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"Report – 2,4,5-T"

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SUMMARY AND RECOMMENDATIONS

Summary

This review of the herbicide, 2,4,5-T, began with an examination of the results from an experimental screening study which implicated it as a potential teratogen. It quickly became evident that examination from such a restricted basis was inadequate. Therefore, the Panel decided to study more broadly important aspects of 2,4,5-T, including details of its chemistry and purity, its domestic uses and their relative importance, the military significance attached to 2,4,5-T as a defoliant, residue levels (in order to estimate probabilities of human exposure), general effects on the environment, as well as its toxicity. In examining the toxicity of 2,4,5-T, the Panel reviewed the information available from the literature (as well as some unpublished documents) which had been considered in the past in establishing policy decisions for the various uses to which this pesticide had been put.

Selection of 2,4,5-T as an example for detailed examination has had a number of advantages. There has been an extensive history of use and experience. 2,4,5-T was first registered on March 2, 1948, by the Amchem Products Company, Ambler, Pennsylvania. Since that time considerable information on its properties and uses has accumulated. Furthermore, it has been the subject of reviews by others in the past. Finally, the problem which brought it to the Panel's attention, suspicion of teratogenicity, appears to be a relatively manageable problem in contrast to many other biological effects, notably tumor production and genetic alterations. This is important since the recommendations which follow can be made with a degree of confidence that cannot be applied to carcinogenic or mutagenic effects. For example, the dose-response characteristic of teratogens is generally restricted to a relatively small range of dosage. Accordingly, a threshold below which no effect would be expected can be assigned with more certainty. Experiments to determine this range of values can be performed in a relatively short time and do not require very large numbers of animals

The Panel is gratified that some of its recommendations are already being carried out, especially further experiments to confirm and extend the results of the original screening that indicated 2,4,5-T to be teratogenic.

In considering the chemistry of 2,4,5-T, our attention was drawn to impurities which can result from the manufacturing process. Particular attention was focused on a single impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin, which occurs in commercial preparations of the herbicide in highly variable amounts unless particular care is taken to exclude it. This impurity is extremely toxic. Its amount depends upon variations in the reaction conditions. Other dioxins can be formed from various impurities in the starting materials. The dioxin impurity came to particular attention when the U.S. herbicide industry was asked to produce larger quantities of 2,4,5-T during the middle 1960's. However, its presence as an impurity and certain of its acute toxic effects had been known since 1957. Its concentration in commercial 2,4,5-T has been greatly reduced in the past year or so.

Analytic methods available for 2,4,5-T are accurate and reliable. With the possible exception of citrus fruits, determination of residues in food has not presented a serious analytic problem.

2,4,5-T is relatively labile in nature. Residues in soils and water are not persistent except under unusual conditions. The herbicide is not stored in plants or animals to a significant extent.

Production of 2,4,5-T in the United States rose rapidly between 1960 and 1968. Civilian use, most of which is for clearing of range land and rights-of-way and for treatment of pastures, declined about 50% between 1964 and 1966. Military use of 2,4,5-T as a defoliant, expressed as number of acres sprayed, rose sharply between 1964 and 1967 but has declined since then. Although accounting for only a small amount of the total usage of 2,4,5-T, its place in control of aquatic weeds is significant. There is a small but important list of agricultural uses where 2,4,5-T is applied to food crops. Potential human exposure is recognized in this direct application to food crops, in range and pasture lands grazed by domestic meat and dairy animals, and possibly, in water supplies derived from treated waterways and streams. The economic importance of the various uses is considerable, but is very much less than that of 2,4-D. Substitution of 2,4-D for 2,4,5-T can be made for certain uses.

Defoliation, using mixtures of 2,4-D and 2,4,5-T, has been employed in Vietnam since 1962, more intensively since 1967.

Although not rigorously demonstrated, its military usefulness has been considered to be very high.

The background of toxicological information on 2,4,5-T is thin. Most of the animal studies have been concerned with acute toxicity (single doses or repeated doses for short periods of time). Based on these experiments, the acute toxicity of 2,4,5-T was found to be low. Little is known of the details of the metabolic handling of the material although rapid excretion in the urine seems to be the rule.

The screening study supported by the National Cancer Institute on the toxicity of certain pesticides and other important industrial chemicals marks an important advance in toxicological testing in that the tests were designed to detect carcinogenic, teratogenic and mutagenic potential. The preparation of 2,4,5-T used in those tests was shown to be teratogenic in both rats and mice. There was no evidence that it was carcinogenic. While this study had a number of limitations which qualified its usefulness, the teratogenic results were sufficiently convincing so that the Panel urged, early in its discussions, that they be repeated and extended using better characterized preparations of 2,4,5-T. Analysis of a sample of the 2,4,5-T preparation used in the original teratogenesis study revealed a dioxin level of about 27 ppm. Such a considerable contamination by this highly toxic material raised the question as to whether the teratogenic effects observed were caused by 2,4,5-T itself, by the dioxin impurity or by other impurities in the commercial preparation tested.

The Panel was aware of press reports of increased birth defects in Vietnam attributed to the use of defoliants. The lack of accurate epidemiological data on the incidence and kinds of birth defects in the Vietnamese population before or since the military use of defoliants precludes any estimate as to whether an increase in birth defects has occurred. Calculations of potential human exposures from sources such as drinking water or direct fallout make this appear unlikely (though theoretically possible).

A review of the environmental effects of 2,4,5-T on nontarget organisms reveals few harmful consequences of its recommended uses. Induced changes in vegetation are followed by alteration in numbers of wild animals. Accelerated erosion of soil may follow the killing of brush with herbicides but mechanical removal causes greater erosion.

Recommendations

1. *Further studies.*

a. *The animal experiments which raised the question of the teratogenic potential of 2,4,5-T should be extended to include a wider range of doses administered to non-inbred strains of animals and to larger numbers of animals.*

b. *The importance of the impurities in 2,4,5-T as potential health hazards should be ascertained.* Recent experiments designed to distinguish between 2,4,5-T and the dioxin impurity have suggested that both the herbicide and the dioxin are potential teratogens in some experimental animals. However, experiments necessary to establish this answer have not been performed. In addition, there may be additional impurities in commercially prepared phenoxy herbicides which may be biologically active.

c. *The metabolism of 2,4,5-T in humans should be determined and compared to that in experimental animals.*

2. *The level of dioxin, a recognized impurity in 2,4,5-T should be rigorously controlled and limited to not more than 0.5 ppm.* A reduction to not more than 0.1 ppm should be urged. Several polychlorinated dioxins have been found to be highly toxic and capable of eliciting teratogenic effects, though they vary widely in toxicity. Since they may reach the environment from multiple sources. control over known sources should be exercised to the extent possible.

3. *A decision to restrict the use of 2,4,5-T should not be based on the isolated finding of toxicity but on the expected exposure following recommended use in relation to dose response effects.*

In general, the imposition of restrictions on the use of a pesticide would appear to be a function of two factors, the potential for human exposure and the nature of the toxic effects. For example, if carefully documented residue information points to little likelihood of exposure, the risk of adverse effects would be less significant than if exposure were widespread.

The Panel found no evidence to suggest that significant residues would result from recommended uses of 2, 4, 5-T on food crops. It is possible for residues to occur in tissues of animals grazing on recently treated pastures and range land. In fact, the only residues which have been identified in the total diet studies have occurred in meat and dairy products. However, the few cases in which residues have been discovered have all been at levels well below those which would be expected to result in significant toxicity for man.

The experimental finding of teratogenesis requires further elaboration before it can be interpreted as a human health hazard.

The risk of teratogenic effects should be placed in perspective. Teratogenesis induced by chemicals is a fetal response at a particularly sensitive period in embryonic development to lower doses of the chemical than are acutely toxic to the mother. Birth defects can be produced in the embryo through many mechanisms of injury when the agents are administered during critical periods of organogenesis. It is generally held that by careful choice of dosage, which may be close to the acutely toxic dose for the mother, most chemicals might be shown to be teratogenic in animals. For a variety of reasons, it is not possible to translate directly the results of experiments in animals to man. There are differences in sensitivity which arise from differences in metabolism. Comparative metabolic studies in man and animals, therefore, are important in interpreting toxicity for man.

The important consideration is not only the demonstation of teratogenicity, which may occur with many chemicals at seeleted dosages, but the estimation of the likelihood of teratogenic effects with the amounts likely to be ingested incident to recommended use. To restrict or ban usage of chemicals on the basis of demonstration of teratogenicity at dose levels which far exceed actual or expected exposures is unreasonable and could well deny usage of chemicals whose benefits far outweigh risks.

4. *Registrations of 2,4,5-T for uses on pastures and range lands should be treated as registrations for food crop uses.*

It is possible for residues of 2,4,5-T to occur in milk and tissues of animals grazing on land recently treated with 2,4,5-T. To date, meat and dairy products have been the only food products in the total diet studies that contained measurable amounts of 2,4,5-T. Use on range and pasture land should be included in registration for use on food crops.

5. *Monitoring of 2,4,5-T residues should be significantly expanded, especially for meat and milk.* In sampling meat and milk, special attention should be given to geographic areas where treatment of pastures and range lands with 2,4,5-T is most common. The 2,4,5-T residues that may occur in meat and milk of animals allowed to graze on pastures and range lands treated according to current recommendations should also be restudied.

6. *As new information is developed on pesticides, it should be disseminated promptly to individuals and organizations that are legitimately concerned as manufacturers, formulators, users and scientific investigators.*

The case of 2,4,5-T is illustrative of inordinate delay in making available new research information as it became known. The screening study of pesticides which was carried out by Bionetics Corporation under contract with the National Cancer Institute was completed about August 1968. It was 14 months later when the Government announced its coordinated actions on restricting the use of 2,4,5-T It was only after an additional several months that the detailed data of the screening study were made publicly available. A centralized mechanism for handling and disseminating new information about pesticides could help alleviate this problem.

7. *A mechanism should be established for restricting the use of a registered pesticide temporarily on the basis of information which implicates the chemical as a possible health hazard pending the collection of more definitive information.*

If a pesticide is already in established use, the decision is particularly difficult. Long established use inevitably implies a dependence upon it by the consumer and a corresponding reluctance by the manufacturer to withdraw it from the market.

At the present time, a registration may be held in abeyance only by cancellation or suspension. Because of the serious import of these actions they are put into effect with considerable reluctance. They were not designed for situations such as the present with respect to 2,4,5-T where temporary withdrawal from use, without cancellation or suspension of registration, might have been a more appropriate action. Such an alternative course of action is not possible under present regulations.

There is need for a mechanism whereby the use of a pesticide or other chemical that may affect human health can be temporarily restricted or held in abeyance. Such action would permit the gathering of more definitive information in time for sufficient consultation to permit a decision that would protect the public health and not impose an undue economic burden on the producers, marketers, and users of a product. Coincident with the imposition of restrictions on a pesticide, a mechanism should be available for informing and educating pesticide users and applicators so as to make them more responsible agents. It is recognized that a change in the law governing pesticides would be necessary to accommodate this mechanism of a temporary restriction.

8. *There is an urgent need for a focus of responsibility in D/HEW to coordinate and monitor the toxicity and health activities related to effects of pesticides.*

Information about the health effects of a pesticide derives from a variety of sources including occupational exposures, residue monitoring, toxicological investigations, clinical experience and epidemiological studies. In the past, there has been no single focus within D/HEW which has been concerned with all of these sources of data and, more important, which has had the authority and responsibility to coordinate new investigative initiatives. The new Advisory Committee on Pesticides to the Secretary of D/HEW can be expected to serve as a source of expert advice but cannot fill the essential need for a focus of responsibility and authority at the level of the Office of the Secretary. Consideration of the functions to be fulfilled and the resources available suggests that this responsibility should be assigned to the Assistant Secretary for Health and Scientific Affairs because the various components of D/HEW concerned most with aspects of the health effects of pesticides report directly to him (National Institutes of Health, Food and Drug Administration, National Communicable Disease Center, Environmental Health Service).*

9. *Information provided in applications for registrations of pesticides should take into account not only the pesticide for which registration is sought but should identify other substances including vehicles used in formulations, "inert" ingredients, and impurities.*

Investigation of the synthesis of 2,4,5-T and examination of the manufacturing process revealed that an extremely toxic impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin, present in variable amounts in commercial preparations of 2,4,5-T, may account for some of the toxicological characteristics assigned to 2,4,5-T itself. The presence of this impurity was recognized as early as 1957. However, the importance of this impurity was not generally recognized in the United States until after 1964. It appears logical that greater specificity in identifying the components and properties of the mixture of materials which are registered under a single name would increase the probability of identification of potentially toxic substances.

10. *Registration procedures should be based on toxicological studies of the particular compounds to be registered rather than extrapolations from studies on related compounds.*

Toxicological studies provided as information in behalf of 2,4,5-T registration were performed on a variety of related compounds (the free acid, several types of esters and a variety of salts). Results of these tests were regarded as being interchangeable and applicable to the related compounds. There is evidence to caution against this concept. The thorough testing of isomers, esters, salts, and related compounds is a very large and expensive task. Nevertheless, information about a potential health hazard may be incomplete unless all of the compounds to be used are tested.

*Since this report was written, the President established, through Reorganization Plan, the Environmental Protection Agency which is to be responsible for broad areas of regulation covering environmental matters. The Environmental Protection Agency will also have the capacity to carry out some research under its name. Hence, we recognize that some of the elements of coordination recommended in this section will be accommodated by this new agency.

INTRODUCTION

In 1964, The National Cancer Institute undertook through a contract a screening study of a number of pesticidal chemicals. Among the results of this screening study was the finding that birth defects could be provoked experimentally in rats and mice by the administration of relatively large doses of the herbicide, 2,4,5-T. By the time these results were reported, 2,4,5-T had been in common use as an herbicide for more than 20 years. Further, it had been employed along with 2,4-D as a defoliant in Vietnam since 1962, although in sizeable quantities only since 1967.

In October 1969 several agencies of Government moved in a coordinated manner to bring about restriction of the use of 2,4,5-T both within the United States and abroad (*1*). In terms of domestic agricultural use, restriction was placed on the use of 2,4,5-T on food crops pending the acquisition of further information that might permit the Food and Drug Administration to grant a tolerance. Use as a defoliant in Vietnam was restricted to non-populated areas.

For a number of reasons, it seemed wise to explore this issue in some detail. The most important of these reasons, perhaps, was the desire to examine the scientific evidence available to stand behind future policies governing the use of 2,4,5-T and to suggest directions for futher experimental research. Accordingly, a panel of experts was assembled by the President's Science Adviser to consider a number of aspects of a variety of herbicides some of which were used as defoliants in Vietnam. The present report represents their review of 2,4,5-T.

This review considers topics which are of concern to those who are faced with policy decisions for 2,4,5-T. We hope that it can serve as an example for the consideration of the health effects and safety of pesticides and other chemicals purposefully placed in the environment.

A number of issues are raised when the utility and safety of an already existing material is questioned. It is elementary but nevertheless true to say that the issues are complex. In a way, their examination can be compared to following a seemingly endless and continuously branching program. The subject of how much assurance of safety should be afforded is important.

Teratogenesis appears to be a more manageable problem than some other health effects (such as tumor production). Prediction of safety can probably be made with reasonable assurance. In addition, experiments to test a suspected substance are reasonably straightforward to conduct.

Among other problems, the purity of the chemical became an issue. In the case of 2,4,5-T separation of biological effects of the principal material from those of the impurities turned out to have unusual importance. For this reason, the resolution and accuracy of analytic methods available and used to detect 2,4,5-T and its impurities had to be evaluated. In ascertaining the probable hazard to man of an agricultural chemical, its toxicity in absolute terms must be related to the probabilities of human exposure. Residue information on 2,4,5-T therefore was explored.

Finally, there remains a series of policy questions which are at least as philosophical as they are technical, the most crucial one being how wide a margin of safety should a society adopt for itself.

The panel also touched on a narrower aspect of this question by posing an additional one. This is the practical problem, in the case of a material already in use, of how the Government should act in the interim between the time of acquisition of preliminary experimental data which reveal a chemical suspect and the performance of more definitive experiments which establish the risk.

REFERENCES

(*1*) Press Release on 2,4,5-T. Office of Science and Technology, October 29, 1969.

The FDA group reported the following toxicities:

TABLE 3.—*After Higginbothan, et al, (12).*

Reactants	No. of Cl atoms in dioxin	Chicken embryo bioassay	
		µg/egg	percent mortality
2,4-dichlorophenol	2	500	70
Chlorinated dibenz-p-dioxin [1]		0.05	100
2,4,5-trichlorophenol	4	0.25	100
2,4,6-trichlorophenol	4	5.0	50
2,3,4,6-tetrachlorophenol	6	1.0	100
Pentachlorophenol	8	5.0	27
Reference toxic fat components	Mixture	3.0	100

[1] Mixed 3 & 4 chloro species.

The tetrachloro species, which will be the important product from the condensation of 2,4,5-trichlorophenol, requires only 0.25 µg for 100% mortality in the chick embryo bioassay. A mixture of the tri- and tetrachloro species was reported in one study to be more toxic than the tetrachloro species alone (*12*). However, more recent unpublished observations by the same authors have pointed toward a singularly high degree of toxicity of the four chlorine members of the family. The conditions required for the production of the tetrachlorodibenz-p-dioxin, TDD, are present in step (1) of the commerical 2,4,5-T synthesis, so this material can be present in the original herbicide. Members of the family of dioxins have been recognized in a variety of environmental situations. The origins of these are not clear in every case (*23*).

The second source of information about the toxicity of dioxin compounds came from observations of occupational exposures in plants manufacturing 2,4,5-T. These are reviewed in another section of this report. One of the diseases reported was a particularly refractory form of skin rash known as chloracne. This was also seen in workers involved in the production of other compounds, The first report of chloracne in 2,4,5-T plant workers was in 1957 (*14*). The authors in this case suggested that the dioxin impurity may have been the factor which caused the chloracne.

In 1964, the Dow Chemical Company (*6*) attempted to increase the production of 2,4,5-T by changing the reaction conditions. Plant operators became affected with chloracne. The Dow Chemical Company closed their facility and, early in 1965, alerted other manufacturers of their problem. The active agent was identified as 2,3,7,8-tetrachlorodibenz-p-dioxin. In addition, an analytical method for its detection was standardized and various methods for removing the impurity were devised. By 1965, sufficient technology was available to allow the manufacture of 2,4,5-T and 2,4,5-trichlorophenol containing no more than 1 ppm of 2,3,7,8-tetrachlorodibenz-p-dioxin. By 1966, a new Dow plant, conforming to those specifications, was

put into operation. TDD levels in technical grade 2,4,5-T from another manufacturer are listed year by year in Table 4. Dioxin levels in 2,4,5-T currently manufactured are reported not to exceed 1 ppm.

TABLE 4.—*History of TDD concentration in technical 2,4,5-T*

[Analysis of material from one manufacturer]

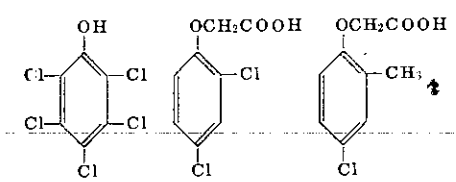
Date	ppm, TDD
1958	11
1959	11
1950	8
1961	5
1962	10
1963	11
1964	12
1965	5–32
1966	3–18
1967	1–25
1968	1–25
1969	<1

Analytical Methods

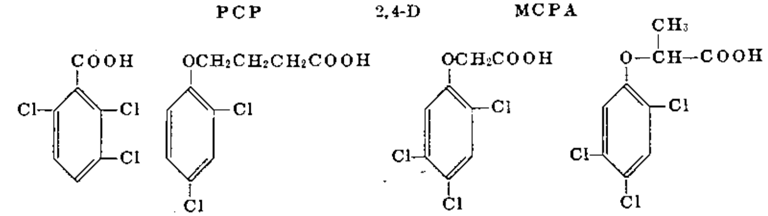
1. *Standard Procedures.*

A typical method for the analysis of herbicide residues in oil seed crops has been described by G. Yip of the FDA (*27*). The method involves extraction of 50 grams of oil with sodium bicarbonate solution, acidification, and extraction of the herbicides with chloroform. The herbicide residues in the acid form are then esterified with diazomethane to produce the methyl esters which are finally analyzed by programmed temperature gas chromatography. Both electron capture and microcoulometric detection schemes are used. The microcoulometric detector consists of a quartz tube condensation chamber where the herbicide is pyrolyzed at 800° C in the presence of oxygen. The HCl formed is carried into a microcoulometric titration cell where the chloride is titrated with silver ion. The sensitivity of this analytical scheme is about 0.01 ppm.

A gas chromatogram obtained from cottonseed oil treated with a mixture of seven herbicides each at 0.02 ppm is shown in Fig. 1. The seven herbicides used in this test of the method included:



PCP 2,4-D MCPA



2,3,6-TBA 2,4-DB 2,4,5-T 2,4,5-TP

(23) U.S. Department of HEW, FDA, personal communication.
(24) U.S. Tariff Commission, "Synthetic Organic Chemicals. U.S. Production and Sales, 1967." Publication No. 295.
(25) WOOTON, J. C. Chem. and Eng. News, 45:10, 1967.
(26) YIP, G. J. Assoc. of Official Agricultural Chemists, 45:367, 1962.
(27) YIP, G. J. Assoc. of Official Agricultural Chemists, 47:116, 1964.
(28) YIP, G. J. Assoc. of Official Agricultural Chemists, 47:343, 1964.
(29) YIP, G., and HEY, R. E., Jr., Weeds 14:167, 1966.

1907

USES AND SIGNIFICANCE

Summary

2,4,5-T has become important in land and waterway management. It has been very useful for brush and weed control. A result has been a growing dependence upon it. The Government itself has encouraged the use of 2,4,5-T through an agricultural cost sharing program.

Nearly 8 million acres were treated with 2,4,5-T in the United States in 1964. The major use was brush control on rangelands, pastures, and rights-of-way. Other uses were on certain food and non-food crops, in aquatic weed control and in forestry.

In 1964 and 1966 almost half of the 2,4,5-T was used on rights-of-way. Over two million acres of rights-of-way were treated in 1964 which is one quarter of the total area treated with this herbicide.

Civilian uses of 2,4,5-T dropped nearly 50% from 1964 to 1966. More recent, unpublished information from the Department of Agriculture suggests that this trend continued through 1968 but may have begun to reverse itself within the past year. This decrease accompanied price increases and shortages of supply associated with the demand for 2,4,5-T as a defoliant and tactical weapon in Vietnam. If acreage decreased proportionately, about four million acres would have been treated in 1966.

To some extent, other herbicides can be substituted for 2,4,5-T (notably 2,4-D). If all alternative herbicides were available the banning of 2,4,5-T would appear to lead to an additional cost of nearly $52 million in land and waterway management or nearly a 100% increase over the current expenditures. These figures assume practices designed to achieve the current level of management and agricultural production. If other phenoxy herbicides are also banned, the additional costs from elimination of 2,4,5-T alone would amount to $172 million or over three times the present investment. Agricultural costs are estimated to rise $32 million and $44 million, respectively, under the two assumptions, while costs of right-of-way management would rise $12 million and $75 million, respectively.

Agricultural production has become dependent upon the use of herbicides. Their use in the United States has increased rapidly during the past few years. They are employed as substitutes for the more costly practices of hoeing, cultivating, mowing, chopping, burning, and various other cultural practices for the control of weeds.

One of the principal uses of the herbicide, 2,4,5-T, is for control of weeds and brush on pasture and rangeland. Large quantities are also used to control brush along roadways and under powerlines. The principal crop use of 2,4,5-T is on hay and pasture.

In forest production, 2,4,5-T has proved useful for selective weed control. 2,4,5-T acts upon deciduous hardwoods leaving the conifers with little injury. This treatment has been helpful in releasing conifers from deciduous hardwood competition in mixed stands.

Production

Total herbicide production in the United States has increased rapidly:

*1960 75,000,000 pounds (*3*)
1965 220,000,000 pounds (*8*)
1968 403,000,000 pounds (*12*)

For 2,4,5-T (acid, esters and salts), production has increased as follows:

1960 7,900,000 pounds (*7*)
1965 13,500,000 pounds (*10*)
1966 18,100,000 pounds (*10*)
1967 27,200,000 pounds (*10*)
1968 42,500,000 pounds (*12*)

A portion of this production is exported and a portion shipped abroad for military use.

Uses

1. *Domestic.*

a. *Farm use.*

In 1964, of the estimated 13,000,000 pounds of 2,4,5-T produced in the United States, only 13 percent or about 1,655,000 pounds were used in agriculture (Table 1). About 40 percent of the quantity employed in agriculture was used for weed control along fence rows, ditch banks, farm roadways, and other non-crop uses. The remaining 60 percent or 979,000 pounds was employed on crops (including hay, pasture and rangeland).

Since 1964, the use on farms has been decreasing. In 1966, 760,000 pounds were applied which is less than 50% of the 1964 amount (*13*). Use on hay, pasture, and rangeland declined 35 percent, and other crop use decreased by 31 percent, whereas non-crop use decreased about 84 percent.

b. *Forestry, Rights-of-way, Aquatic Weeds and Lawn and Turf.*

In 1964, about 888,000 pounds of 2,4,5-T were used in private nonfarm forest management for control of undesirable trees and brush (Table 1); in 1966, this declined to 408,000 pounds (Table 3).

An estimated 4,368,000 pounds were applied to rights-of-way, roadways, fire lanes, and similar areas for tree and brush control in 1964 (Table 1); in 1966, this had decreased to 2,315,000 pounds (Table 3).

About 162,000 pounds were applied to aquatic habitats in 1964 for weed control on about 81,000 acres (Table 1); in 1966, this had dropped to 75,000 pounds (Table 3).

*Changes in the method of reporting after 1960 make this figure difficult to compare with later figures.

TABLE 1—*Estimated use of 2,4,5-T in the United States, 1964* (*13*)

Use category	Land treated per 1,000 acres	Quantities of active 2,4,5-T applied per 1,000 pounds	Proportion of total quantity applied (Percent)
Farm use:[1]			
Hay, pasture, and rangeland	2,441	581	7
Other farm use	[2] 1,010	1,074	12
Total farm use	3,451	1,655	19
Non-farm use:			
Federal Government agencies [3]	296	656	7
Lawn and turf treatment [4]	1,200	600	7
Rights-of-ways [5]	2,175	4,368	49
Private non-farm forests [6]	430	888	10
Aquatic treatment [7]	81	162	2
Other uses [8]	306	583	6
Total non-farm use	4,488	7,257	81
All uses	7,939	[9] 8,912	100

[1] Based on "Quantities of Pesticides Used by Farmers in 1964." AER 131. Farm data excludes Alaska and Hawaii. In some farm uses, all acres in a field were reported treated while only spots actually received 2,4,5-T, thus making the rate per acre seem low.
[2] Sum of the acres of all crops, except hay, pasture, and rangeland treated, plus an acreage estimate for noncropland receiving treatment. The acreage of noncropland was estimated by allocating the quantity of 2,4,5-T used for such purposes at the rate of 2 pounds per acre.
[3] Based on 1969 usage of the Departments of Agriculture, Interior, and Defense; and 1951–69 average usage by the Tennessee Valley Authority.
[4] Based on estimated 500,000 acres of turf and 700,000 acres of lawns treated. Estimates based on "Extent and Cost of Weed Control with Herbicides and an Evaluation of Important Weeds," ARS 34–102; and unpublished data.
[5] Based on sources cited in footnote 4 with rate of application same as for federally treated rights-of-way. Does not include rights-of-way treated by Federal agencies.
[6] Estimated at 4 times the acreage treated and quantities of pesticides applied to public forests.
[7] Based on sources cited in footnote 4 and rates used on federally treated waterways.
[8] Includes governments other than federal and any other usage.
[9] Based on table 28 of the Pesticide Review 1969, Ag. Cons. Stab. Service.

TABLE 2.—*Farm use of 2,4,5-T on crops, by category of use, United States, 1964 and 1966* (*13*) [1]

Use category	Active ingredients per 1,000 pounds		Acres treated per 1,000 acres		Percentage of planted acres treated with 2,4,5-T (percent)	
	1964 [2]	1966 [3]	1964 [2]	1966 [3]	1964 [4]	1966 [5]
Hay, pasture, and rangeland	581	379	2,441	861	0.4	0.1
Corn	72	58	255	337	0.4	0.5
Wheat	16	26	55	59	(6)	0.1
Sorghum	5	6	48	18	0.3	0.1
Rice	(7)	23	(7)	16	(7)	0.8
Other grains	264	34	196	99	0.4	0.2
Other crops	41	127	117	175	0.1	0.2
All crop usage	979	653	3,112	1,565	0.3	0.2

[1] Does not include Alaska and Hawaii. Use in 1964 generally reflects current practices. Use in 1966 was unusually small and not representative of current practices because of domestic shortages due to increased military purchases.
[2] Revised estimates based on Quantities of Pesticides Used by Farmers in 1964. U.S. Dept. Agr., Agr. Econ. Rpt. No. 131, Jan. 1968.
[3] Data from the ERS Pesticide and General Farm Survey, 1966.
[4] Acres treated as a percent of acres grown as reported in Stat. Bul. 384 and *Agricultural Statistics* 1968.
[5] Acres treated as a percent of acres grown as reported in Crop Production, 1967. U.S. Dept. Agr., Cr. Pr. 2-2(7–67).
[6] Less than one-tenth percent.
[7] Included in other grains in 1964 only.

2,4,5-Trichlorophenoxyacetic Acid

(Principal formulations: EC esters; amine salts; Type pesticide: Herbicide and plant regulator)

Use	Tolerance (ppm)	Dosage	Limitations
		lb. actual/A.	
Pastures: Grasses	Extended	3	Heavy brush. Apply when in full leaf and after grass is well established.
		1	Light brush. Apply when leaves are fully expanded.
Rangeland clearance	Extended	4	Apply in spring by airplane when brush is in heavy foliage stage (40–90 days after leaves unfold).
Apples (McIntosh)	Extended	20 ppm spray (acid equivalent).	Preharvest drop control. Apply a single application 4–5 days before drop normally begins.
Blueberries (low bush)	Extended	1.0 (acid equivalent).	Spray on revolving cloth-covered drum held above blueberry foliage. Apply during June and July of season preceding a burn. Do not apply within 2 years of harvesting berries.
Grains, cereal (undesignated).	Extended	0.5	Apply when grain is in tiller to boot stage and weeds are in actively growing condition. Do not apply from boot to milk stage or in seedling stage.
Pastures: Grasses	Extended	3	Heavy brush. Apply when in full leaf and after grass is well established.
		1	Light brush. Apply when leaves are fully expanded.
Rangeland clearance	Extended	4	Apply in spring by airplane when brush is in heavy foliage stage (40–90 days after leaves unfold).
Rice	Extended	1.25	Apply tiller to boot and before flooding (4–8 weeks after rice emerges).
		1.5	Apply after flooding (2–3 weeks) or 7–10 weeks after planting.
Sugarcane	Extended	1	Preemergence use only. (Louisiana). If cane is shaved and off-barred, treat immediately following this operation.
		4.5	Preemergence use only. (Hawaii). Apply just before cane emerges.
		1	Postemergence (weeds in established cane). Apply over row when weeds are growing vigorously. Do not apply after cane is 2 feet tall.
Lakes; Ponds	Extended	4 (with 20 lbs./A. 2,4-D as ester).	Broadcast application in early spring to summer. Do not use treated water for crop irrigation or livestock drinking water.

TABLE 7.—*The nonfood crop uses of 2,4,5-T. The doses listed below are given in pounds of 2,4,5-T and equivalent in 100 gallons of spray using water or oil as the vehicle. (USDA, Pesticide Regulation Division, as amended by Pesticide Regulation Division Notices 70–11 (4/20/70) and 70–13 (5/1/70).*

Non-food crop uses	Pounds 2,4,5-T acid	Comment
Around farm buildings and yards	2	
Farm fence rows, lanes and roads	2–6	
Pine release in hardwood forest	2–6	
Industrial buildings including: Around factories, elevators loading platforms, oil refineries, etc.	12–16	
Industrial sites: Airline beacon stations, airport runways, coalyards, electric transformer stations, lumberyards, parking areas, radio towers, railroad sidings, sawmills.	3–12	
Recreational areas including: Race tracks, wildlife management	5.6–10	
Rights-of-way: Fire lanes, highways, pipelines, powerlines, railroads, telephone and telegraph.	4–20	
Vacant lots	2–4	

2. *Military Uses of 2,4,5-T.*

The phrase, military uses, refers to the employment of 2,4,5-T as a defoliant in operations. Basic research on herbicides proceeded through the period 1941–1947. The work was encouraged by efforts

to develop defoliating agents for use in jungle areas of the South Pacific area during World War II (*2*). A major demonstration of the utility of the mixture now known as Orange (1:1 mixture of the butyl esters of 2,4-D and 2,4,5-T) as a defoliant for military purposes was conducted in 1959 (*1*).

A preliminary series of defoliation trials was conducted in Vietnam between July 1961 and April 1962 (*3*). Operational spraying began in Vietnam in 1962 and increased sharply after 1967. Reviews on this subject are available (*3*, *5*, *6*).

Two herbicide formulations used in military operations in Southeast Asia include 2,4,5-T:

Agent	Composition	Lb./Gal. of active material
Purple	n-butyl ester 2,4-D 50% (wt.) n-butyl ester 2,4,5-T 30% (wt.). Isobutyl ester 2,4,5-T 20% (wt.).	8.9
Orange	n-butyl ester 2,4-D 50% (wt.) n-butyl ester 2,4,5-T 50% (wt.).	8.9

Purple mixture was discarded early because it was found to be no more effective than Orange. Orange is applied at a rate of 24 lb. per acre from both fixed wing aircraft and helicopters. C–123 aircraft fly at 150 feet altitude at 130 knots. A swath of 240 feet per pass is sprayed. Typically, a formation of 3–9 aircraft fly at the same time.

The following table shows the total areas sprayed each year between 1962 and 1968.

TABLE 8.—*Land area in Vietnam to which defoliants have been applied between 1962 and 1968 (15)*

Year	Number of acres sprayed
1962	4,940
1963	24,700
1964	83,486
1965	155,610
1966	741,247
1967	1,486,446
1968	1,267,110

It has been estimated that under usual operating conditions 90% of the released material is confined to a band about 2.0 km wide on either side of the 80 meter spray path (*15*). This figure is based on the spectrum of particle sizes, the direction and speed of the crosswind, and the altitude. Under realistic conditions but with a crosswind at right angles to the flight path, only 0.1% of the spray would be deposited between 1 and 2 km from the center line of the flight path.

As shown in Table 8, the total area sprayed for defoliation in 1968 was less than in 1967. Defoliation was discontinued in April 1970.

Defoliation operations have been carried out to some extent in virtually all sections of South Vietnam. The major use has been within War Zone C, War Zone D, and the Rung Sat Special Zone. These three areas comprise about one-fifth of the total area to which defoliants have been applied.

The Rung Sat Special Zone is an area which surrounds the shipping channel into Saigon. 113,600 acres had been sprayed by the end of January 1968 (*6*). War Zone C is northwest of Saigon between the Song Be River and the Cambodian border. 227,000 acres had been treated in War Zone C by January 1968. War Zone D, in which 405,000 acres were treated, is northeast of Saigon between the Song Be and Song Dong Hai Rivers (*6*). Repeated application was made in some areas.

The general purposes for which defoliation operations have been used include:

a. Defoliation of lines of communication. Sites of frequent ambush have been defoliated to afford better visibility along roads and trails.

b. Defoliation of areas where Vietcong tax collectors customarily exacted payments from the populace.

c. Defoliation of enemy infiltration routes.

d. Defoliation of enemy base camps. The rationale in this case was based on the observation that the enemy tended to move out of a base after the area had been sprayed.

e. Clearing of vegetation around American base camps and fire bases in order to clear fields of fire and improve observation.

Importance of 2,4,5-T as a military defoliating agent.

Systematic studies have not been carried out to quantify the value of defoliation in Vietnam. However, many of those concerned with the program believe that the military advantages are clearly evident (*4, 15*). The following evaluations are extracted from testimony offered by Rear Admiral W. E. Lemos before the Subcommittee on National Security Policy and Scientific Developments of the Committee on Foreign Affairs (*4*).

a. Major defoliation has been accomplished in War Zone C. Prior to defoliation, 7 brigades were necessary to maintain US/GVN presence. After defoliation, only 3 brigades were required.

b. The Commander of Naval Forces in Vietnam in a report to General Abrams stated: "As you know, a major concern is the vegetation along the main shipping channel. Your continuing efforts under difficult and hazardous flying condition, in keeping this area and the adjacent inland areas devoid of vegetation have contributed considerably in denying the protective cover from which to ambush the slow-moving merchant ships and U.S. Navy craft."

c. In 1968, the Commanding General of the First Field Force reported: "Defoliation has been effective in enhancing the success of allied combat operations. Herbicide operations using C–123 aircraft, helicopters, truck mounted and hand sprayers have become an integral part of the II CTZ operations against VC/NVA. The operations are normally limited to areas under VC/NVA control remote from population centers. The defoliation program has resulted in the reduction of enemy concealment and permitted increased use of supply routes by friendly units. Aerial surveillance of enemy areas has improved and less security forces are required to control areas of responsibility. An overall result of the herbicide program has been to increase friendly security and to assist in returning civilians to GVN control."

d. The U.S. Commander in the III CTZ related: "Herbicide operations have contributed significantly to allied combat operations in the III Corps. Defoliation is an important adjunct to target acquisition. Aerial photographs can often be taken from which interpreters can "see the ground" in areas that previously were obscured. Defoliation also aids visual reconnaissance. USAF FAC's (forward air controllers) and U.S. Army aerial observers have discovered entire VC base camps in defoliated areas that had previously been overlooked."

e. In the south in the IV CTZ, C–123 herbicide operations are limited. This is because of the vast areas of valuable crops which are not to be destroyed, even though they may be in enemy hands. Therefore, commander of the IV Corps area in presenting his evaluation cited the value of helicopter operations as follows: "A significant helicopter defoliation mission was conducted in the vicinity of SADEC in August 1968. The target area consisted of 3 main canals which converged and formed a strong VC base. The dense vegetation permitted visibility of only 10–15 meters horizontally and nil vertically. The area was sprayed with approximately 135 gallons of herbicide White and over 90 percent of the area was defoliated. As the result of the defoliation, an ARVIN battalion was able to remain overnight in the area for the first time in five years. Many enemy bunkers were open to observation. Since the defoliation, the VC presence has decreased to the point that only RF/PF forces are now necessary for local security."

f. As a part of the 1968 evaluation report of herbicide operations, the U.S. Senior Advisor in the IV Corps Tactical Zone area reported: "A section of National Highway 4 in Phong Dinh Province was the site for a defoliation operation on 24 June 1968. Since January 1968, a series of ambushes was conducted against SYN convoys and troop movements. Because of the total inability of ground troops to keep the area clear of VC, this area was sprayed using 685 gallons of herbicide White. The target area was primarily coconut palm and banana trees that had been abandoned by their owners for several years. During the period of abandonment the vegetation had become so dense that convoy security elements were not able to see more then five meters into the underbrush and had to rely on reconnaissance by fire to discover the hidden enemy. This method of protection had proven ineffective. Three RF/PF companies with U.S. advisors were used to secure the target for the helicopter operation in addition to an armored cavalry troop. Since the defoliation mission was completed, convoys have used the highway 2 or 3 times a week without attack or harassment. Only one RF platoon has remained in the area to provide local security to the hamlet and highway."

g. In certain instances, we know the VC have been forced to divert tactical units from combat missions to food-procurement operations and food transportation tasks, attesting to the effectiveness of the crop destruction program. In local areas where extensive crop destruction missions were conducted, VC/NVA defections to GVN increased as a result of low morale resulting principally from food shortages.

The most highly valued item of equipment to field commanders in Vietnam is the helicopter. There was some question when the helicopter spray equipment was first procured whether field commanders would divert the use of helicopters from combat operations for herbicide spray operations. The very fact that the commanders have used their helicopter spray equipment to the fullest and have asked for more is certainly proof that herbicide operations have been helpful in protecting the American Soldier and contributing to successful accomplishment of the ground combat mission." *(4)*

Cost Sharing Programs

The Agricultural Stabilization and Conservation Service of the U.S. Department of Agriculture has provided a cost sharing program for farmers in order to encourage desirable farm and conservation practices. Included in this Agricultural Conservation Program are two programs in which 2,4,5-T was used:

1. *Control of competitive shrubs on range and pastures.*

In 1968, $7 million were paid to farmers who treated 1.9 million acres. Approximately one-half of this land was treated with chemicals, a substantial part of which was 2,4,5-T. The remainder was treated by mechanical means.

2. *Control of specified noxious weeds on farmland or biennial weeds on pasture and range lands.*

In 1968, $1.75 million were paid to farmers to treat 700,000 acres. In some cases 2,4,5-T was used, although in most cases, 2,4-D and other chemicals were employed.

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TOXICOLOGY

Summary

Relatively little toxicological information has been available on 2,4,5-T. Most of the experiments prior to the National Cancer Institute screening study were of acute, single-dose or short-term toxicity. The longest period of observation was 90 days. It was assumed (not unreasonably) that the several phenoxy herbicides behaved in similar fashion toxicologically. Hence, an understanding of aspects of the toxicology of 2,4,5-T was inferred from experiments with 2,4-D. The sample sizes used in many of the early toxicity studies were so small that it is difficult to draw statistically valid inferences. The studies indicated that 2,4,5-T is only moderately toxic. Relatively little is known about the mechanisms of toxicity of 2,4,5-T or of its metabolism in man and animals.

The screening study of pesticides carried out under contract for the National Cancer Institute tested the teratogenicity of a number of compounds, including 2,4,5-T. 2,4,5-T appeared to be teratogenic in mice and rats. Subsequent studies have confirmed these observations and in addition indicated that purified 2,4,5-T containing less than 1 ppm of the toxic contaminant tetrachlorodibenz-p-dioxin as well as 2,4,5-T contaminated with 27 ppm dioxin are teratogenic. It has also been reported that dioxin by itself is teratogenic. Tumor production by 2,4,5-T was not found.

Accounts of birth defects in defoliated areas of Vietnam have been reported. The information available does not permit the conclusion that 2,4,5-T used in Vietnam has been the cause of human birth defects.

2,4,5-T is classed as a plant hormone since, in appropriate amounts, it accelerates plant growth. The mechanism of the herbicidal effect is not fully understood. However, it is generally believed that excess growth stimulation and herbicidal properties are related. Herbicidal effects occur when these materials are used in large doses.

A. Acute Toxicological Investigations

Toxicological studies on 2,4-D were first reported in 1944 (*8*). However, there were no published reports of toxicological investigations of 2,4,5-T until 1953. In that year, Drill and Hiratzka (*5*) reported a series of studies of acute and subacute toxicity of 2,4,5-T on dogs. The material used was commercially available 2,4,5-T (presumably the acid) and was administered in capsules mixed in dog food. The single acute dose ranged from 50–400 mg/kg/. Chronic toxicity was studied in this case by oral administration in doses of 2-20 mg/kg/day, 5 days per week over a period of 13 weeks. Observations and measurements made included determination of the number of days until death ensued, changes in body weight, general observations of abnormal physical signs, gross pathology of organs, and selected histology. The number of animals used was small (as few as one per dose and as many as four per dose). In brief, the findings of this study suggested that a single fatal dose for dogs lay somewhere between 100 and 400 mg/kg. It was inferred by the author that the acute LD_{50}* was around 100 mg/kg for dogs. Repeated daily doses of 20 mg/kg led to the death of all four animals tested within 11–75 days. Repeated daily doses of 10 mg/kg did not prove fatal over a 90-day period. There were some overt signs of toxicity including weight loss, stiffness of hind legs, muscular weakness and, occasionally, bleeding from gums.

The Dow Chemical Company, a manufacturer of 2,4,5-T, undertook a series of studies of acute toxicity of this material beginning around 1950. This series included a variety of species of animals and a number of the various salts and esters of 2,4,5-T and several of the various formulations. The details of these studies have never been published in the open literature. A number of them have been submitted as background material for a petition for the granting of a tolerance for the herbicide for uses of food crops. In 1954, a summary of some of this work was published by Rowe and Hymas (*17*). Table 1 lists the various herbicidal agents tested.

*LD_{50}—median lethal dose=the amount of a toxic agent which will be lethal to 50% of the test animals to which it is administered under the conditions of the experiment.

TABLE 5.—*Teratogenic evaluation of 2,4,5-T in mice (2)*

Compound	Vehicle	Dose (mg/kg)	Litters (No.)	Live fetuses per litter (av. No.)	Fetal mortality per litter (percent)	Abnormal litters (percent)	Abnormal fetuses per litter (percent)	Percentage of fetuses per litter with: Cleft palate	Cystic kidney
C57BL/6 strain treated days 6 to 14									
Nontreated	None	None	72	5.8	26	38	11	<1	1
Control	DMSO	(*)	106	5.5	29	42	12	<1	2
Control	Honey	(*)	32	7.1	15	41	14	0	1
2,4,5-T	DMSO	21.5	6	7.7	3	50	12	0	0
2,4,5-T	DMSO	113.0	18	4.4	42	†86	†57	†22	†41
2,4,5-T	Honey	46.4	6	8.5	8	‡100	‡37	2	†33
2,4,5-T	Honey	113.0	12	4.8	†47	†100	†70	†23	†48
C57BL/6 strain treated days 9 to 17									
Nontreated	None	None	8	5.1	36	71	31	0	7
Control	DMSO	(*)	10	6.1	23	30	8	0	0
2,4,5-T	DMSO	113.0	10	7.7	11	†100	†77	†29	†60
AKR strain treated days 6 to 15									
Nontreated	None	None	58	7.1	16	19	5	<1	<1
Control	DMSO	(*)	72	6.9	15	24	4	<1	<1
Control	Honey	(*)	12	8.8	9	0	0	0	0
2,4,5-T	DMSO	113.0	14	6.9	23	†71	†29	†28	1
2,4,5-T	Honey	113.0	7	5.3	‡42	†100	†55	†55	0

*Dose, 100 μl per mouse.
†P=.01.
‡P=.05.

TABLE 6.—*Teratogenic evaluation of 2,4,5-T in rats*

Compound	Vehicle	Dose (mg/kg)	No. of litters	Average No. live fetuses/litter	Percent fetal mortality/litter	Percent abnormal litters	Percent abnormal fetuses/litter	Percent of fetuses per litter with: Enlarged renal pelvis	Cystic kidney
Nontreated	None		7	9.9	11	43	9	9	0
Control	Honey	(†)	14	8.7	1	57	12	12	<1
2,4,5-T	Honey	4.6	8	8.2	12	88	**36	11	21
2,4,5-T	Honey	10.0	7	7.1	***28	86	**46	17	**30
2,4,5-T	Honey	46.4	6	2.7	***59	67	§60	27	§33

†200 μl/rat (2).
**Statistical Significance Level=0.05.
***Statistical Significance Level=0.01.
§The sample size was possibly too small to show a significant difference.

5. It is puzzling that virtually no skeletal malformations were encountered in either controls or test group. Skeletal defects usually account for a substantial part of the easily detectable malformations that occur spontaneously or after treatment in rodent species. Hardly a strain that has been carefully studied in properly cleared and stained specimens has failed to show vertebral and rib variations.

6. There were some known teratogens used in these experiments (trypan blue, 6-aminonicotinamide). It is puzzling to find that these agents failed to produce significant teratogenic and embryo-lethal effects consistantly. This raises questions about the precision with which these teratogenicity tests were performed.

7. 2,4,5-T appeared to be clearly teratogenic in two strains of mice treated with 2,4,5-T at 113 mg/kg via either of the two routes of administration. In rats, 2,4,5-T appeared only equivocally teratogenic at any dosage but clearly embryo-lethal from 10.0–46.4 mg/kg.

8. The lack of an unequivocally defined dose-response relationship renders these results less than completely satisfying.

Reports of Birth Defects Among Humans Following Exposure to 2,4,5-T

Shortly after the report of the teratogenesis screening in experimental animals of pesticides, there appeared a series of articles in the lay press which described the occurrence of birth defects in parts of Vietnam where defoliants had been used. These articles appeared in at least six different newspapers in South Vietnam between June 26 and July 5, 1969. Both congenital abnormalities and hydatid moles* were described. Translations of the articles have alluded to the possibility that defoliants might be responsible for these defects. The implication was offered that these abnormalities had increased in frequency in the recent past. No documentation has been available.

Toxicity of Dioxin

It was pointed out in an earlier section of this report that one of the impurities which arises in the manufacturing process of 2,4,5-T is 2,3,7,8-tetrachlorodibenzo-p-dioxin. This substance has considerable interest because it is highly toxic, because a close relative was a toxic constitutent in chicken feed and because it has caused chloracne, a severe skin disease, among workers engaged in the manufacture of 2,4,5-T.

The dioxin impurity has assumed a further importance as an impurity in commercially available 2,4,5-T. With the observation that production lots of 2,4,5-T containing approximately 27 ppm dioxin could be teratogenic, it became important to ascertain whether it was 2,4,5-T itself or some impurity which was the teratogen. Recent experiments at the National Institute of Environmental Health Services indicate that partially purified 2,4,5-T ($<$0.1 ppm tetrachlorodibenzo-p-dioxin) shows teratogenic activity in the mouse. Pure tetrachlorodibenzo-p-dioxin shows teratogenic activity also, but not at low enough doses to account for the activity of the partially purified 2,4,5-T. We cannot exclude the possibility that other impurities may contribute significantly to the observed teratogenic activity of 2,4,5-T.

*(An abnormality of pregnancy which involves the placenta and the membranes surrounding the fetus.)

In the following paragraphs, the history of the discovery of dioxin is examined in relation to the experimental observation of the teratogenic properties of 2,4,5-T. Secondly, data on the toxicity of this material are presented.

In March 1949, an accident occurred at a 2,4,5-T plant owned by the Monsanto Chemical Company which led to the release of some of the intermediate chemicals into the plant. As a result, 117 cases of a severe skin disease known as chloracne were found among the exposed workers. Chloracne is characterized by comedones, blackheads, inclusion cysts, and pustules with eventual scarring over the neck, back, and chest. In addition to the cases which were traced to the accident, a number of other clinical cases of chloracne were recognized among workers in the 2,4,5-T plant who were not in the vicinity of the accident. (*10*) Kimmig and Schulz (*11*) reported in 1957 that chloracne occurred among workers engaged in the manufacture of 2,4,5-T in Germany. These authors demonstrated that the agent responsible for chloracne was tetrachlorodioxin. In 1964, the demand for 2,4,5-T in the United States began to rise mainly due to its increasing use as a defoliant in Vietnam. A greater demand was placed on each of the domestic manufacturers to produce more herbicide. Coincident with the increased production was the discovery of chloracne among some 60 2,4,5-T workers. (*7*) The Dow Chemical Company reduced its operations substantially for a period of several months in order to investigate the origin of the toxic hazard. It was found that the amount of dioxin formed varied with the temperature and pressure of the early reaction steps. The Dow Chemical Company made its findings known to the other domestic manufacturers. Looking back it is evident that dioxin levels varied widely among commercial 2,4,5-T samples, as seen in Table 4 of Section III. Rigorous control is now exercised to reduce dioxin levels in the final product to less than 1 ppm.

A recent review of occupational disease attributed to dioxin has been prepared by Poland *et al* (*15*). These authors studied 73 male employees in a 2,4,5-T factory, some of whom had been observed six years previously by Bleiberg (*1*) who then noted the prevalence of chloracne. Poland et al (*15*) also found chloracne among the same population although estimates of exposures were not made. Poland attributed the chloracne to the dioxin impurity. They also examined the prevalence of a type of porphyria, thought to be toxic in origin, known as porphyria cutanea tarda (elevated urinary porphyrin excretion, skin fragility and vesicular eruptions). Uroporphyrinuria had been noted during the early visit to the plant by Bleiberg (*1*) but it was not found during the later study. Elevated urinary coproporphyrin levels were noted, however, but there appeared to be no correlation with the severity or presence of chloracne (*1*, *15*). The later series of observations found much of the chloracne still remaining but the porphyria had disappeared. Chloracne was attributed to the dioxin impurity but the origin of the porphyria was less certain.

In order to measure the toxicity of the dioxin impurity, the Dow Chemical Company undertook a series of acute toxicity studies on small animals in 1967 (*16*). Single doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin were administered orally to lots of five animals for each of several doses. The number surviving and the time of death were noted. The animals used were male rats, female rats and female guinea pigs. Table 7 gives the results of these experiments:

TABLE 7.—*Single-dose oral toxicity of dioxin (18)*

Species	LD_{50} mg/kg
Male rat	0.022
Female rat	0.045
Female guinea pig	0.0006

With this background in mind, the purity of the 2,4,5-T material used in the National Cancer Institute screening study assumed a new importance. A sample of this material was submitted to chemical analysis by gas chromatography. The result was the finding of 27± 8 ppm of dioxin.

Testing for Teratogenicity

The study of teratogenic effects in experimental animals is characterized by a great deal of empiricism. However, in general testing for teratogenic potential is a more manageable problem than are a number of other types of biologic testing. There are large numbers of agents known to be teratogenic to animals. In fact, it has been held that virtually any material is potentially teratogenic if administered in an appropriate dose at the critical time in gestation. Interestingly, however, only a few chemicals have been recognized as human teratogens.

In general, an embryo-toxic dose of a material is separated from a maternal toxic dose by a small margin (perhaps no more than a factor of 10). Only slightly below the embryo-lethal or toxic dose is a no-effect dose (separated, perhaps, by a factor of 2). Between the highest level which has no effect on the developing fetus and the embryo toxic level is a steep dose-response relationship. In testing for teratogenicity, the lowest dose on the dose-response curve (threshold for any embryo toxicity) is identified. All doses below this level are, by definition, no effect doses. By accepting an extra margin of safety below this dose (a factor of 1/10–1/100), reasonable freedom from teratogenic effects can be predicted.

A problem arises in extrapolating findings in experimental animals to man. The embryotoxicity of a chemical agent is, in theory, a function renal defects. In rats renal defects and excess fetal mortality were seen but seemed to be related to dioxin content rather than 2,4,5-T.

3. FDA Experiments (unpublished data acquired in the spring of 1970).

Teratogenic studies were performed by giving the test compound by oral intubation to pregnant hamsters on day 6 through 10 of organogenesis. Embryos were removed by caesarian section on day 14 of pregnancy. Samples of 2,4,5-T tested were:

a. "Dow Technical"—contained ≦ 0.5 ppm dioxin.
b. "Dow Technical"—contained ≦ 0.1 ppm dioxin.
c. "Dow Pure"—contained ≦ 0.1 ppm dioxin.
d. Hercules X-17394—contained no detectable dioxin.
e. Monsanto NL-07-020—contained 2.9 ppm dioxin.
f. "K & K" sample—contained about 45 ppm dioxin.
g. Eastman Kodak (recrystallized in FDA)—contained no detectable dioxin.

In addition pure 2,3,7,8-tetracolorodibenzo-p-dioxin (Dow) was administered to hamsters.

These materials were tested in various doses from 40–100 mg/kg. All of the 2,4,5-T samples produced increased embryotoxicity and gastric and/or intestinal hemorrhages. Birth defects consisted chiefly of poor head fusion and absence of eyelids.

The pure tetradioxin produced increased fetal toxicity at 0.31 and 1.43 μg/kg and gastric and/or intestinal hemorrhages at the lowest dose, 0.02 μg/kg.

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excretory route was the kidney. His data (*7*) showing weak but "certain" protein binding of 2,4-D suggest that this may also occur with 2,4,5-T.

5. Food and feed—Results of studies of pesticide chemical residues in total diet samples by the Food and Drug Administration during the period 1964–68 provide convincing evidence that 2,4,5-T residues are not a significant health hazard in food in the United States. During this 5-year period 30 composite samples of about 82 food items each have been collected each year from retail markets in 25 or more cities. Each composite sample, representing a 2-week diet for a 16- to 19-year-old male, is analyzed by multiresidue methods for more than 60 chlorinated organic and organophosphorus insecticides and for herbicides, carbamates, and selected inorganic chemical residues. Examination of these samples is carried out at levels of sensitivity much lower than those normally used for products tested for compliance with tolerances.

Data reported for the period June 1967–April 1968 (*4*) show incidence and levels of 2,4,5-T residues that are typical. Only one sample of diary products was contaminated. About 2 percent of diary products and 1 percent of meat, fish, and poultry were reported to contain residues of 2,4,5-T. All of these residues were at trace levels, below 0.01 ppm. No residues of 2,4,5-T were found in any of the other 10 classes of foods.

It is significant that residues of 2,4,5-T were detected only in foods of animal origin. The most likely source is forage from pastures and rangelands treated with 2,4,5-T for weed or brush control.

Duggan and Lipscomb (*5*) summarized the results of sampling for herbicide residues in prepared food from the totat diel studies over a period of four years. From these studies, they estimated the likely dietary intake of all of the herbicides searched for to below:

Year	Estimated dietary intake (mg/kg/day)
1965	0.0012
1966	0.00022
1967	0.00005
1968	0.00006

Since 2,4,5-T residues are only a small percentage of these total herbicide residues found in foods it seems safe to conclude that there is little likelihood of exposure from this source.

Residues of 2,4-D and 2,4,5-T resulting from recommended uses by the military for defoliation.

All the available evidence indicates that 2,4-D and 2,4,5-T behave similarly in animals with respect to absorption, distribution, metabolism, and elimination. Since mixtures of these herbicides are used in the defoliation program in Vietnam, it does not appear reasonable to consider them separately.

The large-scale application of 2,4-D-2,4,5-T mixtures for defoliating military target areas in Vietnam poses some possibly unique hazards to humans. Application rates of about 26 lbs/acre of a 1:1 mixture of the n-butyl esters of the chemicals are generally heavier than those used for agriculture and brush clearing in the United States. (There have been a few, limited applications of 2,4-D reported to lakes, streams, and reservoirs in the United States at rates of 80–100 lbs/acre for aquatic weed control.) There are other differences. There are vastly greater areas involved in Vietnam. Because of the nature of the military targets—heavily wooded, jungle areas—it is not likely that many crops are grown in or near treated areas. Although defoliation procedures appear to be under rigid control with all reasonable precautions taken to prevent application to crops, the nature of military operations makes it likely that accidents may occur and that mistakes may be made which result in direct applications to non-target areas. In such cases it is possible that food crops and water supplies in restricted localities could receive heavy doses of the 2,4-D-2,4,5-T mixture. A much more likely possibility is drift of relatively small amounts of the herbicides to non-target areas. The n-butyl esters of both 2,4-D and 2,4,5-T are volatile and substantial amounts of both chemicals may vaporize at temperatures prevalent in Vietnam and escape from the target area.

If mixtures of 2,4-D and 2,4,5-T were applied directly to food crops at the rates being used in Vietnam for defoliation (about 26 lbs/acre), it is theoretically possible that amounts potentially hazardous to humans could be deposited on food. The butyl ester of 2,4,5-T applied to an improved pasture at the rate of 2 pounds per acre was found to result in an initial deposit of about 300 ppm on the forage (*12*). Direct application to food crops at 26 pounds per acre could not reasonably be expected to result in initial residues greater than 3900 ppm. Assuming initial residues on food crops as high as 3900 ppm from direct application at the rate of 26 pounds per acre is unrealistic. Such an assumption requires that: 1) surface/volume ratios of food items be similar to that of forage grasses; 2) all portions of food items, e.g., husks of corn, peels of banana, shells of coconut, be consumed; 3) there is no loss of herbicide during preparation and cooking; and 4) translocation of herbicide into portions of the plants used for food, e.g., root crops, banana pulp, and coconut flesh and milk, results in residues as high as if these portions had been treated directly. None of these is true. Some items of food could not be contaminated by direct contact with the spray formulation. Translocation from foliage to underground roots of sweet potato and fruits of peanut and into aboveground frnits such as coconut would be required for these foods to be contaminated.

Table 5 breaks down an estimated diet for a 60-kilogram Vietnamese into three parts according to the rapidity with which 2,4-D and 2,4,5-T might be translocated into the portions consumed for food.

Table 6 estimates the maximum believable concentrations of the herbicides in such a diet as being 3900 ppm initially, 1560 ppm after one week and 910 ppm after 2 weeks (See Table 2 for estimated rates of disappearance of 2,4,5-T residues from forage). The maximum dosage retained, substantially greater than any realistic figures, is about 30/mg/kg/day.

TABLE 5.—*Estimated for a 60-kilogram Vietnamese divided into three groups based on the rapidity with which 2,4-D and 2,4,5-T, might be translocated into portions consumed for food.*[1]

A. Foods into which very rapid movement is possible, not necessarily probable, and maximum concentration is attained immediately

	Dietary portions consumed g/person/day
Leafy green vegetable	52
Other vegetables	196
Condiment vegetable	40
Bananas	107
Other fruits	75
Total	470

B. Foods into which moderately rapid movement is possible, though not necessarily probable, and maximum concentration is attained within 1 week

Cereals	500
Fish and meat	311
Fish sauce	1
Spices	1
Coconut flesh	25
Coconut milk	12
Total	850

C. Foods into which movement is relatively slow and maximum concentration is attained within 2 weeks

Root vegetables	80
Beverage	5
Sugar and vegetable oils	95
Total	180

[1] Components of diet and amounts consumed adapted from "Federation of Malaya Nutrition Survey", Report Interdepartmental Committee for Nutrition for National Defense, 1964, 365 pp. (Modified in consultation with Vietnamese students at Louisiana State University.)

TABLE 6.—*Calculation of upper limits for daily dose of 2,4-D and 2,4,5-T combined when all food is fully exposed to one aerial application at 26 lbs/A.*[1]

[Maximum possible contamination, mg/kg/day]

Food Group	gm/person/day	Immediate	After 1 week	After 2 weeks
Group A	470	30	12	7
Group B	850		22	13
Group C	180			3
Total		30	34	23

[1] See Table 5.

The possibility that any of these assumptions might approach reality is probably nil. Many crops that are treated directly at such high rates show severe damage within a few days and would not be harvested. Translocation or penetration of residues into coconut meat and milk and banana pulp would take some time to occur, if at all, and would probably be at levels much lower than amounts deposited on foilage. The amount of residues of these chemicals that would penetrate into kernels of mature rice through palea and lemma is probably a very small percentage, if any, of the dose applied. There does not appear to be information on these points, however.

Probably the greatest potential hazard from contaminated food would be from ingesting leafy green vegetables, other vegetables, and fruits.

Much of the drinking water in the villages of Vietnam is from shallow, open wells and from rainwater collected from the roofs of thatched houses. Direct application of heavy rates of herbicide to these sources could result in the ingestion of substantial amounts of the chemicals in rainwater collected from roofs of houses. Assuming that about 270 mg of herbicide is applied per sq. ft. of surface, that water supplies are collected from rain falling on a roof having a total area of 200 sq. ft., and that all of the herbicide is washed off immediately after application and collected in containers holding 200 liters, a 60 kg person drinking 3l. of water could get about 10 mg/kg/day. No information is available on the amounts of these chemicals that can be washed off a thatched roof by rainfall. However, data on rates of dissipation of 2,4-D and 2,4,5-T from dead litter material in pastures show rates of disappearance slower than for green tissues (*12*). Thus, it appears reasonable to expect considerably less than 100 percent of the amount applied to be washed off a thatch roof treated with 2,4-D and 2,4,5-T. Even if it were all washed off, it appears to be unreasonable to expect it all to be captured in vessels having a combined capacity of only 200 liters. Therefore, any reasonable assumption would be that a person getting drinking water from such a source contaminated at the maximum rate possible would get much less than 10 mg/kg/day.

Drinking water from a shallow well contaminated at the maximum rate expected from a 26 lb/acre application, assuming a depth of 4 feet for water in the well, would amount to less than 0.5 mg/kg/day, and thus would pose no hazard.

Other possible routes of entry into humans from accidental direct application to non-target areas would be by inhalation and penetration through the skin. There is inadequate information available on these points to form any concept of the potential hazard of residues from these sources. Way (*17*) has suggested that there is little hazard of transport across the skin barrier. However, these materials are fat soluble which might encourage their percutaneous absorption. At least one study of the acute toxicity of the defoliant, Purple, (esters of 2,4-D and 2,4,5-T) suggested that skin absorption of the animals tested occurred but was perhaps 10–20 times slower than absorption from the GI tract (*10*). In the extreme situation of a nude, pregnant female, prone beneath an area of spraying of the defoliant, Orange, the maximum impingement of 20.2 mg/kg of 2,4,5-T on her skin might be expected. This should probably be viewed as equivalent to 2.0 mg/kg of an oral dose.

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1907

SOME ECOLOGICAL EFFECTS

Summary

Elimination of types of vegetation on which mammals, birds, fish, and insects and other species depend, can severely reduce or eliminate them from the treated habitat. However, altering the vegetation makeup may also benefit certain types of wildlife. The elimination of water hyacinth from some water bodies, for example, has proved a necessity for the survival of sport fishery programs.

2,4,5-T has been found to affect non-target organisms in a number of ways:

1. Although chickens, quail and mallard ducks appear to be relatively resistant to 2,4,5-T, the herbicide has been reported to reduce egg production in domestic chickens.
2. Some formulations of 2,4,5-T, such as certain esters, have been found to be quite toxic to fish and oysters.
3. The hydrocyanic acid content of sudan grass was found to increase 70 percent following treatment with 2,4,5-T. Nitrate levels in certain plants may increase up to 36 percent following treatment with 2,4,5-T, making them more toxic to mammals. Increasing the toxicity of plants to wildlife might have important effects in nature.
5. 2,4,5-T may cause some species of microorganisms to decrease in number while having no effect upon other species. In soil, 2,4,5-T has been found to largely disappear in about three months.

2,4,5-T has been found to influence non-target organisms both directly and indirectly through habitat changes. The impact of 2,4,5-T on the principal classes of non-target organisms is presented in the following sections.

Mammals

Roe and Hymas (*12*) presented data to indicate that the acute oral toxicity LD_{50} of 2,4,5-T to various species of mammals was about 500 mg/kg.

2,4,5-T has some repellent action. When cottontail rabbits (*Sylvilagus floridanus*) were given a choice of either 2,4,5-T treated vegetation or untreated, the rabbits consumed almost none of the treated vegetation. (*14*)

1907