Krijgsman (1948)
Housefly Musca domestica			
a. newly emerged adult	2	surface, acetone	Tobias et al. (1946)
b. mature adult	8-21	surface, acetone	"
c. female adult	9	surface, kerosene	David (1946)
d. male adult	6	surface, kerosene	"
Mosquito Aedes aegypti			
a. male adult	5.5	surface, kerosene	"
b. female adult	8.0	surface, kerosene	"
Blowfly Calliphora sp. adult	9-28	surface, kerosene	Tobias et al. (1946)
Human louse Pediculus humanus	27	surface, kerosene	Busvine (1946)
Bedbug Cimex lectularius	63	surface, kerosene	"
Carausius morosus	60	intra-abdominal water-oil emulsion	Dresden and Krijgsman (1948)

at 1 microgram DDT per gram, 75 per cent at 15°, and 88 per cent at 30°; and at 0.5 micrograms per gram, 60 per cent at 15°, and 70 per cent at 30°. The authors consider that this demonstration of a negative temperature coefficient upon immersion in dilute suspensions, and a positive temperature coefficient for injection localizes the negative effect to adsorption of DDT through the cuticle of the body wall, since adsorption is favored by low temperatures.

The selection of the sensory organs of the insect leg (p. 59) as a primary site of action of DDT suggests that insects with highly developed tarsi should be especially susceptible to exposure to DDT residues. Potts and Vanderplank (1945) found that Glossina could be poisoned by contact of the tarsi alone with DDT residues for periods as short as 2 seconds, and particles of DDT could be demonstrated on the pulvilli. In general, the susceptibility of various insects to DDT could be correlated with pulvillar development. This has been questioned by Hickin (1945) who found DDT to act by contact with the tarsi in the lice, Hematopinus suis and Trichodectes latus, in which the pulvilli are slightly developed. Hayes and Liu (1947) compared the histology of the tarsi of Musca domestica, Blatella germanica, and Epilachna varivestis. The two latter insects are very resistant to the action of DDT. The tarsi of the adult housefly possessed abundant chemoreceptors and a thin cuticle, 12.5 to 25 microns in thickness, while no chemoreceptors were found in Blatella germanica, cuticle 60 to 90 microns, or in adults or larvae of E. varivestis, cuticle 25 to 45 microns and 15 to 40 microns, respectively. Burtt (1945) has found that DDT applied as a saturated solution in lanolin to the dorsal integument of the tick, Ixodes ricinus, produced symptoms of intoxication in an average time of about 4 days, while ticks exposed to crystalline residues of DDT or to DDT in lanolin so that only the tarsi contacted the material showed intoxication in about 8 hours. Further experiments in which DDT was applied to the legs above the tarsi produced intoxication in about 48 hours. This was considered to show that the empodia of the tarsi were more readily permeable to DDT than the body cuticle, but may be a reflection of the sensitivity of certain tarsal proprioceptors to DDT.

Physiological experiments on locus of DDT poisoning--The typical symptoms of DDT poisoning in insects clearly demonstrate an effect on the neuromuscular system. Tobias and Kollros (1946) have described the sequence of symptoms in Periplaneta americana as (1) hyperextension of legs, elevation of center of gravity, postural instability, (2) increasing general tremulousness involving head, body, and appendages, (3) ataxic gait, and hyperactivity resulting from stimuli of sound and touch, (4) animal repeatedly falls on back and finally cannot regain feet, (5) leg movements continue with two components, a high frequency tremor, and a slower flexion and extension, (6) fast tremors disappear leaving only isolated motions of body wall, tarsi, palpi, cerci, and antennae, and (7) final sign of life is beating of heart which may continue for a day or more. In this stage, respiration is probably carried out largely by diffusion.

Decapitation before or after DDT poisoning had no effect on the abnormal activity of poisoning nor did section of the ventral nerve cord-connectives, anterior and posterior to the mesothoracic ganglia. Even complete transection of the whole mesothoracic segment had no effect on the leg tremulousness and hyperactivity characteristic of DDT poisoning. Midsagittal sectioning of the ganglion was also without effect. Removal of the ganglion, however, completely prevented the symptoms in almost all cases. Lateral section of the leg nerves also stopped or reduced activity in 65 per cent of the preparations. Experiments with measured doses of DDT indicated that ganglionectomy was less effective in quieting leg movements, the higher the dosage employed, but that this operation in all cases markedly reduced or abolished the high frequency tremor and diminished the slow movements. The application of minute doses of nicotine, which is known to block synaptic transmission centrally as well as peripherally to leg nerves was without effect on the DDT symptoms; but applied to the ganglion caused complete immobility or markedly decreased activity. Thus Tobias and Kollros conclude that in poisoning with low doses of DDT, the hypermotor symptoms result from

afferent impulses reaching the ganglia and transmitted through the intact reflex arc to the motor fibers. Where large doses of DDT are applied, action may take place directly on the motor fibers and not require the reflex arc. It was also possible for DDT to produce motor effects by action on the peripheral nerve system, since symptoms of poisoning occurred in amputated legs, and legs whose nerves had been cut and ganglia destroyed.

Roeder and Weiant (1946) have reached much the same conclusions using amplification and oscillographic methods of measuring nerve potentials in Periplaneta americana. They were unable to detect any change in the electrical activity from the application of 2 per cent DDT solutions or crystals of DDT directly to the isolated nerve cord. Application of emulsions containing 1600 p.p.m. of DDT to the isolated internal muscles of the insect abdomen resulted in isolated twitches and contractures which involved single muscle fibers or parts of fibers, and only appeared several hours after application, indicating a direct action on the muscle fiber or the myo-neural junction. However, the application of DDT to a similar preparation with an intact nerve cord resulted in reflex movements, twitches, and clonic contractions of entire muscles within 10 minutes, but these responses ceased upon removal of the nerve cord, indicating that an intact reflex mechanism was necessary for their production. These data, therefore, indicated that the primary site of DDT action must be on the sensory peripheral nervous system. This was demonstrated by the effect of DDT in increasing to as much as 700 per cent the number of ascending impulses in the nerve cord. Injection of DDT emulsions in concentrations as low as 0.01 p.p.m. into the femur of a leg resulted in persistent trains of afferent impulses which could be detected in the crural nerve of the leg. Each train seemed to involve a single fiber which fired repetitively at a frequency of 300 to 400 per second. This effect was also produced with suspensions of DDT and with a saturated solution of DDT in water. The time interval between application and response was related to the concentration of DDT. Inasmuch as progressive amputation of segments of the leg resulted in disappearance of both normal electrical activity and of DDT responses only when the trochanter was removed, and it has been shown that the campaniform sensillae occur only on the leg segments below the coxa (Pringle, 1938), it is suggested that the DDT symptoms originate in some proprioceptive sense organ perhaps as yet unidentified. Thus the authors conclude that DDT acts on such sensory endings to produce intense trains of afferent impulses which stimulate the motor neurons to general incoordinated activity which is reflected in the tremors and convulsions typical of DDT poisoning.

Bodenstein (1946) has studied the locus of action of DDT, as a 1 per cent emulsion, in larvae and adults of the fruit fly, Drosophila virilis. Injection of the DDT emulsion into the insects produced spasms of the wings and legs much more quickly than in the muscles of the abdominal wall, and the latter continued to contract long after the former were completely paralyzed. By experimenting with isolated portions of the insect body Bodenstein was able to show that abdominal muscles freed from the control of the central nervous system would still twitch under the influence of DDT, thus indicating that DDT produced an effect on the peripheral nervous system or directly on the muscles. It was shown that 1 per cent phenobarbital injected into the insect or applied in a perfusion of a nerve-muscle preparation would completely quiet the insect by a direct paralytic action on the central and peripheral nervous systems. This action was reversible. Flies treated with phenobarbital gave no muscular response to DDT, and tremors in DDT-treated insects were abolished by the action of phenobarbital. This showed that DDT affected the peripheral nerves, probably either at the motor nerves leading to the periphery or at the myo-neural junctions. Imaginal discs from larvae exposed to DDT for 24 hours were transplanted into normal insects and grew and differentiated normally, thus providing further evidence of an effect on the nervous system alone.

Yeager and Munson (1945) performed qualitative experiments on the injection of

DDT in corn oil into various sites in Periplaneta americana and the observation of the resulting nervous disturbances with a view toward determining the site of action. Injection of DDT into the roach leg resulted in symptoms whether (1) the leg was isolated, (2) attached to the intact insect with the heart cauterized to prevent circulation, or (3) in a leg-ganglion preparation. Severance of legs in (2) and (3) had no effect on the tremors, while symptoms in (1) could be caused to cease by sectioning the leg distad to the point of injection. In a second series of experiments, DDT solution was injected into the region of a thoracic ganglion of a cauterized insect or a leg-ganglion preparation. Symptoms resulted only in legs innervated by the ganglion treated, and were abolished by severing the legs. Thus the authors concluded that DDT provoked these symptons in the legs by acting at a site common to both leg and body, such as that part of a motor nerve between ganglionic origin in the central nervous system and the termination of the fibers in the leg, the so-called myo-neural junction. Similar experiments using nicotine (see p. 14) indicated that the mode of action of nicotine differed greatly from that of DDT. Nicotine symptoms were found to mask DDT symptoms in legs, but the latter would reappear in legs upon severance, which abolished the nicotine action.

Welsh and Gordon (1947) have investigated the nature of the action of DDT and certain of its analogues on the nerve axon of Periplaneta americana and certain Crustacea, using electrical stimulation and measurement of the muscular response. It was found that the typical action of DDT on the nerve axon was a multiplication of the nerve impulse to produce a prolonged burst of impulses. This volley of impulses caused a tetanic contraction of the muscle innervated by the axon. The duration of the train of impulses produced in such a manner was directly proportional to the concentration of the toxicant, and in cases of severe poisoning, spontaneous trains of impulses occurred at intervals. These authors found that 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane and 4,4'-dichlorodiphenylacetic acid which are water soluble had no effect on axonal impulses, while 2,2-bis-(p-fluorophenyl)-1,1,1-trichloroethane, 2,2-bis-(p-bromophenyl)-1,1,1-trichloroethane, 2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane, 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane, and 2,2-bis-phenyl-1,1,1-trichloroethane all resembled DDT in action. Similar effects were produced by hexachloroethane, dibenzyl, naphthalene, alpha-bromonaphthalene, paradichlorobenzene, the pyrethrins, veratrine, and nicotine. The authors conclude that all of these substances having a high lipid to water solubility ratio are absorbed on the surface lipids of the nerve axon where they cause the observed toxic effects, perhaps by interfering with the stabilizing action of divalent cations such as calcium. This viewpoint, however, makes it difficult to explain the insecticidal inactivity which results from minor alterations in the chemical structures of DDT, the pyrethrins, and nicotine. For example, 2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene and 2,2-bis-(p-chlorophenyl)-2,1,1,1-tetrachloroethane have about the same degree of lipid solubility as DDT (see table 22) and yet have only a very feeble insecticidal action (see table 21). Munson and Yeager (1946) found that para-dichlorobenzene, hydroquinone, aniline, and phenol when injected into nymphal Periplaneta americana, produced symptoms practically indistinguishable from DDT, and occurring at comparable dosages, yet these substances are of no value as contact insecticides. Läuger et al. (1946) discuss the production of tremors in insects by the injection of simple lipid soluble organic substances. They state that benzene, chlorobenzene, ortho- and para-dichlorobenzene, carbon tetrachloride, and carbon bisulfide produced tremors but chloroform did not. It was determined that compounds which are fat solvents, non-miscible with water, but which are hydrophilic will produce narcosis, while fat solvents, non-miscible with water but hydrophobic will produce tremors. It is also noted that miscible compounds, such as alcohol and acetone (enzyme poisons), will also cause tremors. It is suggested that these tremor-producing substances may cause disturbances in the semi-permeable membranes of cells by a dissolution of lipid structure.

Hartzell (1945) has investigated the histological effects of DDT poisoning on nerve and muscle tissue in moribund Musca domestica. The changes produced by DDT were

much less pronounced than those observed with the pyrethrins (see p. 24), but consisted of a partial dissolution of the nerve fiber tracts and degeneration of the nuclei in the brain and thoracic ganglia and slight clumping of the chromatin of nuclei in muscle fibers. On the other hand, Richards and Cutkomp (1945a) were unable to detect any clear-cut evidence of degeneration in the nerve cord, midgut epithelium, malpighian tubes, thoracic muscles, heart, or nephrocytes of Periplaneta americana dying from DDT poisoning. The nerve cords of these moribund insects still possessed normal optical properties and were capable of transmitting impulses produced by electrical stimulation. Witt (1947) was also unable to detect any histological changes in the central nervous system of Calliphora erythrocephala poisoned with DDT. He believes that DDT does not penetrate into the insect through the thick cuticular plates but rather through the cuticular articulations, spiracles, and sense organs.

Metabolism of DDT in vivo--White and Sweeney (1945) have studied the metabolism of DDT in the rabbit, and have isolated di-(p-chlorophenyl) acetic acid from the urine of rabbits fed DDT. These authors suggest that the detoxification mechanism in vertebrates may follow the degradation process of DDT in alkaline solution, as shown in figure 1; that is, by loss of hydrogen chloride, through the corresponding ethylene to the acetic acid derivative. No similar work has yet appeared on insects, but Läuger et al. (1946) report experiments on flies poisoned with liquid mixtures of DDT-isomers. Extracts from the thoracic ganglia of 50 poisoned flies would poison 5 additional flies. The DDT content of the thoracic ganglia was at a maximum within 30 minutes after poisoning. Using the same technic with the entire gut and malpighian tubes, enough DDT was recovered from 50 flies to kill 30 flies. The conclusion was reached therefore that DDT is excreted into the rectum by the malpighian tubes.

Hansen et al. (1944) have used 2,2-bis-(p-bromophenyl)-1,1,1-trichloroethane synthesized with Br^{82} to produce a radioactive molecule which they traced in adult Periplaneta americana, and larval Tenebrioides mauritanicus, Tenebrio molitor, and Galleria mellonella. The material was applied externally, and radioautographs were obtained which indicated that the material was present in the nerve cord, and brain, as well as other tissues.

Biochemistry of DDT poisoning in insects--Investigations of the mode of action of DDT were directed toward the effects of the poison upon known enzyme systems of the insect nervous system. The acetylcholine-cholinesterase system was studied in detail because of the resemblance between symptoms of DDT poisoning and those produced by eserine and from a potentiation of DDT toxicity by eserine (Tobias et al., 1946a). Several investigators have shown that this system is of fundamental importance to nerve impulse transmission in insects. Corteggiani and Serfaty (1939) measured the free acetylcholine content of several species of whole insects and in the nerve cord of Tenebrio molitor where the content was 100 to 200 micrograms per gram, and in the cerebral ganglia of Gryllus domesticus, 65 micrograms per gram, and Xylocopa violacea, 200 micrograms per gram. Mikalonis and Brown (1941) determined that the nerve cord of Periplaneta americana contained 70 micrograms of bound acetyl choline and 40 to 220 micrograms of free acetyl choline per gram. They also determined that the cholinesterase in 100 mg. of roach nerve cord would destroy 0.12 mg. of acetylcholine in 60 minutes. Tahmisian (1943) found that acetylcholine is formed in the egg of Melanoplus differentialis only during the post-diapause stage. Means (1942) measured the cholinesterase activity of several types of tissues of adult Melanoplus differentialis but his results were very much lower than the values obtained by other investigators.

The enzyme cholinesterase has been shown by many investigators to destroy acetyl choline by hydrolysis to acetic acid and choline:

$$CH_3\overset{O}{\overset{\|}{C}}OCH_2CH_2N + \overset{Cl^-}{(CH_3)_3} \xrightarrow[H_2O]{\text{Cholinesterase}} CH_3COOH + HOCH_2CH_2N + \overset{Cl^-}{(CH_3)_3}$$

acetyl choline chloride — acetic acid — choline — chloride

It is generally agreed that this process is responsible for the chemical mediation of nerve impulses at the synapses where acetyl choline is the mediator and cholinesterase destroys the mediator to clear the way for the next impulse.

Richards and Cutkomp (1945) have measured the activity of cholinesterase in bee brains and the nerve cord of Periplaneta americana using several choline esters as substrates. The values obtained are given in the table where they are compared with similar data for rat brain cholinesterase by Nachmansohn (Nachmansohn and Rothenberg, 1945). It will be noted that the insect cholinesterase is more active against acetyl-beta-methyl choline than against acetyl choline, and that the converse is true of rat brain cholinesterase. Using Nachmansohn's criteria this suggests that acetyl choline may not be the true chemical mediator in insect nerve impulse transmission, but that a related material, such as the beta-methyl ester, may carry out this function.

Table 25--Cholinesterase activity

Substrate	Comparative rates of hydrolysis	
	Bee brain cholinesterase 24-26° C. (Richards and Cutkomp, 1945)	Rat brain cholinesterase (Nachmansohn and Rothenberg, 1945)
Acetyl choline	100	100
Acetyl-beta-methylcholine	305	43
Butyryl choline	24	22
Benzoyl choline	7	0

Richards and Cutkomp (1945) found that DDT did not inhibit the cholinesterase when added to roach cord in vitro. This, however, was not thought to be conclusive evidence because of the insolubility of DDT in aqueous system. Tobias et al. (1946a) made cholinesterase measurements on normal roach nerve cords and on cords from roaches in various stages of DDT poisoning. They found a cholinesterase activity hydrolyzing 320 mg. acetyl choline per gram tissue per hour at 37.6°, and about 520 mg. acetyl-beta-methyl choline. No inactivation of the cholinesterase could be detected in any stages of DDT poisoning.

These same authors found, however, that the amount of acetyl choline in the ventral nerve cord of the American roach or in the housefly increased very markedly in DDT poisoning when the insect reached the prostrate state but not during the tremor of hyperactive stage, from a normal of 33 micrograms per gram to 102 micrograms per gram. It is of interest that this rise in the acetyl choline content occurred in the connectives and not in the ganglia. In the intact housefly a similar rise from a normal of 47 to 131 micrograms per gram was observed following DDT poisoning. In support of the hypothesis that this increased acetyl choline results from a conversion of a bound form, such as an acetyl-choline-lipoprotein complex, to the free ester, it was found that in the normal roach cord about 20 per cent of the acetyl choline was in the bound form, but that in the poisoned prostrate roach, nearly 100 per cent of the ester was in the free form.

No similar rise in acetyl choline content could be demonstrated in DDT-poisoned frogs and rats. Experiments were carried out to determine if DDT accelerated acetyl choline synthesis by the roach nerve cord, in vitro, and it was found that no significant difference between normal and poisoned insects occurred. Anaerobic synthesis of acetyl choline occurred at about 94 micrograms per gram of nerve cord per hour. Dichlorodiphenylacetic acid, a DDT metabolite, was also without effect.

The violent hyperactivity of DDT-poisoned roaches and the prolonged period of prostration intervening before death has suggested that the ultimate effects of DDT-poisoning are metabolic exhaustion. Merrill et al. (1946) have studied the effects of DDT-poisoning on Periplaneta americana. No significant change occurred in water content which was about 68 per cent for both normal and poisoned insects deprived of food and water for 18 to 48 hours, or in the water content of the nerve cord. The total weight loss in both poisoned insects and normal ones deprived of food and water over an equivalent period was 11 to 12 per cent.

In contrast to this, however, 24 hours after DDT-poisoning the body content of the carbohydrates, glycogen, and glucose had fallen to 10 per cent of normal. However, immobilization of poisoned roaches by carbon dioxide or cyclopropane anaesthesia during the hyperactive stage almost completely eliminated the carbohydrate depletion, thus indicating that this condition arises as a result of the hyperactivity rather than from a direct toxic effect of the DDT. It is interesting to note that poisoned insects recovering from anaesthesia exhibited all the symptoms of poisoning even though they had been spared the hyperactivity and loss of carbohydrates and fat.

The total body pyruvate content was also lowered during the course of DDT-poisoning and this could be largely prevented by glucose administration. Attempts to prevent the toxic reaction of DDT-poisoning by glucose administration, however, were not successful, although the glycogen content was kept at a normal level and the glucose content remained at about 70 per cent of normal. The effects of this abnormal glucose metabolism were sufficient to increase the content of acetone bodies from about 1 microgram per gram wet weight to 1.7 micrograms per gram in poisoned roaches.

Gross measurements of the fat content of the roach fat body in normal and poisoned roaches suggest that the fat reserves may undergo some depletion during poisoning, but that this is not always significant. No significant change occurred in the non-protein nitrogen content during poisoning, suggesting that there is little if any cellular disintegration by 24 hours after poisoning.

Ludwig (1946) has made a comprehensive study of the effects of DDT-poisoning on various life stages of the Japanese beetle, Popillia japonica. Embryonic development was not affected, but when eggs were exposed to DDT in peanut oil early in development, hatching was delayed as the larvae appeared to experience difficulty in breaking through the chorion. DDT-poisoned larvae, averaging 196.4 mg. in weight, lost an average of 67.6 mg. before death, this loss being composed of 50.68 mg. water, 2.52 mg. of fat, 1.06 mg. glycogen, 0.42 mg. glucose, and 14.53 mg. feces. The glycogen content of the larvae was almost depleted but there was no loss of protein. The loss of fat, glycogen, and glucose appeared to be associated with the violent muscular activity exhibited by DDT-poisoned larvae. The poisoned larvae had an increased oxygen consumption starting about 2 hours after poisoning and continuing for 5 days, after which it declined until death. This increased consumption, which reaches a maximum of twice the normal rate within 24 hours after poisoning, corresponds with the period of hyperactivity. The respiratory quotient varied from 0.7 to 0.8 during the first two days and then dropped to 0.6 to 0.7. This appears to indicate that carbohydrates and fats are being oxidized during the first two days, following which the fat reserve is being utilized. The author quotes Barron (unpublished) as finding a three- to five-fold increase in oxygen consumption in the meal worm adult, Tenebrio molitor, after DDT-

poisoning which Barron believes is caused by increased activity, since the excessive oxygen consumption was eliminated by the use of narcotics to prevent muscular tremors, and was not found in grasshopper eggs in diapause, exposed to DDT. In studies with adult Japanese beetles, poisoning with DDT resulted in an increase in oxygen consumption which reached 3 to 4 times normal. The rate of increased oxygen utilization appeared to be correlated with the violence of hyperactivity. The poisoned adults lost about 1/10 of their normal weight and this was accompanied by a reduction in water content. DDT-poisoning also appeared to affect the ability of larvae and adults to withstand dessication. This may be correlated with the hyperactivity which may result in a greater production and loss of metabolic water. The author suggests that death from DDT-poisoning follows from the hyperactivity which results in the rapid utilization of the carbohydrate reserve and causes the death of the insect by starvation. He points out as further evidence that unfed larvae survived an average of 30 days, while unfed adults lived only 8 days, and that poisoned larvae survive 5 to 8 days, while poisoned adults live only 2 to 3 days.

Gordon and Welsh (1948) have investigated the basic mechanisms responsible for the repetitive discharges of nerve impulses produced by DDT applied to the crayfish motor axon. This effect was shown to be similar to those produced by calcium-binding agents such as citrate or oxalate or by low calcium or magnesium ion concentrations in the normal nerve, and the duration of the discharge was inversely proportional to the calcium ion concentration. The authors infer that DDT disorders the surface axon structure, perhaps by simple spatial distortion, reducing the affinity of phosphoryl- or other polar groups for calcium ions. This causes a delay in the restoration of calcium ions to this surface complex following the breakage of the chelate linkage during the passage of the initial exciting impulse. Spontaneous activity of the axon results when this depolarization falls below a critical level.

BENZENE HEXACHLORIDE

Introduction--According to Slade (1945) benzene hexachloride was first prepared by Michael Faraday in 1825. Van der Linden in 1912 first established the existence of four isomers. The discovery of the insecticidal properties of the gamma isomer seems to have been made by chemists of Imperial Chemical Industries, England, about 1942, although French workers had been investigating the insecticidal properties of the crude material as early as 1940. Since that time large tonnages of the crude material have been produced which have proven of exceptional value in the control of grasshoppers, cotton insects, wireworms, and other pests.

Chemistry of hexachlorocyclohexane--The chlorination of benzene in the presence of ultraviolet light produces a mixture of the isomers of 1, 2, 3, 4, 5, 6 - hexachlorocyclohexane, more loosely termed benzene hexachloride. At least five chemically distinct isomers are present in the crude insecticidal mixture, and, in addition, small amounts of a heptachloro-cyclohexane, and an octachloro-cyclohexane which are formed by the additive chlorination of monochloro- and dichlorobenzene probably formed during the reaction. Table 26 summarizes the per cent composition of various crude hexachlorocyclohexane mixtures as determined by several methods. In the presence of alcoholic alkali, the alpha, gamma, delta, and epsilon isomers readily dehydrohalogenate, liberating 3 moles of hydrogen chloride per mole to form principally 1,2,4-trichlorobenzene, although lesser amounts of 1,2,3- and 1,3,5-trichlorobenzene are formed (Cristol, 1947; Kauer et al., 1947; Gunther and Blinn, 1947). The beta isomer, although reacting very slowly under these conditions at room temperature (Cristol, 1947), dehydrohalogenates at reflux temperature to produce principally 1,2,4-trichlorobenzene (Gunther and Blinn). Cristol obtained the following approximate rate constants for alkaline dehydrohalogenation at 20° C. according to the following reactions:

$$C_6H_6Cl_6 + OH^- \xrightarrow{k_1} C_6H_5Cl_5 + H_2O + Cl^-$$

$$C_6H_5Cl_5 + OH^- \xrightarrow{k_2} C_6H_4Cl_4 + H_2O + Cl^-$$

$$C_6H_4Cl_4 + OH^- \xrightarrow{k_3} C_6H_3Cl_3 + H_2O + Cl^-$$

	$\times 10^5$ (to conform with table 22)
Alpha - 0.169 liters/sec/mole = k_1	16,900
Beta - 3×10^{-6} = k_1	0.3
Gamma - 0.045 approx. = k_1	4,500
Delta - 0.110 = k_2	11,100

Kauer et al. (1947) give comparative figures for the per cent dehydrohalogenation of the five isomers in alcoholic sodium hydroxide as follows:

Table 25a--Dehydrohalogenation of isomers

Isomer	Per cent reaction to trichlorobenzene	Dehydrohalogenation of isomers of benzene hexachloride in 0.1 N alcoholic NaOH, at 0° C., in 2 hours
		Calculated as mole fraction HCl produced
Alpha	70	2.10
Beta	0	0
Gamma	25	0.75
Delta	50	1.5
Epsilon	19	0.57

Table 26--Composition of technical hexachlorocyclohexanes

Compound	m.p. °C.	Per cent composition		
		Ramsey and Patterson (1946)	Slade (1945)	Kauer et al. (1947)
Alpha-1,2,3,4,5,6-hexachlorocyclohexane	157-8°	65-70	Up to 70	55
Beta-1,2,3,4,5,6-hexachlorocyclohexane	309°	5-6	5	14
Gamma-1,2,3,4,5,6-hexachlorocyclohexane	112°	13	10-12	12
Delta-1,2,3,4,5,6-hexachlorocyclohexane	138-9°	6	7 (by difference)	8
Epsilon-1,2,3,4,5,6-hexachlorocyclohexane	218-19°	--	--	3-4
Heptachlorocyclohexane	85-86°	4		
Octachlorocyclohexane	147-149°	0.6		

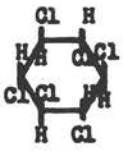
In this series of compounds, as in the case of the DDT derivatives, there is no apparent correlation between the relative rates of dehydrochlorination of the various isomers and their relative toxicities to insects. This again cannot be accepted as an entirely valid objection to the dehydrochlorination mechanism of action theory because of the peculiar stereochemical relations imposed on the molecule which is to act as a competitive metabolite to inositol as will be discussed in a later section.

The gamma isomer of hexachlorocyclohexane is remarkably stable toward oxidation and can be burned and recrystallized from hot nitric acid without appreciable decomposition (Slade, 1945). The vapor pressures of the various isomers are given by Balson (1947) at 20° C. as alpha 2.5×10^{-5} mm. Hg., beta 2.8×10^{-7}, gamma 9.4×10^{-6}, delta 1.7×10^{-5} mm. The values given by Slade at 20° C., i.e., alpha 0.02 mm., beta 0.005 mm., gamma 0.03 mm., and delta 0.02 mm., seem to be entirely too high.

Stereochemical configuration of isomers--The problem of the determination of the structure of the isomers of 1,2,3,4,5,6-hexacyclohexane is complicated by the uncertainty as to the basic form of the cyclohexane ring. Strainless forms can exist either in the "Z or chair form" and the "C or boat form" (Daasch, 1947). Actual structural determinations have been made only on the beta isomer (Dickinson and Bilicke, 1928) which has been shown to be the most symmetrical structure having adjacent chlorine (or hydrogen) atoms trans to each other. Slade, Kauer et al., and Daasch have assigned tentative structures to the other isomers, on the basis of infra-red spectra, stereochemical considerations, and statistical treatments.

Formulae based on a non-planar cyclohexane ring are difficult to express and interpret in line drawings. Slade, however, presents hypothetical structural formulae for the alpha, beta, gamma, and delta isomers based on a planar structure for the cyclohexane ring. This structure has also been proposed for the corresponding hexahydroxycyclohexanes or inositols (Gilman, 1943). The following figure shows these structures and meso-inositol:


alpha


beta


gamma


delta


meso-inositol

Relative toxicity of isomers and related materials--Data for the relative effectiveness of the various isomers are presented in table 27. Slade (1945) obtained the following results with direct sprays against Musca domestica: alpha-isomer at 0.80 per cent - 21 per cent mortality; gamma-isomer at 0.01 per cent - 73 per cent mortality; and delta-isomer at 1.1 per cent - 24 per cent mortality. From the various experiments it appears that the gamma-isomer is at least several hundred times as toxic to insects as any of the other isomers. In this connection, Slade (1945) gives the oral LD_{50}'s of several isomers to rats as alpha- 1.7 g./kg., beta- non-toxic, gamma- 0.19 g./k.g., and delta- 1.0 g./kg. Metcalf (1947) was unable to show that the presence of any of the other isomers alone or in combination had any effect on the toxicity of the gamma-isomer to Heliothrips haemorrhoidalis, at concentrations as high as 1000 times the amount of the gamma-isomer present.

Two interesting attempts have been made to prepare structural analogues (isosters) of gamma-hexachlorocyclohexane which would possess insecticidal properties. McGowan (1947) reasoning that 2,2-bis-(p-methoxyphenyl)1,1,1-trichloroethane is nearly as efficient an insecticide as DDT (see p. 49), prepared the hexamethyl ether of meso-inositol or hexamethoxycyclohexane which should be very similar in size and shape to the gamma-hexachlorocyclohexane molecule, and should possess corresponding lipid-solubilizing properties. However, the compound was not highly insecticidal, killing only 13 per cent of Musca domestica when tested at 0.1 per cent as compared with 59 per cent killed by the gamma-isomer at 0.01 per cent. The pentamethylether-monoacetate of meso-inositol was also ineffective, killing 22 per cent at 0.5 per cent.

Table 27--Relative toxicity of isomers of hexachlorocyclohexane and related materials to insects

Material	Amount required to give about 50% mortality to Calendra granaria (Sitophilus granarius) when applied as dust to grain (Slade, 1945)	Approx. median lethal conc. to Heliothrips haemorrhoidalis (Metcalf, 1947)
		Per cent
Gamma-1,2,3,4,5,6-hexachlorocyclohexane m.p. 112° C.	0.4 p.p.m.	0.0001
Alpha-1,2,3,4,5,6-hexachlorocyclohexane m.p. 157-158°C.	360 p.p.m.	0.1
Beta-1,2,3,4,5,6-hexachlorocyclohexane m.p. 309° C.	Almost non-toxic	>1.0
Delta-1,2,3,4,5,6-hexachlorocyclohexane m.p. 138-139° C.	2200 p.p.m.	1.0
Epsilon-1,2,3,4,5,6-hexachlorocyclohexane m.p. 218-219° C.	---	>1.0
Alpha-1,1,2,3,4,5,6-heptachlorocyclohexane m.p. 140-141° C.	---	>0.1
Beta-1,1,2,3,4,5,6-heptachlorocyclohexane m.p. 256-259° C.	---	>0.1
Beta-1,1,2,3,4,4,5,6-octachlorocyclohexane m.p. 260° C.	---	>0.1
Alpha-1,1,2,2,3,4,5,6-octachlorocyclohexane m.p. 146-150° C.	---	>0.1
Beta-1,1,2,2,3,4,5,6-octachlorocyclohexane m.p. 257-260° C.	---	>0.1
1,1,2,2,3,4,4,5,6-enneachlorocyclohexane m.p. 94-95° C.	---	>0.1
1,2,3,4,5,6-hexabromocyclohexane m.p. 212-213° C.	---	>0.1
1,2,4-trichlorobenzene	---	>0.1

Stringer and Woodcock (1948) prepared hexaethylcyclohexane by hydrogenation of hexaethylbenzene. This compound was also relatively ineffective, 25 mg. resulting in 10 per cent kill of Calandra granaria (Sitophilus granarius) in 96 hours, as compared to 90 per cent killed by 5 mg. of gamma-isomer.

Physiological effects of poisoning--Savit et al. (1946) and Dresden and Krijgsman (1948) point out the approximate equivalence between the LD_{50} for gamma-hexachlorocyclohexane applied externally and injected intra-abdominally to the American cockroach, and emphasize the importance of this remarkable absorptive capacity of the insect body surface in determining the effectiveness of contact insecticides. The symptoms of poisoning in this insect are described by Savit et al. as tremors, followed by ataxia, convulsions, falling and prostration and they appear to occur much sooner after the administration of a toxic dose of gamma-isomer, than after the administration of a comparably toxic dose of DDT. This more rapid lethal action is apparently characteristic of many insects.

Dresden and Krijgsman (1948) injected water-oil emulsions of the gamma, alpha, and delta isomers intra-abdominally into P. americana. The LD_{50} of the gamma isomer was 17 μg./g. body weight, while the other isomers produced no effects at 85 μg./g., the limit of solubility. This indicates that the high effectiveness of the gamma-isomer as compared to the others is not due to a more effective penetration of the insect body.

Biochemistry of poisoning--Tobias et al. (1946a) have studied the effect of poisoning by gamma-hexachlorocyclohexane on the acetyl choline content of the ventral nerve cord of Periplaneta americana and found a definite increase in the prostrate stage of poisoning from a normal of 38 μg./g. of cord to 57 μg./g. for the poisoned roach. DDT poisoning results in a similar increase to about 100 μg./g. cord (see p. 62).

Slade (1945) has mentioned the isosteric resemblance between the molecular configuration of the B-vitamin meso-inositol (Posternak, 1941), a 1,2,3,4,5,6-hexahydroxycyclohexane, and gamma-hexachlorocyclohexane, which was pointed out by Mooney, and believes that the gamma-isomer may act as a competitive metabolite to the meso-inositol and thus block some vital reaction in the organism. This hypothesis has gained support through the work of Kirkwood and Phillips (1946a) who have studied the effect of the addition of isomers of hexachlorocyclohexane to the media for growing the Gebrüder-Meyer strain of Saccharomyces cerevisiae, an organism which must have meso-inositol for normal growth. The alpha and beta isomers produced a slight inhibition of yeast growth at the limits of their solubility, i.e., 40 and 20 μg./ml., while the delta-isomer produced a more marked inhibition at 50 μg./ml. In all three cases the inhibition was not reversed by the addition of inositol. The gamma-isomer markedly inhibited the growth of the yeast at 60 micrograms per ml. and this inhibition was progressively reversed by the addition of 1 to 6 micrograms of inositol per ml., but not completely so. The low molecular inhibition ratio, ci/cm = 30, however, points to a very close structural resemblance between the two compounds (McIlwain, 1942). Similarly, Buston et al. (1946) have found that the addition of the gamma-isomer to culture media curtailed the growth of Nematospora gossypii, while the alpha- and beta-isomers had little effect. Attempts to show an antidotal effect from the administration of inositol to insects poisoned with gamma-hexachlorocyclohexane have so far not proven successful (Metcalf, 1947; Thorpe and de Meillon, 1947), although the latter found that liquid triglycerides such as triolein inhibited the toxicity of the gamma-isomer to fourth instar Culex fatigans larvae and as a contact spray to Cimex lectularius. Dresden and Krijgsman (1948) found that the LD_{50} of injected water-oil emulsions of gamma-isomer to Periplaneta americana was not altered by the simultaneous administration of equal parts, or twice as much meso-inositol. Therefore, they doubt the validity of the Slade theory.

Melander (1946) measured the dipole moments of the isomers of hexachlorocyclohexane and obtained the following values: alpha - 2.2 D, beta - O, gamma - 3.6 D, and delta - O. From a study of other compounds such as DDT, he concluded that there was

a relationship between dipole moment and insecticidal activity, which was greatest for aromatic compounds having moments of the order of 4 D.

Quantitative toxicology of gamma-hexachlorocyclohexane to insects--Table 28 gives data on the quantitative toxicity of the gamma-isomer to several insects.

Table 28--LD_{50} values for gamma-hexachlorocyclohexane to various insects

Insect	LD_{50} in micrograms per gram body weight	Method and site of administration	Reference
Periplaneta americana	5	Body surface, acetone,120 hrs.	Savit et al. (1946)
"	4	Intra-abdominal, acetone,120 hrs.	"
"	17	Intra-abdominal, water-oil emulsion	Dresden and Krijgsman (1948)
Musca domestica	0.4	Newly emerged fly, surface,48 hrs., acetone	Savit et al. (1946)
"	1.0	Older adult, 48 hrs. surface, acetone	"
"	2.0	Male fly surface, kerosene	David (1946)
"	3.0	Female fly surface, kerosene	"
Aedes aegypti (adult)	3.0	Male surface, kerosene	"
" "	3.5	Female surface, kerosene	"
Pediculus humanus	1.5	Surface, kerosene	Busvine (1946)
Cimex lectularius	6.0	" "	"
Calliphora spp.	0.6	Surface, older adults, acetone,48 hrs.	Savit et al. (1946)
Melanoplus differentialis	5-10	Oral in xylene emulsion,24 hrs.	Kearns et al. (1946)
M. differentialis	4.4	Contact	Weinman and Decker (1947) calculated from data on 37% gamma-isomer
M. differentialis	13	Stomach	"

ORGANIC PHOSPHATES

Introduction--The development of the organic phosphates as insecticides apparently resulted from the discoveries of Schrader of Germany immediately prior to the war. Since the facts regarding these materials were intimately connected with chemical warfare research they were not publicized until after the defeat of Germany, and indeed much of the material was apparently unknown in this country until uncovered by Office of Technical Service representatives (Kilgore, 1945; Thurston, 1946). Although these materials have been available for scarcely a year in this country, three have reached commercial status as insecticides, viz., hexaethyl tetraphosphate, tetraethyl pyrophosphate, and 0,0-diethyl-0-p-nitrophenyl-thiophosphate, or parathion.

Chemistry--Schrader (1942, 1943) claimed as the reaction product of three moles triethyl orthophosphate and one mole phosphorus oxychloride at 150° a compound which he called hexaethyl tetraphosphate, HETP, $O = P[OP(OC_2H_5)_2]_3$. The material was described as light yellow to brown liquid, sp. gr. 1.2917/27°, which could not be distilled and decomposes at 145-150°. Thurston (1946) describes a modification of this process using ethyl alcohol in place of triethyl phosphate. Subsequently, Woodstock (1946) patented compositions resulting from the reaction of triethyl phosphate and phosphorous pentoxide at low temperatures. Depending on whether the molecular ratio of the reactants was 1:1, 1:2, 1:2.5, or 1:4, respectively, there were formed ethyl metaphosphate, $C_2H_5O\text{-}P(=O)(-O)$, hexaethyl tetrapolyphosphate; pentaethyl tripolyphosphate, $O = P(OC_2H_5)[OP(OC_2H_5)_2]_2$; or tetraethyl pyrophosphate, TEP, $(C_2H_5O)_2P(=O)\text{-}O\text{-}P(=O)(OC_2H_5)_2$. Recently, Hall and Jacobson (1948) have stated that the principal product of the above reactions is tetraethyl pyrophosphate which can be isolated in amounts up to 40 per cent and distilled. Ethyl metaphosphate, triethyl orthophosphate, and possibly pentaethyl triphosphate are present as impurities. Since these latter compounds are relatively non-toxic it is concluded that tetraethyl pyrophosphate is the active insecticidal ingredient of the hexaethyl tetraphosphate mixtures. By increasing the proportion of triethyl orthophosphate in the Schrader reaction from 3 moles to 5 moles, a product has been obtained which corresponds to the empirical formula for tetraethyl pyrophosphate and is more active insecticidally. A similar increase in activity of the product from the Woodstock process was obtained by heating for one hour at 160° C.

Hansen (1947) has suggested that the active ingredient of HETP is tetraethyl pyroxydiphosphate, $(C_2H_5O)_2P(=O)\text{-}O\text{-}O\text{-}P(=O)(OC_2H_5)_2$ which he states is present in concentrations of 10 to 40 per cent in various commercial HETP products, but this idea appears to lack confirmation.

Tetraethyl pyrophosphate was first prepared by Clermont (1854) by the reaction between ethyl iodide and silver pyrophosphate, and subsequently by Rosenheim et al. (1906), Rosenheim and Pritze (1908), and Arbusow and Arbusow (1932). The pure ester is a water-white, mobile liquid, b.p. 104-110°/0.08 mm., sp. gr. 1.1845/25° (Hall and Jacobson, 1948). It is completely miscible in water and hydrolyzes to produce two equivalents of diethyl orthophosphoric acid, following a first order reaction. The times required for 50 per cent hydrolysis are 6.8 hours at 25°, and 3.3 hours at 38°; and for 99 per cent hydrolysis, 45.2 hours at 25°, and 21.9 hours at 38°. Pure tetraethyl pyrophosphate decomposes to liberate ethylene and metaphosphoric acid when heated to 208° to 213°, and various crude products undergo this decomposition at temperatures of 140° and above (Hall and Jacobson, 1948). All the insecticidal products of this nature are

hygroscopic and care should be exercised to protect them from moisture in order to preserve their insecticidal activity. The hydrolysis products are corrosive, attacking iron, zinc, tin, and some grades of porcelain. Harris (1947) describes the hydrolysis of HETP as the formation of two equivalents of acid per mole of ester immediately upon addition of water, followed by a slow hydrolysis which results in detoxification. The hydrolysis products are mono- and di-ethyl phosphoric acids (Hall and Jacobson, 1948). Fischer (1947) prepared and studied the hydrolysis of several hexa-alkyl-tetraphosphates and obtained the following results:

Table 29--Hydrolysis of tetraphosphates

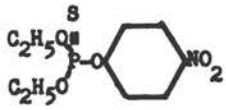
Compound	Approximate time required for 50% hydrolysis of 0.1% solutions at 18° C.
hexamethyltetraphosphate	2 minutes
hexaethyltetraphosphate	5 minutes
hexapropyltetraphosphate	20 minutes
hexabutyltetraphosphate	2 hours

A large portion of the physiological work summarized was carried out using di-isopropylfluorophosphonate, $FP\text{-}[OCH(CH_3)_2]_2$, or DFP; a material which was apparently among the first of the highly toxic phosphoric acid esters to be discovered (Lange and Kreuger, 1932). The compound is a colorless liquid, sp. gr. 1.055, soluble to 1.54 per cent in water at 25° (Comroe et al., 1946). Its action on vertebrates and invertebrates is of the same nature as that of tetraethylpyrophosphate (Mazur and Bodansky, 1946; Mazur, 1946). Schrader (1947) found that diethylfluorophosphonate, b.p. 72° at 12 mm. was a very promising insecticide for the control of flies, gnats, bedbugs, lice, caterpillars, aphids, and scale insects. It has considerable fumigant as well as contact insecticidal action. The dialkylfluorophosphonates can be prepared by reacting the corresponding dialkylchlorophosphonates with sodium or potassium fluoride in aqueous solution or in non-aqueous solvents (Schrader, 1947). Mazur (1946) found the relative rates of hydrolysis of dialkylfluorophosphonates in water to fluoride ion and dialkylphosphoric acids to be:

Table 30--Hydrolysis of fluorophosphonates

Compound	Relative rates of hydrolysis at 38° C.
dimethylfluorophosphonate	10
diethylfluorophosphonate	2
diisopropylfluorophosphonate	1
ethylmethylfluorophosphonate	2

Parathion, or 0,0-diethyl-0-para-nitrophenylthiophosphate,



was apparently first prepared by Schrader (1947) (see also Thurston, 1946), by reacting phosphorus thiotrichloride with sodium ethoxide, and the product with sodium para-nitrophenate. The compound is a dark brown to yellow liquid, theoretical b.p. 375°, sp. gr. 1.26/25°, and vapor pressure about 0.0006 mm. Hg. at 24°. (Gleissner et al., 1947) It is water soluble to about 20 p.p.m. and hydrolyzes in alkaline solutions but not in water or lime water, forming para-nitrophenol and diethyl-ortho-thiophosphoric acid. The related ester, 0,0-diethyl-0-para-nitrophenylphosphate, $(C_2H_5O)_2\overset{O}{\overset{\|}{P}}OC_6H_4NO_2$, b.p. 173°/1 mm. is a yellow liquid, soluble in water to about 0.1 per cent and more readily hydrolyzable in the presence of alkali than parathion. It is also considerably more toxic to warm-blooded animals than parathion, and is a very effective insecticide (Schrader, 1947).

Relation of chemical structure to toxicity--Little has been published concerning the relation of chemical structure to insecticidal action of the organic phosphates. Table 31 presents data on simple phosphoric acid esters. Schrader (1947) has shown that bis-(dimethylamido)-phosphoryl fluoride $[(CH_3)_2N]_2\text{-}\overset{O}{\overset{\|}{P}}F$, and pyrophosphoryl-tetra-(dimethylamide) $[(CH_3)_2N]_2\text{-}\overset{O}{\overset{\|}{P}}\text{-O-}\overset{O}{\overset{\|}{P}}\text{-}[N(CH_3)_2]_2$ have insecticidal action comparable to the related compounds, diethylfluorophosphonate, and tetraethylpyrophosphate. These phosphoryl-amides do not hydrolyze in water and according to Schrader possess the remarkable property of being absorbed by and translocated in plant tissues rendering the latter poisonous to insects.

Table 31--Relation of chemical structure of simple phosphate esters to toxicity (data from Ludvik and Decker, 1947)

Compound	Approximate median lethal concentration to:	
	Myzus persicae	Myzus porosus
triethylphosphate	>0.2%	
acid ethylphosphate	>0.2	
diethylphosphite	>0.2	
triethylphosphite	>0.2	
tetraethylpyrophosphate		0.0025%
tetrapropylpyrophosphate		0.0025
tetrabutylpyrophosphate		<0.01
hexaethyltetraphosphate		0.01
hexapropyltetraphosphate		<0.025
hexabutyltetraphosphate		0.01
hexa-(2-ethylhexyl)-tetraphosphate		0.1

The substitution of -P=S for the -P=O group common to all these phosphates does not greatly change the insecticidal action, apparently lowering it in some cases and enhancing it in others. However, the presence of the -P=S group results in a considerable decrease in the toxicity to warm-blooded animals as was shown by Schrader (1947) for parathion and its oxygen analogue, and by Smith et al. (1932) for tri-ortho-cresylphosphate and tri-ortho-cresylthiophosphate. The introduction of the -P=S group into tetraethylpyrophosphate to produce tetraethyl-monothiopyrophosphate, $(C_2H_5O)_2\overset{S}{\overset{\|}{P}}\text{-O-}\overset{O}{\overset{\|}{P}}(OC_2H_5)_2$, results in a compound which does not hydrolyze in water, while the introduction of two such groups to produce tetraethyl-di-thiopyrophosphate, $(C_2H_5O)_2\overset{S}{\overset{\|}{P}}\text{-O-}\overset{S}{\overset{\|}{P}}(OC_2H_5)_2$, results in a compound which will not hydrolyze in lime water. Both of these compounds are nearly as insecticidal as tetraethylpyrophosphate and are considerably less toxic to warm-blooded animals (Schrader, 1947). Brauer (1948) has shown that such thiophosphates inhibit esterases in the same manner as do the corresponding phosphates.

In all the classes of toxic phosphates mentioned above, viz., the pyrophosphates, the fluorophosphonates, and the aromatic-substituted phosphates of the parathion-type, it appears that maximum activity is associated with the presence of the $(C_2H_5O)_2\overset{\|}{P}\text{-}$ or $[(CH_3)_2N]_2\overset{\|}{P}\text{-}$ groupings. These two structures have approximately the same molecular dimensions and it is interesting to note that their replacement by methyl- or propyl-groups in the case of the ethyl-phosphates, or by hydrogen atoms or ethyl-groups in the case of the bis-(dimethylamido)-phosphoryl fluoride, results in decreases in toxicity (Schrader, 1947; Thurston, 1946; Brauer, 1948). The isopropyl-esters closely resemble the ethyl-esters in activity as might be expected from the similarities in structure. A further example of this critical size relationship is illustrated by the antiesterase activity of tri-ortho-cresylphosphate and the inactivity of tri-para-cresylphosphate (Brauer, 1948). From a study of the molecular models of these two compounds it may readily be seen that the tri-ortho-cresylphosphate molecule has certain spatial dimensions, from phosphorous atom to terminal methyl group, similar to those of the active dialkylphosphoric acid esters.

In addition to the considerations discussed above, the activities of compounds related to parathion are also determined by the nature and position of the substituents of the aromatic ring. The available data are shown in table 32.

Quantitative toxicology--Chadwick and Hill (1947) determined the LD_{50} of hexaethyltetraphosphate to P. americana as 6.75 mg./kg., and of diisopropylfluorophosphonate as 5.2 mg. by the injection of aqueous solutions into the thoracic cavity.

Physiological studies--The outward symptoms of intoxication with HETP and DFP in P. americana are indistinguishable from those produced by injecting physostigmine (Chadwick and Hill, 1947). They consist of hyperexcitability and hyperactivity developing almost immediately after injection of the toxicant, followed by exaggerated tonus, muscular incoordination, clonic and tonic convulsions, paralysis, and death. Where DFP was employed, insects which reached the convulsion state seldom survived, but with HETP, recoveries were more frequent. Roeder et al. (1947) found that the application of DFP at 6×10^{-4} M to the eviscerated abdomen of Periplaneta americana caused a strong after discharge following preganglionic stimulus in the giant fibers of the ventral nerve cord. A single stimulus was capable of setting off a discharge lasting 15 to 30 seconds. This condition was frequently followed by synaptic block lasting 30 to 60 seconds. DFP did not cause a permanent synaptic block, but the effects could not be reversed by frequent washing in saline. The conduction of the axons of the giant fibers were not affected by DFP at concentrations as high as 5×10^{-3} M. Acetyl choline applied to a preparation had no effect, but when applied at 5×10^{-6} M after pretreatment

Table 32--Relation of chemical structure of parthion derivatives to insecticidal action (data from Schrader, 1947)

Compound	Concentration in per cent of spray solution for indicated mortality to aphids (species unknown) 50%	100%
0,0-diethyl-0-phenylphosphate	0.2	
0,0-diethyl-0-phenylthiophosphate	0.2	
0,0-diethyl-0-para-nitrophenylphosphate	0.001	0.005
0,0-diethyl-0-para-nitrophenylthiophosphate		0.001
0,0-diethyl-0-ortho-nitrophenylphosphate		0.005
0,0-diethyl-0-meta-nitrophenylphosphate	ca. 0.2	
0-ethyl-N-(dimethylamido)-0-ortho-nitrophenylphosphate	0.2	
N,N-bis-(dimethylamido)-0-para-nitrophenylphosphate	0.05	
0-ethyl-0-ortho-nitrophenylmethanephosphonate	0.005	0.05
0-ethyl-0-phenylmethanephosphonate	0.05	
0,0-diethyl-0-ortho-chlorophenylphosphate	0.05	0.2
0,0-diethyl-0-para-chlorophenylphosphate	0.02	0.2
0-propyl-0-2,4-dinitrophenylmethanephosphonate		0.2
0-ethyl-0-2-nitro-4-methylphenylmethanephosphonate		0.005
0-ethyl-0-ortho-chlorophenylmethanephosphonate		0.05
0-propyl-0-para-chlorophenylmethanephosphonate		0.2
0-ethyl-0-para-carboethoxymethanephosphonate		0.05
0,0-diethyl-0-ortho-carboethoxyphosphate		0.05
N,N-bis-(dimethylamido)-0-para-aminophenylphosphate	>0.2	
N,N-bis-(dimethylamido)-0-ortho-aminophenylphosphate	>0.2	

with DFP at 10^{-5} M caused an immediate block in synaptic but not in axonic transmission. HETP at 2.5 x 10^{-7} to 7.5 x 10^{-7} M produced similar effects on the synapse, while axonic conduction was not impaired until a concentration of 7.5 x 10^{-5} M was reached (Roeder, as quoted by Chadwick and Hill, 1947).

Biochemistry of action--Mazur and Bodansky (1946) have studied the mechanism of action of DFP and have found that it irreversibly inhibits the action of cholinesterase in mammals. Therefore, it seemed probable that the phosphate insecticides would have similar action in insects. Dubois and Mangun (1947) found that HETP produced 58 per cent inhibition of the cholinesterase in the homogenized thorax of P. americana when applied at 1 x 10^{-7} M at 38°, while Chadwick and Hill (1947), using homogenized nerve cords of the same insect at 25°, found that HETP at 1 x 10^{-6} M produced 100 per cent inhibition of cholinesterase with a sharp decrease in effectiveness at about 5 x 10^{-7} M.

DFP produced 100 per cent inhibition at 1 x 10^{-4} M and 50 per cent inhibition at 4 x 10^{-6} M. In comparison, physostigmine produced complete inhibition at 1 x 10^{-4} M, and 50 per cent inhibition at 1 x 10^{-8} M. These authors reason that the sharp drop in effectiveness of HETP is due to its rapid rate of hydrolysis. These values are not greatly different from those obtained using cholinesterase from various mammalian tissues (Koppanyi et al., 1947). In in vivo experiments by Chadwick and Hill (1947) the injection of DFP at 18 micrograms per roach produced 100 per cent inhibition of nerve cord cholinesterase, while 4.5 micrograms produced 53 per cent inhibition. With HETP, 500 micrograms per roach were required to produce 100 per cent inhibition, while 25 micrograms produced an average of 56 per cent inhibition. Doses of 12.5 micrograms produced immediate prostration and were lethal in all cases, but resulted in an average of only 13 per cent cholinesterase inhibition. Thus it seems possible that HETP may have other toxic action in addition to cholinesterase inhibition. From a consideration of the relative amounts of DFP necessary to produce equivalent inhibitions of nerve cholinesterase in vitro and in vivo, it was found that at low concentrations a considerable portion of the DFP was either inactivated or failed to reach the nerve cord cholinesterase, and it may be that an enzyme is present in the roach which detoxifies DFP by hydrolysis, as Mazur (1946) found in mammalian tissue. DFP inhibition of cholinesterase is not readily reversible in the roach tissue, the enzyme showing continued inhibition for several days. Activities as low as 20 per cent of normal cholinesterase activity were found in specimens showing normal behavior. Thus, Chadwick and Hill conclude that although the possibility of other toxic mechanisms of action is not excluded, the toxicity of DFP and HETP is largely a function of their anticholinesterase activity. This confirms studies with DFP in mammals where Nachmansohn and Feld (1947) have shown that animals surviving DFP poisoning always have a small amount of cholinesterase activity remaining in the active state, while in those fatally poisoned, cholinesterase activity is completely abolished. They, therefore, suggest that the activity of DFP is due solely to its anticholinesterase effects.

Considerable popular publicity has been given a recent attempt to prepare HETP containing radio-phosphorus, P^{32} (Anonymous, 1947, 1947a) by conversion to P_2O_5 and reaction with triethylphosphate. Brauer (1948) has shown that upon interaction of this radio HETP with human plasma esterase no P^{32} could be found in the protein precipitated by ethanol, nor in the precipitate or the supernatant portions of such preparations after dialysis. Therefore, he concluded that a stable combination does not take place between the esterase and a phosphorus containing portion of the HETP. It would seem of interest to prepare HETP from radio-phosphorus incorporated in triethylphosphate, since the behavior of the phosphorus atoms in this portion of the molecule might be very different from that of the atom contributed by P_2O_5.

Theories of toxic action--Hansen (1947) theorizes that TEP may hydrolyze in the insect to diethylphosphoric acid, which then might interfere with phosphate metabolism by interaction with adenosine triphosphate, an essential metabolite which also has two ester linkages connecting phosphorus with other groups. Martin (1947) has suggested that an important toxophoric grouping is $-O\overset{\parallel}{P}(OC_2H_5)_2$. It seems that a theory of the mode of action of the phosphates should be able to explain the activities of diisopropylfluorophosphonate, (and the diethyl ester which has similar action - Mazur, 1946) tetraethylpyrophosphate, and 0,0-diethyl-0-para-nitrophenylthiophosphate, all of which have been reported as highly active anticholinesterases. The work of Mazur (1946) on the enzymatic hydrolysis of diisopropylfluorophosphonate to fluoride ion and diisopropylphosphoric acid suggests that a common reaction of these three phosphates might be the in vivo hydrolysis to dialkylphosphoric acid. Brauer (1948) studied the effects of a number of phosphate esters on esterase activity in human plasma and erythrocytes. It was found that phosphorus compounds of varying structures were active esterase inhibitors and that the only structural feature common to all of these compounds was the presence of the grouping $\equiv P\text{-}O\text{-}R$ (where R is aryl or alkyl). However, the author states that the presence of this grouping is not sufficient for anti-esterase activity as illustrated by

such pairs of compounds as (1) triethylphosphate (inactive) and tetraethylpyrophosphate (active), (2) trimethylphosphate (inactive) and dimethylfluorophosphonate (active), and (3) tri-p-cresylphosphate (inactive) and tri-o-cresylphosphate (active). He notes that in each case the active compound contains a structural arrangement which might be expected to possess a high degree of free energy. As a result of his studies, Brauer postulates the following mechanism of inhibition:

$$\equiv POR + EH \longrightarrow \equiv POH + ER$$

or

$$\equiv PX + EOH \longrightarrow \equiv POH + EX$$

where $\equiv POR$ or $\equiv PX$ is inhibitor, EH or EOH is active enzyme, ER or EX is inactivated enzyme, R is alkyl or aryl group, and X is halide.

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