Chemical Residues and Additives in Foods of Plant Origin

ROBERT N. GOODMAN, PH.D.*

WE have all read accounts of residues of particular chemical compounds in foods or seen reviews of the whole subject of residues and chemical additives. Some were perhaps limited in scope or so broad that many important details and the rationale for the use of these substances were obscured or omitted entirely.

As a horticultural pathologist I have certain obligations to those concerned with the production of plants as food and, to some extent, as fiber. However, as a regular consumer of this produce, I have obligations to myself, to my family and to society regarding our health and welfare. As a researcher in a non-profit institution, one which is State owned and operated with additional funds made available by the Federal Government, I also have a duty to that segment of the public which includes industries allied with agriculture. Of frequent concern here is the evaluation of chemicals developed for the improvement of crop production.

In my capacity I am able to accomplish this without excessive bias, having nothing to gain by either the success or failure of a given chemical. This is not to say that I am without pressures from the producer who hears of a new wonder chemical to improve yields, or the manufacturer who would like to have a seal of State approval given to his product. I am sure that many members of the medical profession have found themselves in a position where the patient has tried to dictate the prescription and/or where pressure from the manufacturer of a compound were equally great. Fortunately for us both, the preponderance of these compounds accomplish what the manufacturer says they should and in time become a part of our economy, helping to maintain the rate of progress and efficiency of our society. There are of course, certain moral, financial, as well as legal safeguards that limit the number of harmful chemicals that reach the consumer.

One frequently reads or hears comments on the food value and quality of fruits and vegetables of yesteryear. Varieties of apple, like Peewaukee and Ben Davis, which did not have to be sprayed simply because no self-respecting insect or disease would single them out for a meal. What is often forgotten is that these varieties were also not particularly fit for man either. Thus horticulturists have selected and bred varieties which may appear to be botanical monstrosities, but have the eating qualities desired by man. Then we hear, for example, our vegetables have less food value now than in days gone by and, in addition, are covered with harmful chemical pesticides. Time does not permit me to document a refutation to this type of comment. However, I might indicate that standard tomato varieties, such as Rutgers and Marglobe, contain about 15 to 20 mg. ascorbic acid per 100 gm. of fresh weight whereas the content of this vitamin in many new varieties is 50 to 70 mg. per 100 gm.[1] This is an increase of over 200 per cent. Carotene content of the standard sweet potato varieties containing 4 mg. per 100 gm. fresh weight has been raised to 12 mg. per 100 gm. fresh weight, about a

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* Associate Professor of Horticulture, University of Missouri, Columbia, Missouri.

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FIG. 1. Conventional ground sprayer.

FIG. 2. Aerial dusting.

300 per cent increase.[2] In addition, the ascorbic acid content of this vegetable has also been increased, approximately 40 per cent.

If one is permitted to generalize at this point, it would seem that concomitant with an improvement in quality of some of our fruits and vegetables we have had an increase in cultural problems which have in many instances been solved by the use of pesticidal chemicals. The question would of course resolve itself by our return to the inferior fruits and vegetables of former days and, in many instances, varieties that yielded less per acre.

Actually I am not so sure that many of the ills that we cure or prevent today were not with us in earlier times; they just went unnoticed or ignored. They were perhaps accepted as the blight that comes occasionally on potatoes, causing mass migration and famine, or that periodically destroys the pear industry whenever conditions are conducive to the development of the bacterium, or the annual loss of grapes to mildew. There was in most instances no alternative, as the means to protect the plants against these disorders were not at hand.

The following definitive statements indicate the position I have taken with regard to these chemicals. First, there is little doubt in my mind that many of the chemicals used today to combat insects, disease, weed plants, or that alter the metabolism of higher plants, can be toxic to man if ingested, inhaled, or absorbed in sufficiently high concentration. It is well to keep in mind that despite important morphologic and physiologic differences between plants, animals, insects, and microorganisms, their metabolic processes at the cellular level may be essentially the same. Thus a compound which interferes with respiration in one species is likely to do the same in others. Whether or not it does is contingent upon concentration and/or ready access to the site of activity. I should like also to state that the necessary legal safeguards of the public's safety have been, for the most part, already erected. Some may need modification, or require increased personnel to insure their enforcement and to interpret astutely the available research data. I believe, however, that time, patience and logic will obviate these disparities.

The types of equipment used to apply these materials are illustrated in Figures 1 and 2. The pictures illustrate the exposure hazard to which the food producer is subjected. Further, this gives one an appreciation of the quantities of pesticides that can be tolerated by man. Put another way, the laws that protect the spray operator are the same ones that protect the consumer. We should now consider the types of chemicals that are used regularly in the production of fruits and vegetables.

TYPES OF COMPOUNDS

Perhaps the most acutely toxic materials used in crop production are the insecticides. Presented here (Fig. 3) are representative types of the phosphate, chlorinated hydrocarbon and heavy metal compounds. Of these

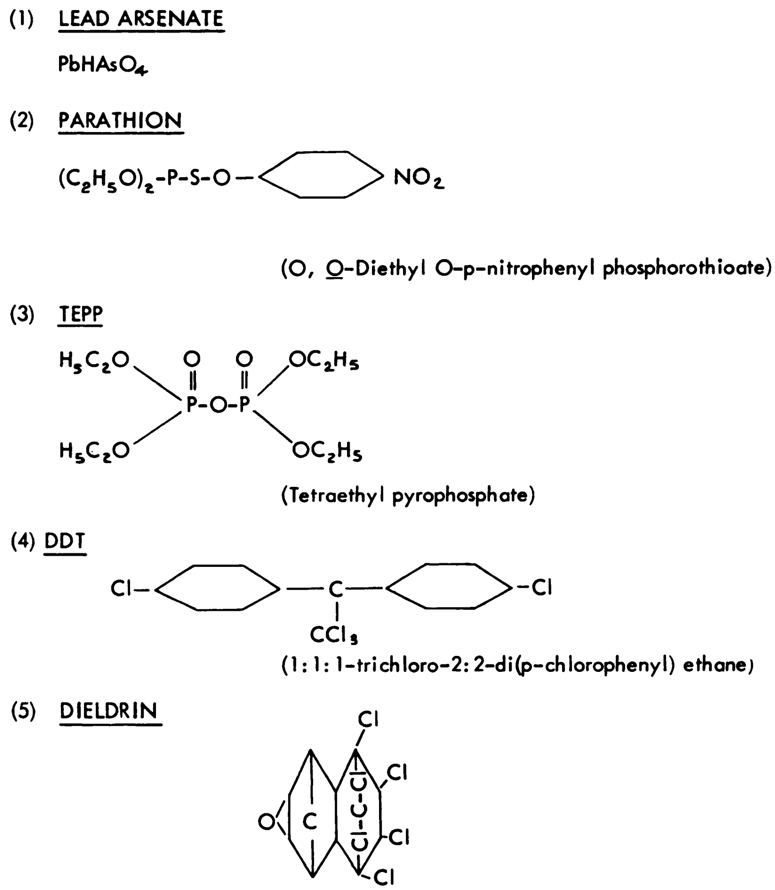

FIG. 3. Typical insecticides.

the phosphate derivatives, e.g., Parathion and TEPP are believed to be the most toxic to warm blooded species, of which man is one. The toxicity of both is due to the inhibition of the cholinesterase enzymes. Depression of these enzymes in both the erythrocyte and plasma fraction of the blood has been documented.[3,4] The enzymes' physiologic function is to catalyze the hydrolysis of choline esters. One of these, acetylcholine, is believed to be the transmitting agent of nerve impulses. Actually the activity of these compounds against the insect, their primary target, is much the same as their effect upon mammals. The literature contains innumerable accounts of fatalities directly assessable to these compounds.[5,6]

It may be said that the effect of the clorinated hydrocarbons, e.g., DDT and dieldrin, is on the central nervous system of the insect. Although there is no clear evidence of their inducing permanent toxic effects in man, 10 mg. per kg. has caused acute illness manifested as headache, nausea, dizziness, and convulsions.[5,7]

The role of herbicides (Fig. 4) or plant hormones in human physiology is at present a highly speculative one. The term carcinogen has been linked to these compounds via l'affaire—cranberry and amino triazole. These substances are used to kill weeds in row crops, as well as dandelions in lawns and poison ivy in back of the house, de-blossom fruit trees, increase fruit "set" (increase the number of fruit developing per cluster), suppress sprouting of onions and potatoes in storage, suppress growth and induce rooting of cuttings. Their place in agriculture becomes more firmly entrenched daily. With such a multitude of effects upon metabolism of plants one should

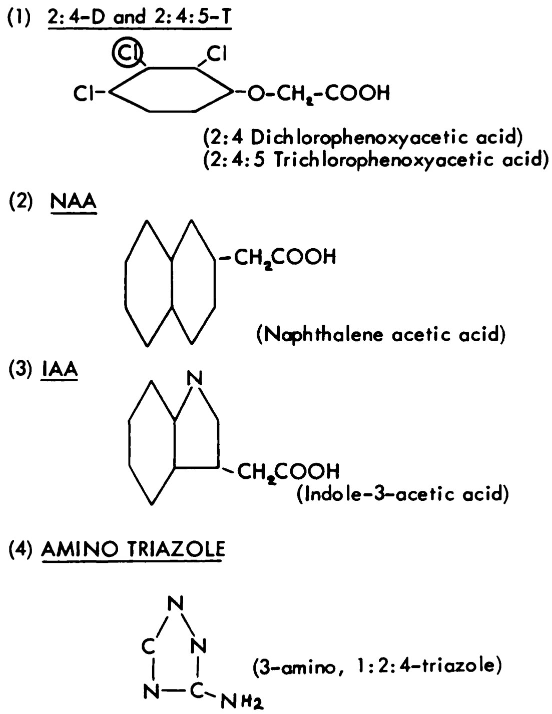

FIG. 4. Typical herbicides (plant hormones).

not wonder that they could deleteriously affect man.

Nitrated and chlorinated phenols are used as herbicides as well as fungicides and it is held by some that their toxic effect upon man may be in their ability to interfere with the production of high energy phosphate compounds which are so essential to cell respiration.

Of the fungicides, perhaps the mercurials, both organic and inorganic ones (e.g., phenyl mercuric acetate and mercuric oxide) are the most hazardous to man (Fig. 5). They seem to combine with and immobilize sulfhydryl groups which are so important in many enzyme reactions. It would seem, however, that the organic fungicides in general have a low order of mammalian toxicity. The bactericides which seem to be the most effective and promising at this time are the antibiotics and we shall dwell at some length on these compounds later on.

Of the final group, the nematocides (Fig. 6) and here we might also include chloropicrin (tear gas), the most hazardous is methyl bromide. These are all applied or at least become active as gases, thus their activity on or in foods of plant origin would be dissipated in a relatively short period of time.

It is apparent now from this brief discussion that the chemical compounds which are used in the production of foods of plant origin are varied and I can assure you almost astronomical in number.

TOXICOLOGY

Of particular concern now, more so than in previous years, is the existence of these compounds within the plant tissues as well as on their external surfaces. It is the realization that some relatively large organic molecules can be absorbed by both the aerial and below ground portions of the plant that has made this problem of residues more disconcerting. In addition, a number of these compounds are actually translocated by the vascular system of the plant. This may not sound particularly startling or unusual to those who are familiar with translocatory systems of members of the animal kingdom, but it is to the plantsman.

Here we find the rationale for the concern for some of the growth regulators which are rapidly absorbed by plant foliage. They have been termed systemic, able to move in the system of the plant. This is equally true for a number of the organic phosphorous insecticides and some antibiotics. The systemicity of these compounds is to a certain extent related to their water solubility. On the other hand the chlorinated hydrocarbons are considered lipoid or fat soluble and their movement into the plant system is limited. One might also ponder the fate of these chemicals, once inside the plant.

We might digress here for a moment to discuss the manner in which these large organic compounds penetrate the cuticular layer of the plant's epidermis. In our laboratory we have obtained almost irrefutable evidence that direct penetration of the cuticular layer of the plant is possible.[8,9] A closer look at the barrier and its morphology and chemical constituents affords the opportunity to visualize how this penetration is effected. There are two hydrophyllic lamellae, consisting of cellulose and pectins which are parallel and wax molecules which

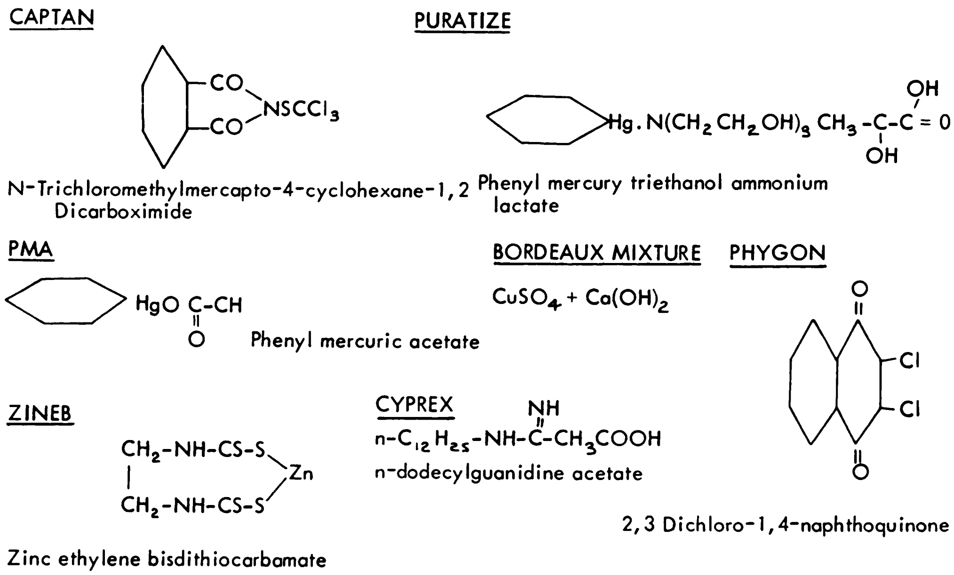

FIG. 5. Typical fungicides.

form in layers radially. Between the two in random orientation is the amorphous polar cutin. The cellulose and pectins are located predominantly in the inner regions of the wall, whereas the waxes are the most numerous towards the outer surface. The cutin contains both hydrophyllic (OH and COOH) and hydrophobic (CH_3) groups, the former are oriented towards the cellulose and the latter towards the wax.[9] This arrangement provides a convenient pathway by which water molecules and water soluble substances may penetrate the epidermis of the plant.

It is apparent now that these chemicals might be located within the plant tissues beyond removal by any washing or leaching procedure. Thus the terms residue and tolerance assume a new dimension. The tolerance for a chemical residue must be calculated in a way which insures its degradation or dilution to a quantity which is nontoxic by the time it reaches the consumer. The fruit growers residue guide assists the grower in keeping his residues within specific limits (Table 1).

Since I am least of all, a toxicologist, I do not believe it is my province to dwell on the matter of safe levels of residues in general. Similarly, regulatory decisions are not mine to make, yet as one concerned with the production of food I should like to describe what has seemed to me and my colleagues to be a logical stepwise procedure in the development of an agricultural chemical. I might add that this method is generally followed for most agricultural chemicals in order to insure effectiveness of the compound for the plantsman, safety to the consuming public as well as financial success to the manufacturer.

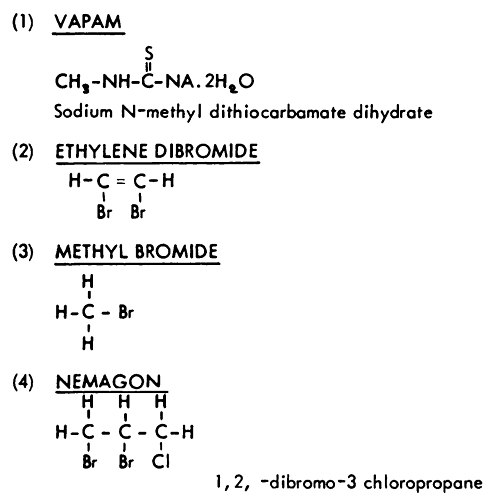

FIG. 6. Typical nematocides.

In the spring of 1952 sprays with two antibiotics, streptomycin and thiolutin, appeared

TABLE I
Fruit Growers' Residue Guide (Partial List)[18]

Compound	Tolerance (μg. per ml.)	Suggested Interval (days)
Bordeaux	Exempt	14
Captan	100	No limit
DDT	0.75	45
Dieldrin	0.1	35
Elgetol 318	0	Dormant only
Lead arsenate	3.5 arsenic; 7 combined lead	45
Naphthaleneacetic acid	1	14
Parthion	1	14
Streptomycin	0	No later than 1st cover
TEPP	0	3
Zineb	7	14
2, 4-D	5	7

in our field experiments to reduce the intensity of a bacterial blight of apple and pear. This disease had all but obliterated the pear industry in most areas east of the Rocky mountains. Further it had wreaked sporadic havoc with the Jonathan and other apple varieties, frequently reducing a crop to less than half and limiting production the following year. In 1953 crude spray formulations of streptomycin and Terramycin® at concentrations from 50 to 500 μg. per ml. were evaluated. It was determined from these experiments that 50 to 100 p.p.m. was an effective dosage. At about the same time we developed a simple microbiologic assay which was designed primarily to provide us with information concerning minimum inhibitory concentrations necessary to inhibit the bacterium in plant tissue.[10] With a single spray of streptomycin at 100 p.p.m., a residue in the leaf of 16 p.p.m. was detected after twenty-seven days. At the end of the season, at harvest, the apple fruit was assayed for streptomycin activity, the fruit having been sprayed four times with 100 p.p.m. of the antibiotics. No residue was detected. Although all antibiotics on foods in detectable quanitities were deemed an adulteration within the meaning of Section 402 of the Federal Food and Drug Act, and as such were given a zero tolerance, the absence of any residue cleared these drugs for this specific purpose.

RESIDUES

In 1956 Dr. H. S. Goldberg, of the Microbiology Department of the School of Medicine, and I were awarded a research grant by the National Institute of Health to study "The Public Health Significance of Antibiotic Residues in the Edible Portions of Horticultural Crops." Our initial area of investigation, accomplished with the assistance of J. T. Logue, M.D., was to determine whether or not the spray operators had developed a significant hypersensitivity to streptomycin. In this work a patch test was used. Our results here were negative and induced us to utilize the more sensitive tuberculin-type intradermal skin test using 0.1 ml. streptomycin at 5 μg. per ml. with a suitable control in the opposite arm. A portion of the results from this study has already been reported.[11,12] Approximately 6 per cent of our thirty-two subjects in Missouri, more than fifty in California orchards and 103 agricultural workers in the truck crop area of Florida gave positive reactions. This is approximately the same as was found in the case of a group of nurses[13] and substantially the figure obtained from our group of unexposed control subjects. At present, we believe that streptomycin as used in agricultural sprays is not a particularly allergenic substance.

Streptomycin, oxytetracycline and chlortetracycline were also evaluated in our laboratories in postharvest applications to suppress bacterial soft-rot of leafy vegetables such as spinach and cabbage.[14] In these experiments it was noted that momentary dips of these vegetables into solutions containing the antibiotics significantly delayed the onset of decay. However it was apparent from our bioassays that significant residues of all antibiotics, especially of streptomycin remained in the tissues. Spinach treated with streptomycin at 1,000 μg. per ml. withstood a live steam blanching and rinsing operation and still retained as much as 40 μg. per ml. antibiotics activity.[15] The extreme stability noted for this drug in fruit and foliage of apple, and reports of its stability in pear trees for a full year, cast some doubt as to the desirability of this antibiotic for extending shelf-life of fresh vegetables.

As residual levels in treated horticultural crops were established these quantities were fed to laboratory animals in treated crops and in drinking water in long-term experiments. Using mice, guinea pigs, and rabbits over a two-year period the following conclusions relative to the emergence of a resistant intestinal flora have been drawn: (1) Following long-term, low-level feeding of streptomycin, 5 to 40 per cent of all coliforms emerge resistant to levels of 10,000 μg. per ml. of this drug. (2) Chlortetracycline did not cause detectable emergence of resistance by coliform bacteria. (3) Blood serum levels of chlortetracycline as a result of long-term, low-level feeding in rabbits resulted in levels that were $^1/_{100}$ attained by a therapeutic dose. (4) It was concluded from these and other studies that public health problems such as emergence of antibiotic resistance, anticipated by widespread nontherapeutic usage of antibiotics are less likely with the tetracycline antibiotics than with streptomycin.[16,17]

It remains now to be seen if similar results will be obtained from long-term, low-level feeding experiments in man. We currently have a study in progress with 50 human volunteer subjects at the Missouri State Penitentiary. Half of the men have been getting 10 mg. of oxytetracycline (Terramycin) and half have been receiving sugar placebos daily since November 1, 1959. Blood and stool samples are obtained at monthly intervals. The blood samples are assayed in my laboratory for oxytetracycline; the stool samples are examined in the Department of Microbiology using the replica plating technic for the emergence of resistance coliforms. In addition, we have been looking for the development of any hypersensitivity to this drug.

What I have tried to convey in this final portion is the general procedure followed in the development of most agricultural chemicals that may find their way into the foods we eat. Our studies did not include the toxicologic tests that usually accompany chemicals in their developmental stages. Obviously, these were not necessary since they had been performed when the drugs were first developed for therapeutic purposes. I might also add that tests with human volunteer subjects are less frequently performed than may be desirable.

I should like to reiterate that the general public has three safeguards for its health and welfare. First, there is Federal legislation that is implemented through the Department of Health, Education and Welfare and the Department of Agriculture. Second, are the efforts of State and Federally endowed research institutions, such as our State Agricultural Experiment Stations and the Agricultural Research Service of the Department and finally, the integrity of our chemical industries. It should be apparent that the reputation of the manufacturer is at stake with each product he endeavors to place before the public.

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